Title: PEPTIDES AND SUPPORTED PEPTIDES FOR TREATING SKIN DISEASES

VEGF
3rd Round with C7C library
Acid eluted

Consensus INP W

(57) Abstract: The present invention provides peptides and supported peptides for treating proliferative diseases. In particularly preferred embodiments, the present invention provides peptides and supported peptides for treating diseases of the skin, such as rosacea. In some particularly preferred embodiments, the supported peptides of the present invention are anti-VEGF peptides. In alternative particularly preferred embodiments, the anti-VEGF peptides are expressed on a scaffold protein. In some most preferred embodiments, the scaffold proteins is BBI.
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:
- without international search report and to be republished upon receipt of that report
PEPTIDES AND SUPPORTED PEPTIDES
FOR TREATING SKIN DISEASES


Field of the Invention

The present invention provides peptides and supported peptides for treating proliferative diseases. In particularly preferred embodiments, the present invention provides peptides and supported peptides for treating diseases of the skin, such as rosacea. In some particularly preferred embodiments, the supported peptides of the present invention are anti-VEGF peptides. In alternative particularly preferred embodiments, the anti-VEGF peptides are expressed on a scaffold protein. In some most preferred embodiments, the scaffold proteins is BBI.

Background of the Invention

Angiogenesis is the development of a blood supply to a given area of tissue. Angiogenesis is part of normal embryonic development and revascularization of wound beds, as well as due to the stimulation of vessel growth by inflammatory or malignant cells. Angiogenesis is also the process through which tumors or inflammatory conditions derive a blood supply through the generation of microvessels.

Angiogenesis is regulated in normal and malignant cancer tissues by the balance of angiogenic stimuli and angiogenic inhibitors that are produced in the target tissue and at distant sites (See, Fidler et al., [1998]; and McNamara et al., [1998]). Vascular endothelial growth factor-A (VEGF, also known as vascular permeability factor, "VPF") is a primary stimulant of angiogenesis. VEGF is a multifunctional cytokine that is induced by hypoxia and oncogenic mutations and can be produced by a wide variety of tissues (See, Kerbel et al., [1998]; and Mazure et al., [1996]).

The recognition of VEGF as a primary stimulus of angiogenesis in pathological conditions has led to various attempts to block VEGF activity. Inhibitory anti-VEGF receptor antibodies,
soluble receptor constructs, antisense strategies, RNA aptamers against VEGF and low molecular weight VEGF receptor tyrosine kinase (RTK) inhibitors have all been proposed for use in interfering with VEGF signaling (See, Siemeister et al., [1998]). In fact, monoclonal antibodies against VEGF have been shown to inhibit human tumor xenograft growth and ascites formation in mice (See, Kim et al., [1993]; Asano et al., [1998]; Mesiano et al., [1998]; Luo et al., [1998a] and [1998b]; and Borgstrom et al., [1996] and [1998]).

RTKs comprise a large family of transmembrane receptors for polypeptide growth factors with diverse biological activities. The intrinsic function of RTKs is activated upon ligand binding, which results in phosphorylation of the receptor and multiple cellular substrates, and subsequently in a variety of cellular responses. (See, Ullrich & Schlessinger, Cell 61:203-212 [1990]).

Angiogenesis, involving VEGF and RTKs is not only involved in cancer development, as many other diseases or conditions affecting different physiological systems are angiogenesis-dependent, such as arthritis and atherosclerotic plaques (bone and ligaments), diabetic retinopathy, neovascular glaucoma, macular degeneration, ocular herpes, trachoma and corneal graft neovascularization (eye), psoriasis, scleroderma, rosacea, hemangioma and hypertrophic scarring (skin), vascular adhesions and angiofibroma (blood system).

VEGF is an angiogenesis factor of major importance for skin vascularization (Detmar [2000]). VEGF expression is upregulated in the hyperplastic epidermis of psoriasis (Detmar and Yeo et al. [1995]), in healing wounds and in other skin diseases characterized by enhanced angiogenesis (Detmar [2000], supra). Targeted overexpression of VEGF in the epidermis of transgenic mice was reported to result in enhanced skin vascularization with equal numbers of tortuous and leaky blood vessels (See e.g., Brown et al., [1998]). Also, chronic synthesis of VEGF in mouse skin leads to the first histologically equivalent murine model of human psoriasis (Xia et al., [2003]) that is reversible by binding agents specific for VEGF.

The Bowman-Birk protease inhibitor (BBI) is a designation of a family of stable, low molecular weight trypsin and chymotrypsin enzyme inhibitors found in soybeans and various other seeds, mainly leguminous seeds and vegetable materials. BBI comprises a family of disulfide bonded proteins with a molecular weight of about 8 kD (See e.g., Chou et al., Proc. Natl. Acad. Sci. USA 71:1748-1752 [1974]; Yavelow et al., Proc. Natl. Acad. Sci. USA 82:5395-5399 [1985]; and Yavelow et al., Cancer Res. (Suppl.) 43:2454s-2459s [1983]). BBI has a pseudo-symmetrical structure of two tricyclic domains each containing an independent native binding loop, the native loops containing binding sites for both trypsin and chymotrypsin (See, Liener, in Summerfield and Bunting (eds), Advances in Legume Science, Royal Bot. Gardens, Kew, England). These binding sites each have a canonical loop structure, which is a motif found in a variety of serine proteinase inhibitors (Bode and Huber, Eur. J. Biochem., 204:433-451 [1992]). Commonly, as in one of the

STI inhibits the proteolytic activity of trypsin by the formation of a stable stoichiometric complex (See e.g., Liu, Chemistry and Nutritional Value of Soybean Components, *In: Soybeans, Chemistry, Technology and Utilization*, pp. 32-35, Aspen Publishers, Inc., Gaithersburg, Md., [1999]). STI consists of 181 amino acid residues with two disulfide bridges and is roughly spherically shaped (See e.g., Song et al., J. Mol. Biol., 275:347-63 [1998]). The trypsin inhibitory loop lies within the first disulfide bridge. The Kunitz-type soybean trypsin inhibitor (STI) has played a key role in the early study of proteinases, having been used as the main substrate in the biochemical and kinetic work that led to the definition of the standard mechanism of action of proteinase inhibitors.

Eglin C is a small monomeric protein that belongs to the potato chymotrypsin inhibitor family of serine protease inhibitors. The proteins that belong to this family are usually small (60-90 amino acid residues in length) and contain no disulfide bonds. Eglin C, however, is highly resistant to denaturation by acidification or heat regardless of the lack of disulfide bonds to help stabilize its tertiary structure. The protein occurs naturally in the leech *Hirudo medicinalis*.

**Summary of the Invention**

The present invention provides peptides and supported peptides for treating proliferative diseases. In particularly preferred embodiments, the present invention provides peptides and supported peptides for treating diseases of the skin, such as rosacea. In some particularly preferred embodiments, the supported peptides of the present invention are anti-VEGF peptides. In alternative particularly preferred embodiments, the anti-VEGF peptides are expressed on a scaffold protein. In some most preferred embodiments, the scaffold proteins is BBI.

In some preferred embodiments, the present invention provides cosmetic and/or pharmaceutical compounds suitable for improving the appearance of skin. The present invention further provides peptides that block binding of a protein. In some preferred embodiments, the protein is VEGF. In some particularly preferred embodiments, the peptide is expressed in a protease-resistant scaffold. In some especially preferred embodiments, the scaffold is a protease inhibitor (e.g., BBI, STI, or Eglin chymotrypsin inhibitor). In some most preferred embodiments, the protease inhibitor is BBI.
In some embodiments, the present invention provides cosmetic and/or pharmaceutical compounds for improving the appearance of skin comprising at least one polypeptide or a peptide. In some preferred embodiments, the polypeptide or peptide binds to VEGF. In alternative embodiments, the binding of the polypeptide or peptide to VEGF blocks the downstream activity of VEGF. In some embodiments, the compounds comprise at least one peptide, while in other embodiments, the compounds comprise at least one polypeptide. In some preferred embodiments, the peptide has an amino acid sequence selected from the group consisting of SEQ ID NOS:1-7. In additional preferred embodiments, the peptide has a conserved binding sequence, the sequence being XXLWPXWC (SEQ ID NO:15). In some preferred embodiments, the sequence comprises SEQ ID NO:15. In further embodiments, the sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:8-14, 16 and 17. In further embodiments, the sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:22-24. In alternative preferred embodiments, the compounds have a sequence, the sequence being at least 70%, preferably 80%, more preferably 90%, and most preferably 95% homologous to the sequences set forth herein. In some preferred embodiments, the polypeptide has a molecular weight that is preferably between 500 Daltons and 30,000 Daltons, more preferably between 1000 Daltons and 10,000 Daltons, and most preferably from 1500 Daltons to 8,000 Daltons.

In some preferred embodiments, the compounds find use in the improvement of skin in an organism (i.e., subject) having a skin disorder. In some preferred embodiments, the skin disorder is an angiogenic skin disorder. In additional preferred embodiments, the skin disorder is at least one selected from the group consisting of psoriasis, venous ulcers, acne, rosacea, warts, eczema, hemangiomas and lymphangiogenesis, etc. In some particularly preferred embodiments, the skin disorder is rosacea.

In other preferred embodiments, the present invention provides cosmetic and/or pharmaceutical compounds for improving the appearance of skin. In these preferred embodiments, the compounds comprise at least one peptide or polypeptide and at least one scaffold, the peptide or polypeptide being expressed in the scaffold. In some particularly preferred embodiments, the at least one peptide or polypeptide is a loop. In other particularly preferred embodiments, the loop is closed by a disulfide bond. In some preferred embodiments, the polypeptide or peptide binds to VEGF. In alternative embodiments, the binding of the polypeptide or peptide to VEGF blocks the downstream activity of VEGF. In some particularly preferred embodiments, the peptide is expressed in a protease-resistant scaffold. In some especially preferred embodiments, the scaffold is a protease inhibitor (e.g., BBI, STI, or Eglin chymotrypsin inhibitor). In some most preferred embodiments, the protease inhibitor is BBI.
In some preferred embodiments, the compounds further comprise at least one peptide. Preferably, the peptide has an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-7, 16 and 17. In alternative embodiments, peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 8-14. Most preferably, the compounds comprise an amino acid sequence selected from the group consisting of SEQ ID NOS: 22-24. In some preferred embodiments, the peptide has a conserved binding sequence, the sequence being XXLWFXWC (SEQ ID NO:15). In some preferred embodiments, the compounds have a sequence, the sequence being at least 70%, preferably 80%, more preferably 90%, and most preferably 95% identical to the sequences set forth herein. The peptide molecular weight is preferably between 500 Daltons and 45,000 Daltons, more preferably between 1000 Daltons and 12,000 Daltons, and most preferably from 1500 Daltons to 10,000 Daltons. In some preferred embodiments, the compounds comprise at least one polypeptide.

The present invention provides compositions comprising at least one peptide selected from the group consisting of SEQ ID NOS: 1-14, 16 and 18, wherein the peptide binds to a vascular endothelial growth factor. In some preferred embodiments, the peptide is expressed in a protease resistant scaffold. In alternative preferred embodiments, the scaffold is a protease inhibitor. In some more preferred embodiments, the protease inhibitor is selected from the group consisting of Bowman-Birk Inhibitor, soybean trypsin inhibitor, and Eglin chymotrypsin inhibitor. In some most preferred embodiments, the scaffold is Bowman-Birk inhibitor. In still further embodiments, the protease resistant scaffold and the peptide comprise a fusion protein. In some particularly preferred embodiments, the composition comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 22-24. In additional embodiments, the scaffold comprises the amino acid sequence set forth in SEQ ID NO:19. In still further embodiments, the scaffold comprises an amino acid sequences set forth in SEQ ID NOS: 20 and 21. In yet additional embodiments, the amino acid sequences set forth in SEQ ID NOS: 20 and 21 are replaced by at least one peptide having an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-8, 16 and 17.

The present invention also provides cosmetic and/or pharmaceutical compositions comprising the at least one peptide that binds to a vascular endothelial growth factor. In some embodiments, the composition is capable of modulating angiogenesis. In additional embodiments, the composition further comprises a scaffold comprising a protease inhibitor. In some preferred embodiments, the protease inhibitor is selected from the group consisting of Bowman-Birk Inhibitor, soybean trypsin inhibitor, and Eglin chymotrypsin inhibitor. In some preferred embodiments, the scaffold is Bowman-Birk inhibitor. In some particularly preferred embodiments, the scaffold comprises the amino acid sequence set forth in SEQ ID NO:19. In some alternative
embodiments, the scaffold comprises the amino acid sequences set forth in SEQ ID NOS:20 and 21. In further preferred embodiments, the amino acid sequences set forth in SEQ ID NOS:20 and 21 are replaced by at least one peptide having an amino acid sequence selected from the group consisting of SEQ ID NOS:1-8, 16 and 17.

The present invention also provides methods for modulating angiogenesis comprising: i) providing a composition comprising a peptide contained within a scaffold; ii) providing a subject to be treated; and iii) applying the composition to the subject in an area in which angiogenesis modulation is desired. In some embodiments, the peptide binds to a vascular endothelial growth factor (VEGF). In some preferred embodiments, the vascular endothelial growth factor (VEGF) is VEGF-A. In further preferred embodiments, the scaffold is selected from the group consisting of Bowman-Birk inhibitor, soybean trypsin inhibitor, and Eglin chymotrypsin inhibitor. In some particularly preferred embodiments, the scaffold is Bowman-Birk inhibitor. In some further embodiments, the scaffold comprises the amino acid sequence set forth in SEQ ID NO:19. In still further embodiments, the scaffold comprises the amino acid sequences set forth in SEQ ID NOS:20 and 21. In some particularly preferred embodiments, the amino acid sequences set forth in SEQ ID NOS:20 and 21 are replaced by at least one peptide having an amino acid sequence selected from the group consisting of SEQ ID NOS:1-8, 16 and 17. In still further particularly preferred embodiments, the scaffold and the peptide are encoded by an amino acid sequence selected from the group consisting of SEQ ID NOS:22-24.

The present invention also provides methods for decreasing the activity of a vascular endothelial growth factor comprising the steps of: i) providing a subject; and ii) administering the composition comprising at least one peptide that binds to the vascular endothelial growth factor to the subject, under conditions such that the activity of the vascular endothelial growth factor is decreased. In some embodiments, the vascular endothelial growth factor (VEGF) is VEGF-A. In some particularly preferred embodiments, the composition comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:22-24.

In some additional preferred embodiments, the compounds are used for the improvement of skin in an organism (i.e., a subject) having a skin disorder. In additional preferred embodiments, the skin disorder is at least one selected from the group consisting of psoriasis, venous ulcers, acne, rosacea, warts, eczema, hemangiomas and lymphangiogenesis, etc. In some particularly preferred embodiments, the skin disorder is rosacea.

In yet further embodiments, the present invention provides cosmetic and/or pharmaceutical compositions comprising at least one polypeptide or peptide, as set forth herein, and a physiologically acceptable carrier or excipient. Preferably, the compound is present in an amount of about 0.0001% to about 5% by weight based on the total weight of the composition. Also
preferably, the compound is present in an amount of about 0.001% to about 0.5% by weight based on the total weight of the composition. The composition may be in the form of an emulsified vehicle, such as a nutrient cream or lotion, a stabilized gel or dispersion system, a treatment serum, a liposomal delivery system, a topical pack or mask, a surfactant-based cleansing system such as a shampoo or body wash, an aerosolized or sprayed dispersion or emulsion, a hair or skin conditioner, styling aid, or a pigmented product such as makeup, as well as other suitable make-up and cosmetic preparations. In some embodiments, the carrier is preferably at least one selected from the group consisting of water, propylene glycol, ethanol, propanol, glycerol, butylene glycol and polyethylene glycol.

In yet further embodiments, the present invention provides means for decreasing VEGF activity and/or levels. In some preferred embodiments, the VEGF activity and/or levels are decreased in the epidermis. In some embodiments, the method comprising applying an effective amount of at least one of the compounds described herein to an organism in need thereof.

In additional embodiments, the present invention provides applications for hair and/or skin treatment, as well as applications wound healing, treatment of proliferative diseases, etc. Thus, the present invention provides compositions and methods suitable for application in/on humans and other animals.

Description of the Figures

Figure 1 provides a sequence summary of VEGF binding phage clones (SEQ ID NOS:1-14). Twenty-four phage clones were sequenced after 3 rounds of panning. The sequence alignment tree indicates a highly conserved sequence motif ACXLWPXXWC (SEQ ID NO:18). The number in parentheses represents the frequency of that sequence within the 24 clones sequenced after the third round of panning.

Figure 2 provides results of a phage ELISA to demonstrate the binding of unique clones to VEGF and not to BSA. Equivalent amounts of phage were evaluated to determine their relative binding affinity to hVEGF165. The clone number and randomized sequence are indicated below each symbol. Target-bound phage were detected with anti-M13-HRP. The HRP was monitored with ABTS substrate at an A405nm after 30 minutes (n=3).

Figure 3 provides results of a BIACORE binding analysis of VEGF binding peptides. Binding curves were obtained as described in the Examples. Data were fit to a two state reaction model with conformation change: Analyte (A) binds to ligand (B) to form complex AB. Complex AB changes to AB* which cannot dissociate directly to A + B. Panel A provides results for biotinylated peptide CK37281. ka1=2.84e3 M⁻¹s⁻¹ kd1=0.0122 s⁻¹ ka2=1.5e-3 s⁻¹ kd2=3.36e⁻³ s⁻¹ K_D=1.92e⁻⁶ M. Panel B provides results for CK37283 (6000 RU VEGF, 3500 RU TNF□ no
buffer only subtraction); $k_{a1} = 1.24 \times 10^4 \text{M}^{-1} \text{s}^{-1}$, $k_{d1} = 0.318 \text{s}^{-1}$, $k_{a2} = 6.34 \times 10^{-3} \text{s}^{-1}$, $k_{d2} = 1.23 \times 10^3 \text{s}^{-1}$, $K_D = 4.90 \times 10^{-6} \text{M}$. Panel C provides results for v114 control peptide (1000 RU VEGF, 850 RU TNFα). Data were fit to a 1:1 Langmuir binding $k_{a1} = 7.51 \times 10^2 \text{M}^{-1} \text{s}^{-1}$, $k_{d1} = 0.167 \text{s}^{-1}$, $K_D = 2.23 \times 10^{-7} \text{M}$. Figure 4 provides plasmid maps used in the Examples. Panel A provides the map for pCB04 WT expression phagemid for expression of C-terminal His6X tagged beta-lactamase. Panel B provides the map for pME22 N-terminal stuffer phagemid for cloning using BbsI restriction sites. Panel C provides the map for pCM01 N-terminal aVEGF-BLA fusion expression phagemid. Figure 5 provides a summary of N-terminal fusion cloning strategy using BbsI cloning sites (SEQ ID NOS:32-42).

Figure 6 provides an SDS-PAGE gel of His-tag purified beta-lactamase fusions with peptides. IMAC purified BLA versions and different peptides were concentrated and loaded onto an SDS PAGE gel (4-12%). Lanes 1 & 10: MW markers. Lane 2: pCB04 (WT with 6Xhis tag); Lanes 3,4,5,6: pCM01 aVEGF-BLA N-terminal fusion protein scaffold; and Lanes 7,8: pCM02 achaemotrypsin-BLA N-terminal fusion protein.

Figure 7 provides a graph showing that aVEGF peptide-BLA fusion binds specifically to VEGF. Increasing concentrations of pCM01 (aVEGF peptide-BLA fusion) and pCB04 (WT) were added to VEGF coated wells of a microtiter plate. Residual bound nitrocefin activity was measured after washing 5X with nitrocefin assay buffer (0.125% n-octyl-beta-D-glucopyranoside in PBS).

Figure 8 provides a graph showing inhibition of VEGF-induced HUVEC proliferation by anti-VEGF peptide (filled circles). Proliferation was monitored by radioactive incorporation of $^3$H thymidine ($n = 3$). Anti-VEGF antibody (open circles) was used as a positive control, as described in the Examples.

Figure 9 provides the BBI gene sequence (SEQ ID NO:19) designed for efficient cloning. The protein signal sequence is italicized while the trypsin loop (CTKSNPPQC; SEQ ID NO:20) and chymotrypsin loop (CALSYPAPQC; SEQ ID NO:21) are highlighted in bold.

Figure 10 provides an SDS PAGE gel showing the results of refolding anti-VEGF BBI. Anti-VEGF BBI was refolded in the presence or absence of subtilisin BPN’ Y217L. The lanes are as follows: Lane 1: Hampton Foldit 11, refolding buffer, -subtilisin; Lane 2: Hampton Foldit 11 refolding buffer, +subtilisin; Lane 3: Hampton Foldit 13 refolding buffer, -subtilisin; Lane 4, Hampton Foldit 13 refolding buffer, + subtilisin; and Lane 5, Molecular Weight Markers.

Figure 11 provides a graph showing that BBI-VEGF1 (SEQ ID NO:22) binds specifically to VEGF.

Figure 12 provides a graph showing HUVEC results for designated peptides.
Figure 13 provides sequences of three BBI-VEGF fusions, BBI-VEGF1 (SEQ ID NO:22), BBI-VEGF2 (SEQ ID NO:23) and BBI-VEGF12 (SEQ ID NO:24). Fusions BBI-VEGF1 and BBI-VEGF2 have only one of the binding loops replaced; fusion BBI-VEGF12 has both of the binding loops replaced.

Description of the Invention

The present invention provides peptides and supported peptides for treating proliferative diseases. In particularly preferred embodiments, the present invention provides peptides and supported peptides for treating diseases of the skin, such as rosacea. In some particularly preferred embodiments, the supported peptides of the present invention are anti-VEGF peptides. In alternative particularly preferred embodiments, the anti-VEGF peptides are expressed on a scaffold protein. In some most preferred embodiments, the scaffold proteins is BBI.

Unless otherwise indicated, the practice of the present invention involves conventional techniques commonly used in molecular biology, microbiology, and recombinant DNA, which are within the skill of the art. Such techniques are known to those of skill in the art and are described in numerous texts and reference works (See e.g., Sambrook et al., "Molecular Cloning: A Laboratory Manual", Second Edition (Cold Spring Harbor), [1989]); and Ausubel et al., "Current Protocols in Molecular Biology" [1987]). All patents, patent applications, articles and publications mentioned herein, both supra and infra, are hereby expressly incorporated herein by reference.

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. For example, Singleton and Sainsbury, Dictionary of Microbiology and Molecular Biology, 2d Ed., John Wiley and Sons, NY (1994); and Hale and Marham, The Harper Collins Dictionary of Biology, Harper Perennial, NY (1991) provide those of skill in the art with a general dictionaries of many of the terms used in the invention. Although any methods and materials similar or equivalent to those described herein find use in the practice of the present invention, the preferred methods and materials are described herein. Accordingly, the terms defined immediately below are more fully described by reference to the Specification as a whole. Also, as used herein, the singular "a," "an," and "the" includes the plural reference unless the context clearly indicates otherwise. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. It is to be understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these may vary, depending upon the context they are used by those of skill in the art.
Furthermore, the headings provided herein are not limitations of the various aspects or embodiments of the invention which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification as a whole. Nonetheless, in order to facilitate understanding of the invention, a number of terms are defined below.

**Definitions**

As used herein, the term "vascular endothelial growth factor" (VEGF) refers to proteins with the ability to stimulate vascular growth, including those designated "VEGF-A" known to those of skill in the art.

As used herein, the term "anti-VEGF" (aVEGF) refers to peptides and other compositions that recognize (i.e., bind) to VEGF. In preferred embodiments, these peptides/compositions modulate VEGF activity.

The term "angiogenesis" refers to the biological processes which result in the development of blood vessels and/or increase in the vascularity of tissue in an organism. In particular embodiments herein, the term refers to the process through which tumors or other rapidly proliferating tissue derive a blood supply through the generation of microvessels.

The terms "angiogenic disease," "angiogenic disorder," and "angiogenic skin disorder," are used in reference to a disorder, generally a skin disorder or related disorder which occurs as a consequence of or which results in increased vascularization in tissue. Often times, the etiology of the angiogenic disease is unknown. However, whether angiogenesis is an actual cause of a disease state or is simply a condition of the disease state is unimportant, but the inhibition of angiogenesis in treating or reversing the disease state or condition is an important aspect of the present invention. Thus, it is not intended that the present invention be limited to any particular mechanisms of action. Examples of angiogenic skin disorders which are suitable for treatment utilizing compounds of the present invention include, but are not limited to psoriasis, acne, rosacea, warts, eczema, hemangiomas and lymphangiogenesis, Sturge-Weber syndrome, neurofibromatosis, tuberous sclerosis, chronic inflammatory disease, and arthritis. Any skin disorder which has as a primary or secondary characterization, increased vascularization, is considered an angiogenic skin disorder herein. Thus, the compounds provided by the present invention find use in treatment of a wide variety of diseases and/or conditions.

The term "rosacea" is used to describe acne, rosacea, or erythematosa characterized by vascular and follicular dilation typically involving the nose and contiguous portions of the cheeks. Rosacea may vary from very mild but persistent erythema to extensive hyperplasia of the sebaceous glands with deep-seated papules and pustules and be accompanied by telangiectasia at
the affected erythematous sites. This condition is also referred to as “hypertrophic rosacea” or “rhinophyma,” depending upon the severity of the condition. It is intended that the term encompass all of the various forms of the condition.

The term "wart" is used to describe a small, usually hard growth on the skin. Also known as a “verruca,” warts are flesh-colored growths of the skin which are characterized by circumscribed hypertrophy of the papillae of the corium, with thickening of the malpighian, granulation and keratin layers of the epidermis. Verruca vulgaris, a subset of warts or verruca, is characterized by infection of the keratinocytes with human papillomavirus.

The term "psoriasis" is used to describe a skin condition which is characterized by the eruption of circumscribed, discrete and confluent, reddish, silvery-scaled maculopapules. Although it is not intended that the present invention be limited to any particular body area, psoriatic lesions typically occur on the elbows, knees, scalp and trunk. Microscopically, these lesions demonstrate characteristic parakeratosis and elongation of rete ridges.

The term "acne" is used to describe a condition of the skin characterized by inflammatory follicular, papular and pustular eruptions involving the sebaceous apparatus. Although there are numerous forms of acne, the most common form is known as acne simplex or acne vulgaris which is characterized by eruptions of the face, upper back and chest and is primarily comprised of comedones, cysts, papules and pustules on an inflammatory base. The condition occurs primarily during puberty and adolescence due to an overactive sebaceous apparatus which is believed to be affected by hormonal activity.

The term "eczema" is a generic term used to describe acute or chronic inflammatory conditions of the skin, typically erythematous, edematous, papular, vesicular and/or crusting. These conditions are often followed by lichenification, scaling and occasionally, by duskeness of the erythema and, infrequently, hyperpigmentation. Eczema is often accompanied by the sensation of itching and burning. Eczema vesicles form due to intraepidermal spongiosis. Eczema is sometimes referred to colloquially as “tetter,” “dry tetter,” and “scaly tetter.” There are numerous subcategories of eczema, all of which are treated by one or more of the compounds according to the present invention.

As used herein, “CK” followed by an integer refers to a specific peptide. Peptide sequences can be found as described herein (See e.g., Figure 1). As an example, CK37281 refers to the peptide sequence “ACYNLYGWTCGG” (SEQ ID NO:1), as shown in Figure 1.

As used herein, in some embodiments, the “compound” comprises the “complete” protein, (i.e., in its entire length as it occurs in nature (or as mutated)), while in other embodiments it comprises a truncated form of a protein. Thus, in some embodiments, the compounds of the present invention are either truncated or be “full-length.” In addition, in some embodiments, the
truncation is located at the N-terminal end, while in other embodiments the truncation is located at the C-terminal end of the protein. In further embodiments, the compound lacks one or more portions (e.g., sub-sequences, signal sequences, domains or moieties), whether active or not.

The term "organism" is used throughout the specification to describe an animal, preferably a human, to whom treatment, including prophylactic treatment, with the compounds according to the present invention is provided. For treatment of those infections, conditions or disease states which are specific for a specific animal such as a human patient, the term organism refers to that specific animal.

The "host cells" used in the present invention generally are prokaryotic or eukaryotic hosts which contain an expression vector and/or gene of interest. Host cells are transformed or transfected with vectors constructed using recombinant DNA techniques. Such transformed host cells are capable of either replicating vectors encoding the protein variants or expressing the desired protein variant. In the case of vectors which encode the pre- or prepro-form of the protein variant, such variants, when expressed, are typically secreted from the host cell into the host cell medium.

The term "effective amount" is used throughout the specification to describe concentrations or amounts of compounds according to the present invention which may be used to produce a favorable change in the disease or condition treated, whether that change is hair growth or prevention of hair growth.

As used herein, "vitamin B₃ compound" means a compound having the formula:

```
\[ \text{N} \longrightarrow \text{R} \]
```

wherein R is - CONH₂ (i.e., niacinamide), - COOH (i.e., nicotinic acid) or - CH₂OH (i.e., nicotinyl alcohol); derivatives thereof; and salts of any of the foregoing.

As used herein, "non-vasodilating" means that an ester does not commonly yield a visible flushing response after application to the skin in the subject compositions. It is contemplated that the majority of the general population would not experience a visible flushing response, although such compounds may cause vasodilation not visible to the naked eye.

As used herein, "retinoid" includes all natural and/or synthetic analogs of Vitamin A and/or retinol-like compounds which possess the biological activity of Vitamin A in/on the skin, as well as the geometric isomers and stereoisomers of these compounds.

As used herein, the term "bioactivity" refers to a cause and effect relationship between a composition and a biological system. Thus, the term is used as by those skilled in the art of
biotechnology and biological sciences as the phrase that describes a cause and effect relationship between a molecular composition and living biological matter (e.g., tissue, cells, etc.).

As used herein as a noun, the term "bioactive" refers a composition that exhibits bioactivity upon administration to living biological matter (e.g., tissue, cells, etc.). The term is used synonymously with "bioactive compound."

As used herein, "silicone gum" means high molecular weight silicones having an average molecular weight in excess of about 200,000 and preferably from about 200,000 to about 4,000,000. It is intended that the definition encompass non-volatile polyalkyl and polyaryl siloxane gums.

As used herein, the term "polypeptide" refers to a compound made up of a single chain of amino acid residues linked by peptide bonds. The term "protein" herein may be synonymous with the term "polypeptide" or may refer, in addition, to a complex of two or more polypeptides. The exact meaning is that known to those in the art.

As used herein, the terms "expression cassette" and "expression vector" refer to nucleic acid constructs generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid sequence to be transcribed and a promoter. The term "expression cassette" may be used interchangeably herein with "DNA construct" and its grammatical equivalents.

As used herein, the terms "vector" and "cloning vector" refer to nucleic acid constructs designed to transfer nucleic acid sequences into cells.

As used herein, the term "expression vector" refers to a vector that has the ability to incorporate and express heterologous DNA fragments in a foreign cell. Many prokaryotic and eukaryotic expression vectors are commercially available. Selection of appropriate expression vectors is within the knowledge of those of skill in the art.

As used herein, the term "plasmid" refers to a circular double-stranded (ds) DNA construct used as a cloning vector, and which forms an extrachromosomal self-replicating genetic element in some eukaryotes or integrates into the host chromosomes.

As used herein, the term "expression" refers to the process by which a polypeptide is produced based on the nucleic acid sequence of the gene or the chemical synthetic peptide. The process includes both transcription and translation of the gene to produce polypeptide/protein.

As used herein, the term "gene" means the segment of DNA involved in producing a polypeptide chain that may or may not include regions preceding or following the coding region.
As used herein, the terms "nucleic acid molecule" and "nucleic acid sequence" include sequences of any form of nucleic acid, including, but not limited to RNA, DNA and cDNA molecules. It will be understood that, as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding a given protein may be produced, in addition to mutant proteins.

As used herein, "codon" refers to a sequence of three nucleotides in a DNA or mRNA molecule that represents the instruction for incorporation of a specific amino acid into a polypeptide chain.

As used herein, the term "disulfide bridge" or "disulfide bond" refers to the bond formed between the sulfur atoms of cysteine residues in a polypeptide or a protein. In this invention, a disulfide bridge or disulfide bond may be non-naturally occurring and introduced by way of point mutation.

As used herein, the term "salt bridge" refers to the bond formed between oppositely charged residues, amino acids in a polypeptide or protein. In this invention, a salt bridge may be non-naturally occurring and introduced by way of point mutation.

As used herein, an "enzyme" refers to a protein or polypeptide that catalyzes at least one chemical reaction.

As used herein, the term "activity" refers to any activity associated with a particular protein, such as enzymatic activity associated with a protease. In some embodiments, the activity is biological activity. In further embodiments, activity encompasses binding of proteins to receptors which results in measurable downstream effects (e.g., VEGF binding to its cognate receptor). "Biological activity" refers to any activity that would normally be attributed to that protein by one skilled in the art.

As used herein, the term "protease" refers to an enzyme that degrades peptide bonds.

As used herein, "peptide bond" refers to the chemical bond between the carbonyl group of one amino acid and the amino group of another amino acid.

As used herein, "wild-type" refers to a sequence or a protein that is native or naturally occurring.

As used herein, "point mutations" refers to a change in a single nucleotide of DNA, especially where that change results in a sequence change in a protein.

As used herein, "mutant" refers to a version of an organism or protein where the version is other than wild-type. The change may be effected by methods well known to one skilled in the art, for example, by point mutation in which the resulting protein may be referred to as a mutant.

As used herein, "mutagenesis" refers to the process of changing a composition (e.g., protein) from a wild-type composition (e.g., protein) into a mutant or variant composition (e.g., protein).
As used herein, "substituted" and "substitutions" refer to replacement(s) of an amino acid residue or nucleic acid base in a parent sequence. In some embodiments, the substitution involves the replacement of a naturally occurring residue or base.

As used herein, "modification" and "modify" refer to any change(s) in an amino acid or nucleic acid sequence, including, but not limited to deletions, insertions, interruptions, and substitutions. In some embodiments, the modification involves the replacement of a naturally occurring residue or base.

As used herein, "functional portion of a secreted polypeptide" and its grammatical equivalents refers to a truncated secreted polypeptide that retains its ability to fold into a normal, albeit truncated, configuration. In some embodiments, it is contemplated that sufficient residues of a domain of the naturally secreted polypeptide must be present to allow it to fold in its normal configuration independently of the desired polypeptide to which it is attached. However, in most cases, the portion of the secreted polypeptide are both correctly folded and result in increased secretion as compared to its absence. Similarly, in most cases, the truncation of the secreted polypeptide means that the functional portion retains a biological function. In a preferred embodiment, the catalytic domain of a secreted polypeptide is used, although other functional domains may be used, for example, the substrate binding domains. Additionally preferred embodiments utilize the catalytic domain and all or part of the linker region.

As used herein, "loop" refers to a sequence of amino acids, for example 3-20 amino acids, more preferably 5-15 amino acids, even more preferably 5-10 amino acids, and most preferably 7-9 amino acids, which connects structural elements of a protein. Such elements include, but are not limited to beta sheets and helical elements and the connecting loop of a beta-hairpin. In some embodiments, the loop is further stabilized through the use of covalent linkages. In some preferred embodiments, the covalent linkages comprise disulfide bonds, especially as provided herein. In alternative embodiments, the loops are stabilized by the use of other means, including but not limited to amides, hydrogen bonds, and/or salt bridges. In most embodiments, the loops are located on the surface of proteins and may be altered, as provided herein, to confer additional (e.g., desirable) properties to the requisite proteins.

As used herein, "oligonucleotide" refers to a short nucleotide sequence which may be used, for example, as a primer in a reaction used to create mutant proteins.

As used herein, the terms "an oligonucleotide having a nucleotide sequence encoding a gene" and "polynucleotide having a nucleotide sequence encoding a gene," means a nucleic acid sequence comprising the coding region of a gene or in other words the nucleic acid sequence which encodes a gene product. The coding region may be present in either a cDNA, genomic DNA or RNA form. When present in a DNA form, the oligonucleotide or polynucleotide may be single-
stranded (i.e., the sense strand) or double-stranded. Suitable control elements such as enhancers/promoters, splice junctions, polyadenylation signals, etc. may be placed in close proximity to the coding region of the gene if needed to permit proper initiation of transcription and/or correct processing of the primary RNA transcript. Alternatively, the coding region utilized in the expression vectors of the present invention may contain endogenous enhancers/promoters, splice junctions, intervening sequences, polyadenylation signals, etc. or a combination of both endogenous and exogenous control elements.

As used herein, the term "primer" refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is induced, (i.e., in the presence of nucleotides and an inducing agent such as DNA polymerase and at a suitable temperature and pH). The primer is preferably single stranded for maximum efficiency in amplification, but may alternatively be double stranded. If double stranded, the primer is first treated to separate its strands before being used to prepare extension products. Preferably, the primer is an oligodeoxynucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent. The exact lengths of the primers will depend on many factors, including temperature, source of primer and the use of the method.

As used herein, the term "probe" refers to an oligonucleotide (i.e., a sequence of nucleotides), whether occurring naturally as in a purified restriction digest or produced synthetically, recombinantly or by PCR amplification, which is capable of hybridizing to another oligonucleotide of interest. A probe may be single-stranded or double-stranded. Probes are useful in the detection, identification and isolation of particular gene sequences. It is contemplated that any probe used in the present invention will be labelled with any "reporter molecule," so that is detectable in any detection system, including, but not limited to enzyme (e.g., ELISA, as well as enzyme-based histochemical assays), fluorescent, radioactive, and luminescent systems. It is not intended that the present invention be limited to any particular detection system or label.

As used herein, the term "target," when used in reference to the polymerase chain reaction, refers to the region of nucleic acid bounded by the primers used for polymerase chain reaction. Thus, the "target" is sought to be sorted out from other nucleic acid sequences. A "segment" is defined as a region of nucleic acid within the target sequence.

As used herein, the term "polymerase chain reaction" ("PCR") refers to the method well-known in the art (See e.g., U.S. Patent Nos. 4,683,195 4,683,202, and 4,965,188, hereby incorporated by reference), for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. This process for amplifying the target
sequence consists of introducing a large excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence, followed by a precise sequence of thermal cycling in the presence of a DNA polymerase. The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, the mixture is denatured and the primers then annealed to their complementary sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing and polymerase extension can be repeated many times (i.e., denaturation, annealing and extension constitute one "cycle"; there can be numerous "cycles") to obtain a high concentration of an amplified segment of the desired target sequence. The length of the amplified segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the "polymerase chain reaction" (hereinafter "PCR"). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified."

As used herein, the terms "PCR product," "PCR fragment," and "amplification product" refer to the resultant mixture of compounds after two or more cycles of the PCR steps of denaturation, annealing and extension are complete. These terms encompass the case where there has been amplification of one or more segments of one or more target sequences.

As used herein, the term "amplification reagents" refers to those reagents (deoxyribonucleotide triphosphates, buffer, etc.), needed for amplification except for primers, nucleic acid template and the amplification enzyme. Typically, amplification reagents along with other reaction components are placed and contained in a reaction vessel (test tube, microwell, etc.).

As used herein, the term "RT-PCR" refers to the replication and amplification of RNA sequences. In this method, reverse transcription is coupled to PCR, most often using a one enzyme procedure in which a thermostable polymerase is employed, as described in U.S. Patent No. 5,322,770, herein incorporated by reference. In RT-PCR, the RNA template is converted to cDNA due to the reverse transcriptase activity of the polymerase, and then amplified using the polymerizing activity of the polymerase (i.e., as in other PCR methods).

As used herein, the term "hybridization" refers to the process by which a strand of nucleic acid joins with a complementary strand through base pairing, as known in the art.

As used herein, "maximum stringency" refers to the level of hybridization that typically occurs at about Tm-5°C (5°C below the Tm of the probe); "high stringency" at about 5°C to 10°C below Tm; "intermediate stringency" at about 10°C to 20°C below Tm; and "low stringency" at about 20°C to 25°C below Tm. As will be understood by those of skill in the art, a maximum
stringency hybridization can be used to identify or detect identical polynucleotide sequences while an intermediate or low stringency hybridization can be used to identify or detect polynucleotide sequence homologs. The phrases "substantially similar and "substantially identical" in the context of two nucleic acids or polypeptides typically means that a polynucleotide or polypeptide comprises a sequence that has at least 75% sequence identity, preferably at least 80%, more preferably at least 90%, still more preferably 95%, most preferably 97%, sometimes as much as 98% and 99% sequence identity, compared to the reference (i.e., wild-type) sequence. Sequence identity may be determined using known programs such as BLAST, ALIGN, and CLUSTAL using standard parameters. (See e.g., Altschul, et al., J. Mol. Biol. 215:403-410 [1990]; Henikoff et al., Proc. Natl. Acad. Sci. USA 89:10915 [1989]; Karin et al., Proc. Natl Acad. Sci USA 90:5873 [1993]; and Higgins et al., Gene 73:237 - 244 [1988]). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. Also, databases may be searched using FASTA (Pearson et al., Proc. Natl. Acad. Sci. USA 85:2444-2448 [1988]).

As used herein, "equivalent residues" refers to proteins that share particular amino acid residues. For example, equivalent residues may be identified by determining homology at the level of tertiary structure for a protein (e.g., VEGF) whose tertiary structure has been determined by x-ray crystallography. Equivalent residues are defined as those for which the atomic coordinates of two or more of the main chain atoms of a particular amino acid residue of the protein having putative equivalent residues and the protein of interest (N on N, CA on CA, C on C and O on O) are within 0.13 nm and preferably 0.1 nm after alignment. Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the proteins analyzed. The preferred model is the crystallographic model giving the lowest R factor for experimental diffraction data at the highest resolution available, determined using methods known to those skilled in the art of crystallography and protein characterization/analysis.

In some embodiments, modification is preferably made to the "precursor DNA sequence" which encodes the amino acid sequence of the precursor enzyme, but can be by the manipulation of the precursor protein. In the case of residues which are not conserved, the replacement of one or more amino acids is limited to substitutions which produce a variant which has an amino acid sequence that does not correspond to one found in nature. In the case of conserved residues, such replacements should not result in a naturally-occurring sequence. Derivatives provided by the present invention further include chemical modification(s) that change the characteristics of the protein.
In some preferred embodiments, the protein gene is ligated into an appropriate expression plasmid. The cloned protein gene is then used to transform or transfect a host cell in order to express the protein gene. In some embodiments, this plasmid replicates in the hosts, in the sense that it contains the well-known elements necessary for plasmid replication or the plasmid may be designed to integrate into the host chromosome. The necessary elements are provided for efficient gene expression (e.g., a promoter operably linked to the gene of interest). In some embodiments, these necessary elements are supplied as the gene's own homologous promoter if it is recognized, (i.e., transcribed, by the host), a transcription terminator (a polyadenylation region for eukaryotic host cells) which is exogenous or is supplied by the endogenous terminator region of the protein gene. In some embodiments, a selection gene such as an antibiotic resistance gene that enables continuous cultural maintenance of plasmid-infected host cells by growth in antimicrobial-containing media is also included.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

As used herein, the term "recombinant DNA molecule" as used herein refers to a DNA molecule which is comprised of segments of DNA joined together by means of molecular biological techniques.

The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule which is expressed from a recombinant DNA molecule.

The term "native protein" as used herein to indicate that a protein does not contain amino acid residues encoded by vector sequences; that is the native protein contains only those amino acids found in the protein as it occurs in nature. A native protein may be produced by recombinant means or may be isolated from a naturally occurring source.

As used herein the term "portion" when in reference to a protein (as in "a portion of a given protein") refers to fragments of that protein. The fragments may range in size from four amino acid residues to the entire amino acid sequence minus one amino acid.

As used herein, the term "fusion protein" refers to a chimeric protein containing the protein of interest (i.e., VEGF and fragments thereof) joined to an exogenous protein fragment (the fusion partner which consists of a non-VEGF protein). In some embodiments, the fusion partner enhances solubility of the VEGF protein as expressed in a host cell, may provide an affinity tag to allow purification of the recombinant fusion protein from the host cell or culture supernatant, or both. If desired, the fusion protein may be removed from the protein of interest (i.e., VEGF and/or fragments thereof) by a variety of enzymatic or chemical means known to the art.
The terms "in operable combination," "in operable order," and "operably linked" as used herein refer to the linkage of nucleic acid sequences in such a manner that a nucleic acid molecule capable of directing the transcription of a given gene and/or the synthesis of a desired protein molecule is produced. The term also refers to the linkage of amino acid sequences in such a manner so that a functional protein is produced.

As used herein the term "coding region" when used in reference to structural gene refers to the nucleotide sequences which encode the amino acids found in the nascent polypeptide as a result of translation of a mRNA molecule. The coding region is bounded, in eukaryotes, on the 5' side by the nucleotide triplet "ATG" which encodes the initiator methionine and on the 3' side by one of the three triplets which specify stop codons (i.e., TAA, TAG, TGA).

As used herein, the term "structural gene" refers to a DNA sequence coding for RNA or a protein. In contrast, "regulatory genes" are structural genes which encode products which control the expression of other genes (e.g., transcription factors).

As used herein, the term "purified" or "to purify" refers to the removal of contaminants from a sample. For example, recombinant VEGF or aVEGF polypeptides are expressed in host cells and the polypeptides are purified by the removal of host cell proteins; the percent of recombinant VEGF or aVEGF polypeptides is thereby increased in the sample.

The term "isolated" when used in relation to a nucleic acid, as in "an isolated oligonucleotide" or "isolated polynucleotide" refers to a nucleic acid sequence that is identified and separated from at least one contaminant nucleic acid with which it is ordinarily associated in its natural source. Isolated nucleic acid is such present in a form or setting that is different from that in which it is found in nature. In contrast, non-isolated nucleic acids as nucleic acids such as DNA and RNA found in the state they exist in nature. For example, a given DNA sequence (e.g., a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences, such as a specific mRNA sequence encoding a specific protein, are found in the cell as a mixture with numerous other mRNA s which encode a multitude of proteins. However, isolated nucleic acid encoding a VEGF protein includes, by way of example, such nucleic acid in cells ordinarily expressing a VEGF protein where the nucleic acid is in a chromosomal location different from that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in nature. The isolated nucleic acid, oligonucleotide, or polynucleotide may be present in single-stranded or double-stranded form. When an isolated nucleic acid, oligonucleotide or polynucleotide is to be utilized to express a protein, the oligonucleotide or polynucleotide will contain at a minimum the sense or coding strand (i.e., the oligonucleotide or polynucleotide may single-stranded), but may contain both the sense and anti-sense strands (i.e., the oligonucleotide or polynucleotide may be double-stranded).
As used herein, "cosmetic composition" refers to compositions that find use in the cosmetics. The Food Drug and Cosmetic Act (FD&C Act) definition is used herein. Thus, cosmetics are defined by their intended use, as articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body for cleansing, beautifying, promoting attractiveness, or altering appearance. These compositions provide non-therapeutic benefits and are not regulated as pharmaceuticals. However, in some situations, cosmetic compositions are incorporated into pharmaceutical compositions to provide cosmetic benefits (e.g., products that treat skin or hair diseases, but also contain cosmetic compositions for their coloring or other benefits). Also, it is intended that the present invention encompass the use of cosmetics on animals other than humans.

As used herein, the terms "pharmaceutical compositions" and "therapeutic compositions" refer to compositions such as drugs that provide medical benefits, rather than solely cosmetic benefits. In the United States, pharmaceutical and therapeutic compositions are approved by the Food and Drug Administration for treatment and/or prevention of particular conditions.

As used herein, the term "drug" is defined as it is in the FD&C Act definition. Thus, drugs are defined as articles intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease, and articles (other than food) intended to affect the structure or any function of the body of man or other animals.

As used herein, the term "cosmetic benefit" refers to a desired cosmetic change that results from the administration of a personal care composition. Cosmetic benefits include but are not limited to improvements in the condition of skin, hair, nails, and the oral cavity. In preferred embodiments, at least one cosmetic benefit is provided by the skin care, hair care, nail care, and makeup compositions of the present invention.

As used herein, "skin care composition" refers to compositions that are applied to skin in order to provide beneficial properties, including but not limited to wrinkle minimizing, wrinkle removal, decoloring, coloring, skin softening, skin smoothing, depilation, cleansing, etc. In some particularly preferred embodiments, the present invention provides skin care compositions that improve skin tone. In these embodiments, the improvement comprises lessening of wrinkles, smoothing skin texture, modifying skin coloration, and other desired cosmetic benefits.

As used herein, "hair care composition" refers to compositions that are applied to hair to provide beneficial properties such as thickening, thinning, coloring, decoloring, cleansing, conditioning, softening, shaping, etc.

As used herein, "makeup compositions" refer to cosmetic preparations that are used to beautify, caring for, maintaining, or augment the appearance of a human or other animal. "Makeup
compositions” include, but are not limited to color cosmetics, such as mascaras, lipsticks, lip liners, eye shadows, eye-liners, rouges, face powders, foundations, blushes, and nail polish.

As used herein, the term “dispersed phase” is used as by those of skill in the art of emulsion technology as the phase that exists as small particles or droplets suspended in and surrounded by a continuous phase. The dispersed phase is also known as the “internal” or “discontinuous” phase.

As used herein, “penetration enhancers” refer to compositions that facilitate penetration through the upper stratum corneum barrier to the deeper skin layers. Examples of penetration enhancers include, but are not limited to, propylene glycol, azone, ethoxydiglycol, dimethyl isosorbide, urea, ethanol, dimethyl sulfoxide, microroemulsions, liposomes, and nanoemulsions.

As used herein, the terms “emulsifier” and “surfactant” refer to compounds that disperse and suspend the dispersed phase within the continuous phase of a material. Surfactants find particular use in products intended for skin and/or hair cleansing. In particular embodiments, the term “surfactant(s)” is used in reference to surface-active agents, whether used as emulsifiers or for other surfactant purposes such as skin cleansing.

Detailed Description of the Invention

The present invention provides peptides and supported peptides for treating proliferative diseases. In particularly preferred embodiments, the present invention provides peptides and supported peptides for treating diseases of the skin, such as rosacea. In some particularly preferred embodiments, the supported peptides of the present invention are anti-VEGF peptides. In alternative particularly preferred embodiments, the anti-VEGF peptides are expressed on a scaffold protein. In some most preferred embodiments, the scaffold proteins is BBI.

In some preferred embodiments, the present invention provides cosmetic and/or pharmaceutical compounds suitable for improving the appearance of skin. The present invention further provides peptides that block binding of a protein. In some preferred embodiments, the protein is VEGF. In some particularly preferred embodiments, the peptide is expressed in a protease-resistant scaffold. In some especially preferred embodiments, the scaffold is a protease inhibitor (e.g., BBI, STI, or Eglin chymotrypsin inhibitor). In some most preferred embodiments, the protease inhibitor is BBI.

In some embodiments, the present invention provides cosmetic and/or pharmaceutical compounds for improving the appearance of skin comprising at least one polypeptide or a peptide. In some preferred embodiments, the polypeptide or peptide binds to VEGF. In alternative embodiments, the binding of the polypeptide or peptide to VEGF blocks the downstream activity of VEGF. In some embodiments, the compounds comprise at least one peptide, while in other
embodiments, the compounds comprise at least one polypeptide. In some preferred embodiments, the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:1-7, 16, and 17. In additional preferred embodiments, the peptide has a conserved binding sequence, the sequence being XXLWPXWC (SEQ ID NO:15). In some preferred embodiments, the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:22-24. In alternative preferred embodiments, the compounds have a sequence, the sequence being at least 70%, preferably 80%, more preferably 90%, and most preferably 95% homologous to the sequences set forth herein. In some preferred embodiments, the polypeptide has a molecular weight that is preferably between 500 Daltons and 30,000 Daltons, more preferably between 1000 Daltons and 10,000 Daltons, and most preferably from 1500 Daltons to 8,000 Daltons.

In some preferred embodiments, the compounds find use in the improvement of skin in an organism (i.e., subject) having a skin disorder. In some preferred embodiments, the skin disorder is an angiogenic skin disorder. In additional preferred embodiments, the skin disorder is at least one selected from the group consisting of psoriasis, venous ulcers, acne, rosacea, warts, eczema, hemangiomas and lymphangiogenesis, etc. In some particularly preferred embodiments, the skin disorder is rosacea.

In other preferred embodiments, the present invention provides cosmetic and/or pharmaceutical compounds for improving the appearance of skin. In these preferred embodiments, the compounds comprise at least one peptide or polypeptide and at least one scaffold, the peptide or polypeptide being expressed in the scaffold. In some particularly preferred embodiments, the at least one peptide or polypeptide is a loop. In other particularly preferred embodiments, the loop is closed by a disulfide bond. In some preferred embodiments, the polypeptide or peptide binds to VEGF. In alternative embodiments, the binding of the polypeptide or peptide to VEGF blocks the downstream activity of VEGF. In some particularly preferred embodiments, the peptide is expressed in a protease-resistant scaffold. In some especially preferred embodiments, the scaffold is a protease inhibitor (e.g., BBI, STI, or Eglin chymotrypsin inhibitor). In some most preferred embodiments, the protease inhibitor is BBI.

In some preferred embodiments, the compounds further comprise at least one peptide. Preferably, the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-7, 16, and 17. In some alternative the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 14). In some embodiments, the compounds comprise an amino acid sequence selected from the group consisting of SEQ ID NOS:22-24. Most preferably, the compounds comprise SEQ ID NO:22. In some preferred embodiments, the peptide has a conserved binding sequence, the sequence being XXLWPXWC (SEQ ID NO:15). In some preferred embodiments, the compounds have a sequence, the sequence being at least 70%,
preferably 80%, more preferably 90%, and most preferably 95% identical to the sequences set forth herein. The peptide molecular weight is preferably between 500 Daltons and 45,000 Daltons, more preferably between 1000 Daltons and 12,000 Daltons, and most preferably from 1500 Daltons to 10,000 Daltons. In some preferred embodiments, the compounds comprise at least one polypeptide.

In some preferred embodiments, the compounds are used for the improvement of skin in an organism (i.e., a subject) having a skin disorder. In additional preferred embodiments, the skin disorder is at least one selected from the group consisting of psoriasis, venous ulcers, acne, rosacea, warts, eczema, hemangiomas and lymphangiogenesis, etc. In some particularly preferred embodiments, the skin disorder is rosacea.

In yet further embodiments, the present invention provides cosmetic and/or pharmaceutical compositions comprising at least one polypeptide or peptide, as set forth herein, and a physiologically acceptable carrier or excipient. Preferably, the compound is present in an amount of about 0.0001% to about 5% by weight based on the total weight of the composition. Also preferably, the compound is present in an amount of about 0.001% to about 0.5% by weight based on the total weight of the composition. The composition may be in the form of an emulsified vehicle, such as a nutrient cream or lotion, a stabilized gel or dispersion system, a treatment serum, a liposomal delivery system, a topical pack or mask, a surfactant-based cleansing system such as a shampoo or body wash, an aerosolized or sprayed dispersion or emulsion, a hair or skin conditioner, styling aid, or a pigmented product such as makeup, as well as other suitable make-up and cosmetic preparations. In some embodiments, the carrier is preferably at least one selected from the group consisting of water, propylene glycol, ethanol, propanol, glycerol, butylene glycol and polyethylene glycol.

In yet further embodiments, the present invention provides means for decreasing VEGF activity and/or levels. In some preferred embodiments, the VEGF activity and/or levels are decreased in the epidermis. In some embodiments, the method comprising applying an effective amount of at least one of the compounds described herein to an organism in need thereof.

In additional embodiments, the present invention provides applications for hair and/or skin treatment, as well as applications wound healing, treatment of proliferative diseases, etc. Thus, the present invention provides compositions and methods suitable for application in/on humans and other animals.

In additional preferred embodiments, the present invention is directed to at least one peptide or polypeptide, at least one loop and at least one protease-resistant scaffold. Flexible native loops are found on the surface of most protein modules and exist as short stretches of amino acids that connect regions of defined secondary structure. Although crystallographic and NMR
(nuclear magnetic resonance) studies show that native loops are usually less well defined than
alpha-helices and beta-sheets, their conformational freedom is normally restricted substantially
compared with free peptides. Consequently, the binding activities of native loops in proteins
usually differ significantly from those of the corresponding linear amino acid sequence. However,
it is not intended that the present invention be limited to any specific mechanism.

The loops provided by the present invention bind proteins such as VEGF (e.g., VEGF-A).
Binding the loop to the protein prevents the protein from binding to its target. Thus, binding
interactions are thought to be disrupted by binding the loop to the protein. As a result, bioactivity
can be altered as desired. However, it is not intended that the present invention be limited to any
particular mechanism.

The present invention further provides scaffolds to stabilize the loops. STI, BBI and Eglin
C have native loops that bind to and inhibit proteases. In some embodiments, STI and BBI native
loops are replaced with the polypeptides and/or peptides of the invention. In some embodiments,
these sequences are replaced with inhibitors or enhancers of any VEGF, while in other
embodiments, the sequences are replaced with inhibitors or enhancers of VEGF-A. In alternative
embodiments, the sequences are replaced with inhibitors of FGF-5, TGFB-1 and TGFB-2, as well
as inhibitors of the complement pathway such as C2, C3, C4 or C5 inhibitors and Compstatin, etc.
In additional embodiments, STI and BBI native loops are replaced with sequences that have been
isolated using various techniques known in the art (e.g., phage display), such as the VEGF binding
proteins described herein.

In some embodiments, a native loop is replaced with a loop which is 3 to 20 amino acids in
length, preferably 5 to 15 amino acids in length, and more preferably 5 to 10 amino acids in length.
In alternative embodiments, longer sequences find use, as long as they provide binding and/or
inhibition. In addition, peptides suitable for use as replacements of the native loop(s) can form
constrained loops (i.e., a loop formed by the presence to a disulfide bond between two cysteine
residues). In some particularly preferred embodiments, the peptides are between 7 and 9 amino
acids in length.

There are several advantages to using scaffolds to stabilize peptide sequences. In some
preferred embodiments, the biological activity of the peptide is higher and/or effectively modulates
biological function as a result of limiting peptide flexibility and reducing the entropic cost of fixing
the polypeptide sequence in the bioactive conformation. In addition, structural information
obtained by x-ray crystallography finds use in guiding affinity maturation. Furthermore, in some
embodiments, the sequence presented on a structural scaffold is more resistant to proteolytic
degradation in different biological applications. In still further embodiments, the chimeric
construction is obtained in large amount in low cost biological expression systems for industrial applications.

BBI represents a class of protein scaffolds whose binding to proteases is mediated by an exposed native loop that is fixed in a characteristic canonical conformation and which fits into the active site in a manner thought to be similar to that of a substrate (Laskowski and Kato, Ann. Rev. Biochem., 49:593-626 [1980]; and Bode & Huber, supra). The native loop is frequently constrained by the presence of disulfide bridges and/or extensive hydrogen-bonding networks that act to lock the structure into the correct canonical structure. The sequence of this loop determines the specificity of the inhibition, which mirrors the specificity of proteases for their substrates. For example, most trypsin inhibitors have Arg or Lys as their P1 residue. Inhibitors of the BBI family have a network of conserved disulfide bridges that help form a symmetrical structure of two tricyclic domains (Chen et al., supra; Werner and Wemmer, supra; and Liu et al., supra), each containing an independent serine protease binding site. The native binding loop is contained within a region joined by disulfide bridges formed between cysteine residues. The identity of the amino acid residue at the P1 site on each domain is the main determinant of the serine protease inhibited. Native domains possess lysine or arginine for trypsin, leucine or tyrosine for chymotrypsin and alanine for elastase (Tsunogae et al., J. Biochem. (Tokyo) 100:243-246 [1986]). In addition, serine is highly conserved at the P1 position and proline at the P3 position. The individual native loop regions of BBI are well suited for protein loop grafting of previously identified cysteine constrained peptides that bind to targets selectively, as described herein.

Numerous isoforms of BBI have been characterized. For example, the sequence DDESKPCCDQCACHTKNQPPQCRCSMDLNCSCHACKSCICALSYPAQFCVVIDTFDCYE PCKPSEDDKEN (SEQ ID NO:25) provides the amino acid sequence of a BBI backbone described herein. In addition, in some embodiments BBI is truncated with as many as 10 amino acid residues being removed from either the N- or C- terminal. Any of the isoforms described herein, as well as those additional ones known in the art, find use as scaffolds in the present invention.

The present invention provides several advantages over creation of, for example, chimeric proteins. Transfer of an entire protein can be difficult when, for example, a protein domain of interest carries more than one important biological activity. Maintaining one activity (e.g. functionally significant domain-domain interactions) while altering another (e.g. high affinity binding to a co-factor or receptor) can be problematic. The present invention, as indicated herein, overcomes that limitation, as in preferred embodiments the loops are transferred, instead of entire domains.
In addition, in some embodiments, the compounds of the present invention comprise at least one mutation in addition to those set out above. Other mutations, such as deletions, insertions, substitutions, transversions, transitions and inversions, at one or more other locations, also find use in the present invention.

In some embodiments, the compounds of the present invention also comprise a conservative substitution that may occur as a like-for-like substitution (e.g., basic for basic, acidic for acidic, polar for polar etc.). In additional embodiments, non-conservative substitutions are provided (i.e., from one class of residue to another or alternatively involving the inclusion of unnatural amino acids such as ornithine, diaminobutyric acid ornithine, norleucine ornithine, pyrrolalanine, thienylalanine, naphthylalanine and phenylglycine).

In some embodiments, the sequences also have deletions, insertions and/or substitutions of amino acid residues that produce a silent change and result in a functionally equivalent substance.

In some embodiments, deliberate amino acid substitutions are made on the basis of similarity in amino acid properties (e.g., polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues) and it is therefore useful to group amino acids together in functional groups. Amino acids can be grouped together based on the properties of their side chain alone. However it is more useful to include mutation data as well. The sets of amino acids thus derived are likely to be conserved for structural reasons. These sets can be described in the form of a Venn diagram (See e.g., Livingstone and Barton, Comput. Appl Biosci., 9:745-756 [1993]; and (Taylor, J. Theor. Biol., 119:205-218 [1986]). In some embodiments, conservative substitutions are made, for example according to the table below that describes a generally accepted Venn diagram grouping of amino acids.

<table>
<thead>
<tr>
<th>Set</th>
<th>Sub-set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophobic</td>
<td>FWYHKMLVAGC</td>
</tr>
<tr>
<td></td>
<td>Aromatic</td>
</tr>
<tr>
<td></td>
<td>FWYH</td>
</tr>
<tr>
<td></td>
<td>Aliphatic</td>
</tr>
<tr>
<td></td>
<td>ILV</td>
</tr>
<tr>
<td>Polar</td>
<td>WYHKRED CSTNQ</td>
</tr>
<tr>
<td></td>
<td>Charged</td>
</tr>
<tr>
<td></td>
<td>HKRED</td>
</tr>
<tr>
<td></td>
<td>Positively charged</td>
</tr>
<tr>
<td></td>
<td>HKR</td>
</tr>
<tr>
<td></td>
<td>Negatively charged</td>
</tr>
<tr>
<td></td>
<td>E D</td>
</tr>
<tr>
<td>Small</td>
<td>VCAGSPTN D</td>
</tr>
<tr>
<td></td>
<td>Tiny</td>
</tr>
<tr>
<td></td>
<td>AGS</td>
</tr>
</tbody>
</table>

In some embodiments, variant amino acid sequences of the present invention also include suitable spacer groups inserted between any two amino acid residues of the sequence including alkyl groups such as methyl, ethyl or propyl groups in addition to amino acid spacers such as
glycine or β-alanine residues. A further form of variation involves the presence of one or more amino acid residues in peptoid form.

In some embodiments, homology comparisons find use in identifying homologous sequences that find use in the present invention. Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. Available computer programs can calculate the percent homology between two or more sequences. Additionally, percent homology may be calculated over contiguous sequences (i.e., one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence one residue at a time). This is called an “ungapped” alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalizing unduly the overall homology score. This is achieved by inserting “gaps” in the sequence alignment to try to maximize local homology.

However, these more complex methods assign “gap penalties” to each gap that occurs in the alignment, so that for the same number of identical amino acids, a sequence alignment with as few gaps as possible (i.e., reflecting higher relatedness between the two compared sequences) will achieve a higher score than one with many gaps. “Affine gap costs” are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is one of the most commonly used gap scoring system. High gap penalties will of course produce optimized alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (See e.g., Devereux et al., Nuc. Acids Res., 12:387 [1984]). Examples of other software packages than can perform sequence comparisons include, but are not limited to, the BLAST package FASTA, and the GENEWOKS suite of comparison tools, all of which are well-known to those in the art. Both BLAST and FASTA are available for offline and online searching. However, for some applications, it is
preferred to use the GCG Bestfit program. The BLAST 2 Sequence package is also available for comparing protein and nucleotide sequences.

Although the final percent homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pair-wise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied. For some applications, it is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Alternatively, percentage homologies may be calculated using the multiple alignment feature in DNASIS™ (Hitachi Software), based on an algorithm, analogous to CLUSTAL (See e.g., Higgins and Sharp, Gene 73:237-244 [1988]).

Once the software has produced an optimal alignment, it is possible to calculate the percent of homology, and more preferably, the percent of sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

In some embodiments, the present invention provides nucleic acids encoding any of the compounds described herein, as well as complements thereof. In additional preferred embodiments, the invention provides vectors comprising a compound, as disclosed herein, cells comprising the compound and methods of expressing the compound.

Those of skill in the art appreciate the relationship between nucleic acid sequences and polypeptide sequences, in particular as relate to the genetic code and the degeneracy of this code, and will be able to construct such nucleic acids without difficulty. For example, one skilled in the art is aware that for each amino acid substitution in a sequence there may be one or more codons that encode the substitute amino acid. Accordingly, it is evident that, depending on the degeneracy of the genetic code with respect to that particular amino acid residue, one or more nucleic acid sequences may be generated corresponding to that polypeptide sequence.

Mutations in amino acid sequence and nucleic acid sequence may be made by any of a number of techniques, as known in the art. In particularly preferred embodiments, the mutations are introduced into parent sequences by means of PCR (polymerase chain reaction) using appropriate primers. In some embodiments, the parent enzymes are modified at the amino acid level, while in other embodiments, the enzymes are modified at the nucleic acid level, in order to generate the sequences described herein. In some preferred embodiments, the present invention provides for the generation of compounds by introducing one or more corresponding codon changes in the nucleotide sequence encoding a compound. It will be appreciated that the above
codon changes will find use in various nucleic acid sequences of the present invention. For example, in some embodiments, sequence changes are made to any of the homologous sequences described herein.

As indicated above, in some embodiments, the “compound” comprises the “complete” protein, (i.e., in its entire length as it occurs in nature (or as mutated)), while in other embodiments it comprises a truncated form of a protein. Thus, the compounds of the present invention are either truncated or be “full-length.” In addition, in some embodiments, the truncation is located at the N-terminal end, while in other embodiments the truncation is located at the C-terminal end of the protein. In further embodiments, the compound lacks one or more portions (e.g., sub-sequences, signal sequences, domains or moieties), whether active or not.

In yet further alternative embodiments, the nucleotide sequences encoding the compounds are prepared synthetically by established standard methods (e.g. the phosphoroamidite method described by Beaucage et al., Tetrahedr. Lett., 22:1859-1869 [1981]; or the method described by Matthes et al., EMBO J., 3:801-805 [1984]). In the phosphoroamidite method, oligonucleotides are synthesized (e.g., in an automatic DNA synthesizer), purified, annealed, ligated and cloned in appropriate vectors.

In some embodiments of the present invention, the nucleotide sequences are either of mixed genomic and synthetic origin, mixed synthetic and cDNA origin or mixed genomic and cDNA origin, prepared by ligating fragments of synthetic, genomic or cDNA origin, in accordance with standard techniques. Each ligated fragment corresponds to various parts of the entire nucleotide sequence. In some embodiments, the DNA sequence is prepared by polymerase chain reaction (PCR) using specific primers, as known in the art.

In some embodiments, the nucleotide sequences described here and suitable for use in the methods and compositions described here include within them synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include, but are not limited to methylphosphonate and phosphorothioate backbones and/or the addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. However, it is not intended that the present invention be limited to any particular method, as any suitable method known to those in the art for modifying nucleotide sequences find use in the present invention. In some embodiments, these modifications are performed in order to enhance the in vivo activity and/or life span of nucleotide sequences.

In some preferred embodiments, the present invention provides nucleotide sequences and methods for using nucleotide sequences that are complementary to the sequences presented herein, as well as derivatives and/or fragments of these sequences.
In some embodiments, the polynucleotides of the present invention find use in the production of primers and/or probes. Thus, in some embodiments, the polynucleotide sequences are used to produce PCR primers, primers for other amplification methods as known in the art, labeled probes, and/or for cloning methods. In preferred embodiments, these primers, probes and other fragments are at least 15, preferably at least 20, and in some more preferable embodiments, at least 25, 30 or 40 nucleotides. In addition, these primers, probes and fragments are encompassed by the term “polynucleotide.”

In some embodiments, polynucleotides such as DNA polynucleotides and probes are produced recombinantly, while in other embodiments they are produced synthetically. In additional embodiments, these sequences are cloned using standard methods. In general, primers are produced by synthetic means, involving a stepwise manufacture of the desired nucleic acid sequence one nucleotide at a time. Techniques for accomplishing this using automated techniques are readily available in the art. However, it is not intended that the present invention be limited to production of polynucleotides using any particular method, as any suitable method known to those in the art finds use in the present invention.

In some embodiments, longer polynucleotides are generally be produced using recombinant means, for example using PCR cloning techniques, as known in the art. In such embodiments, the primers are typically designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be readily cloned into a suitable cloning vector. Preferably, the variant sequences are at least as biologically active as the sequences presented herein.

In some preferred embodiments, sequences that are provided that are complementary to the compound or sequences that are capable of hybridizing to the nucleotide sequences of the compounds (including complementary sequences of those presented herein), as well as nucleotide sequences that are complementary to sequences that can hybridize to the nucleotide sequences of the compounds (including complementary sequences of those presented herein). In some preferred embodiments, polynucleotide sequences that are capable of hybridizing to the nucleotide sequences presented herein under conditions of intermediate to maximal stringency are provided.

In some preferred embodiments, nucleotide sequences that can hybridize to the nucleotide sequence of the compound nucleic acid, or the complement thereof, under stringent conditions (e.g., 50°C and 0.2xSSC) are provided. More preferably, the nucleotide sequences can hybridize to the nucleotide sequence of the compound, or the complement thereof, under more highly stringent conditions (e.g. 65°C and 0.1xSSC).

In some embodiments, it is desirable to mutate the sequence in order to prepare a compound. Accordingly, in some embodiments, mutants are prepared from the compounds provided herein. In some embodiments, mutations are introduced using synthetic oligonucleotides.
These oligonucleotides contain nucleotide sequences flanking the desired mutation sites. Various methods known in the art find use in this embodiment (See e.g., Morinaga et al., Biotechnol., 2:646-649 [1984]; Nelson and Long, Anal. Biochem., 180:147-151 [1989]; and Sarkar and Sommer, Biotechnol., 8:404-407 [1990]). However, additional methods find use in the present invention and it is not intended that the present invention be limited to any particular method.

In some preferred embodiments, the sequences used in the methods and compositions described herein is a recombinant sequence (i.e., a sequence that has been prepared using recombinant DNA techniques produced using any suitable method known in the art.

In additional embodiments, the present invention provides vectors comprising the compound, cells comprising the compound, and methods of expressing the compound. In some embodiments, the nucleotide sequences used in the methods and compositions described herein are incorporated into a recombinant replicable vector. In some embodiments, the vector is used to replicate and express the nucleotide sequence, in enzyme form, in and/or from a compatible host cell. In some embodiments, expression is controlled using control sequences (e.g., regulatory sequences). In some embodiments, proteins produced by a host cell by expression of the nucleotide sequence are secreted (i.e., into the growth medium), while in other embodiments, the proteins are contained intracellularly within the host cell. In some embodiments, the coding sequences are designed to include signal sequences which direct secretion of the substance coding sequences through a particular prokaryotic or eukaryotic cell membrane. In further embodiments, polynucleotides are incorporated into a recombinant replicable vector. In additional embodiments, the vector is used to replicate the nucleic acid in a compatible host cell. In preferred embodiments, the vector comprising the polynucleotide sequence is transformed into a suitable host cell. While any suitable host finds use in the present invention, in some preferred embodiments, the hosts are selected from the group consisting of bacterial, yeast, insect, fungal, and mammalian cells.

In some embodiments, compounds and their polynucleotides are expressed by introducing a polynucleotide into a replicable vector, introducing the vector into a compatible host cell, and growing the host cell under conditions which bring about replication of the vector. In some embodiments, the vector is recovered from the host cell.

In additional embodiments, the compound nucleic acid is operatively linked to transcriptional and translational regulatory elements active in the host cell. In some embodiments, the compound nucleic acid also encodes a fusion protein comprising at least one signal sequence (e.g., those derived from the glucoamylase gene from Schwanniomyces occidentalis, α-factor mating type gene from Saccharomyces cerevisiae and the TAKA-amylase from Aspergillus oryzae). In further alternative embodiments, the compound nucleic acid encodes a fusion protein comprising a membrane binding domain.
In some preferred embodiments, the compound is expressed at the desired levels in a host organism using an expression vector. It is contemplated that any expression vector comprising a compound nucleic acid that is capable of expressing the gene encoding the compound nucleic acid in the selected host organism will find use in the present invention. The choice of vector depends upon the host cell into which it is to be introduced. Thus, in some embodiments, the vector is an autonomously replicating vector (i.e., a vector that exists as an episomal entity, the replication of which is independent of chromosomal replication, such as, for example, a plasmid, a bacteriophage or an episomal element, a minichromosome or an artificial chromosome). Alternatively, in some embodiments, the vector integrates into the host cell genome and replicates together with the chromosome.

In some preferred embodiments, the expression vector includes the components of a cloning vector, including but not limited to such components as an element that permits autonomous replication of the vector in the selected host organism and one or more phenotypically detectable markers for selection purposes. In preferred embodiments, the expression vector further comprises control nucleotide sequences encoding a promoter, operator, ribosome binding site, translation initiation signal, and optionally, a repressor gene or one or more activator genes. Additionally, in some embodiments, the expression vector comprises a sequence coding for an amino acid sequence capable of targeting the compound to a host cell organelle such as a peroxisome or to a particular host cell compartment. Such a targeting sequence includes but is not limited to the sequence SKL. For expression under the direction of control sequences, the nucleic acid sequence encoding the compound is operably linked to the control sequences in proper manner with respect to expression.

In some preferred embodiments, the polynucleotide in a vector is operably linked to a control sequence that is capable of providing for the expression of the coding sequence by the host cell (i.e., the vector is an expression vector). In some embodiments, the control sequences are modified (e.g., by the addition of further transcriptional regulatory elements) in order to make the level of transcription directed by the control sequences more responsive to transcriptional modulators. In some preferred embodiments, the control sequences comprise promoters.

In some preferred embodiments of the vectors, the nucleic acid sequence encoding for the compound is operably combined with a suitable promoter sequence. The promoter can be any DNA sequence having transcription activity in the host organism of choice and can be derived from genes that are homologous or heterologous to the host organism. Examples of suitable promoters for directing the transcription of the modified nucleotide sequence, such as compound nucleic acids, in a bacterial host include, but are not limited to the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus
licheniformis α-amylase gene (amyL), the aprE promoter of Bacillus subtilis, the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquifaciens α-amylase gene (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes and a promoter derived from a Lactococcus sp.-derived promoter including the PI70 promoter. When the gene encoding the compound is expressed in a bacterial species such as E. coli, a suitable promoter can be selected, for example, from a bacteriophage promoter including a T7 promoter and a phage lambda promoter. For transcription in a fungal species, examples of useful promoters are those derived from the genes encoding the Aspergillus oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral α-amylase, A. niger acid stable α-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase, and A. nidulans acetamidase. Examples of suitable promoters for the expression in a yeast species include but are not limited to the Gal 1 and Gal 10 promoters of Saccharomyces cerevisiae and the Pichia pastoris AOX1 or AOX2 promoters.

Examples of suitable bacterial host organisms are Gram positive species, including, but not limited to members of the Bacillaceae, (e.g., B. subtilis, B. licheniformis, B. lentus, B. brevis, B. stearothermophilus, B. alkalophilus, B. amyloliquifaciens, B. coagulans, B. lautus, B. megaterium and B. thuringiensis), Streptomyces species (e.g., S. murinus and S. lividans) lactic acid bacteria (e.g., Lactococcus spp. such as Lactococcus lactis; Lactobacillus spp. including Lactobacillus reuteri; Leuconostoc spp.; Pediococcus spp.; and Streptococcus spp. Alternatively, strains of Gram-negative species belonging to Enterobacteriaceae (e.g., E. coli) or members of the Pseudomonadaceae find use in the present invention.

In some embodiments, a suitable yeast host organism is selected from various biotechnologically useful yeasts species, including but not limited to Pichia sp., Hansenula sp or Kluyveromyces, Yarrowinia, Saccharomyces (e.g., Saccharomyces cerevisiae), Schizosaccharomyce (e.g., S. pombe). In some embodiments, strains of the methylotrophic yeast species Pichia pastoris are used as the host organism, while in other embodiments, the host organism is a Hansenula species. Suitable host organisms among filamentous fungi include species of Aspergillus (e.g., A. niger, A. oryzae, A. tubiengis, A. awamori and Aspergillus nidulans). Alternatively, strains of Fusarium species (e.g. F. oxysporum) and Rhizomucor (e.g., Rhizomucor miehei) find used as the host organism. Additional suitable strains include, but are not limited to Thermomyces and Mucor species.

In some preferred embodiments, host cells comprising polynucleotides are used to express polypeptides, such as the compounds disclosed herein, fragments, homologues, variants or derivatives thereof. Host cells are cultured under suitable conditions which allow expression of the proteins. In some embodiments, expression of the polypeptides is constitutive (i.e., the peptides
are continually produced), while in other embodiments, expression is inducible. In the case of inducible expression, protein production is initiated when required by addition of an inducer substance to the culture medium (e.g., dexamethasone or IPTG). Polypeptides can be extracted from host cells by a variety of techniques known in the art, including enzymatic, chemical, and/or osmotic lysis and physical disruption. Indeed, it is not intended that the present invention be limited to any particular means of harvesting expressed polypeptides.

In alternative embodiments, polypeptides are produced recombinantly in any suitable (including commercially available) in vitro cell-free system, such as the TntT™ (Promega) rabbit reticulocyte system.

In additional preferred embodiments, the present invention provides cosmetic and/or pharmaceutical compositions comprising at least one polypeptide or peptide, as set forth herein, and a physiologically acceptable carrier or excipient. Preferably, the compound is present in an amount of about 0.0001% to about 5% by weight, based on the total weight of the composition. Also preferably, the compound is present in an amount of about 0.001% to about 0.5% by weight based on the total weight of the composition. The composition may be in the form of an emulsified vehicle, such as a nutrient cream or lotion, a stabilized gel or dispersion system, a treatment serum, a liposomal delivery system, a topical pack or mask, a surfactant-based cleansing system such as a shampoo or body wash, an aerosolized or sprayed dispersion or emulsion, a hair or skin conditioner, styling aid, or a pigmented product such as makeup. Preferably, the carrier is at least compound selected from the group consisting of water, propylene glycol, ethanol, propanol, glycerol, butylene glycol and polyethylene glycol.

In some liposomal embodiments, the liposomes comprise water and one or more ingredients capable of forming lipid bilayer vesicles that can hold one or more functional or active ingredient(s). Non-limiting examples of ingredients capable of forming lipid bilayer vesicles include: phospholipids, hydrogenated phosphatidylcholine, lecithin, cholesterol and sphingolipids. Preferred liposomes include, without limitation: a) lipid liposome 0003 (composed of water and lecithin and glycerin); b) lipid liposome 0300 (composed of water and phosphatidylcholine); c) lipid liposome 0111 (composed of water, Ginkgo biloba leaf extract, denatured alcohol, hydrogenated lecithin and cholesterol); d) anti-irritant liposomes (composed of water, cola acuminata seed extract, bisabolol and phospholipids); e) vitamin C and E liposomes (composed of water, phospholipids, tocopheryl acetate and ascorbyl palmitate); f) firming liposomes (composed of water, butylene glycol, pyrus malus (Apple) fruit extract, phospholipids, tocopheryl acetate and carboxer); and g) moisturizing liposomes (composed of water, sodium PCA, tocopheryl acetate, xanthan gum, arginine, lysine, glycine and proline).
Non-limiting examples of functional or active ingredients that can be delivered via liposomes include: vitamins and their derivatives, antioxidants, proteins and peptides, keratolytic agents, bioflavinoids, terpenoids, phytochemicals, and extracts of plant, marine or fermented origin. In a preferred embodiment, the composition further comprises a skin care or hair care active. Active ingredients can include any of a wide variety of ingredients such as are known in the art. (See e.g., McCutcheon’s Functional Materials, North American and International Editions, (2003), published by MC Publishing Co.). Preferably, such actives include but are not limited to antioxidants, such as tocopheryl and ascorbyl derivatives, bioflavinoids, terpenoids, synthetics and the like, vitamins and vitamin derivatives, hydroxy- and polyhydroxy acids and their derivatives, such as AHAs and BHAs and their reaction products, peptides and polypeptides and their derivatives, such as glycopeptides and lipophilized peptides, heat shock proteins and cytokines, enzymes and enzymes inhibitors and their derivatives, such as proteases, MMP inhibitors, catalases, glucose oxydase and superoxide dismutase, amino acids and their derivatives, bacterial, fungal and yeast fermentation products and their derivatives, including mushrooms, algae and seaweed and their derivatives, phytosterols and plant and plant part extracts and their derivatives and phospholipids and their derivatives, anti-dandruff agents such as zinc pyrithione and delivery systems containing them, as provided herein and/or known in the art.

In some preferred embodiments, the skin care active is selected from the group consisting of a Vitamin B3 component, panthenol, Vitamin E, Vitamin E acetate, retinol, retinyl propionate, retinyl palmitate, retinoic acid, Vitamin C, theobromine, alpha-hydroxyacid, farnesol, phytrantriol, salicylic acid, palmityl peptapeptide-3 and mixtures thereof. In some preferred embodiments, the Vitamin B3 component is niacinamide. In some embodiments, the compositions provided herein comprise a skin care active at a level from about 0.0001% to about 20%, preferably from about 0.001% to about 5%, more preferably from about 0.01% to about 2%, by weight.

Exemplary derivatives of the foregoing vitamin B3 compounds include nicotinic acid esters, including non-vasodilating esters of nicotinic acid, nicotinyl amino acids, nicotinyl alcohol esters of carboxylic acids, nicotinic acid N-oxide and niacinamide N-oxide. Suitable esters of nicotinic acid include nicotinic acid esters of C1-C22, preferably C1-C16, more preferably C1-C6 alcohols. In these embodiments, the alcohols are suitably straight-chain or branched chain, cyclic or acyclic, saturated or unsaturated (including aromatic), and substituted or unsubstituted. The esters are preferably non-vasodilating.

Non-vasodilating esters of nicotinic acid include tocopherol nicotinate and inositol hexanicotinate; tocopherol nicotinate are preferred. A more complete description of vitamin B3 compounds is provided in WO 98/22085. Preferred vitamin B3 compounds include niacinamide and tocopherol nicotinate.
In additional embodiments, the skin care active comprises at least one retinoid. The retinoid is preferably retinol, retinol esters (e.g., C₂ - C₂₂ alkyl esters of retinol, including retinyl palmitate, retinyl acetate, retinyl propionate), retinal, and/or retinoic acid (including all-trans retinoic acid and/or 13-cis-retinoic acid), more preferably retinoids other than retinoic acid. These compounds are well known in the art and are commercially available from a number of sources (e.g., Sigma and Boehringer Mannheim). Preferred retinoids include retinol, retinyl palmitate, retinyl acetate, retinyl propionate, retinal, retinoic acid and combinations thereof. More preferred are retinol, retinoic propionate, retinoic acid and retinyl palmitate. In some embodiments, the retinoid is included as a substantially pure material, while in other embodiments, it is provided as an extract obtained by suitable physical and/or chemical isolation from natural (e.g., plant) sources. When a retinoid is included in the compositions herein, it preferably comprises from about 0.005% to about 2%, preferably from about 0.01% to about 1% retinoid. Retinol is preferably used in an amount of from about 0.01% to about 0.15%; retinol esters are preferably used in an amount of from about 0.01% to about 2% (e.g., about 1%).

In some embodiments, the compositions of the present invention comprise safe and effective amounts of a dermatologically acceptable carrier that is suitable for topical application to the skin or hair within which the essential materials and optional other materials are incorporated to enable the essential materials and optional components to be delivered to the skin or hair at an appropriate concentration. Thus, in some embodiments, the carrier acts as a diluent, dispersant, solvent or the like for the essential components, ensuring that these components can be applied and distributed evenly over the selected target at an appropriate concentration.

In further embodiments, an effective amount of one or more compounds described herein is also be included in compositions to be applied to keratinous materials such as nails and hair, including but not limited to those useful as hair spray compositions, hair styling compositions, hair shampooing and/or conditioning compositions, compositions applied for the purpose of hair growth regulation and compositions applied to the hair and scalp for the purpose of treating seborrhoea, dermatitis and/or dandruff.

In yet additional embodiments, an effective amount of one or more compounds described herein is included in compositions suitable for topical application to the skin or hair. These compositions are provided in any suitable form, including but not limited to creams, lotions, gels, suspensions dispersions, microemulsions, nanodispersions, microspheres, hydrogels, emulsions (e.g., oil-in-water and water-in-oil, as well as multiple emulsions), and multilaminar gels and the like (See e.g., Schlossman et al., The Chemistry and Manufacture of Cosmetics, 1998, incorporated by reference, herein). In some embodiments, the compositions are formulated as
aqueous or silicone compositions, while in other embodiments they are formulated as emulsions of one or more oil phases in an aqueous continuous phase (or an aqueous phase in an oil phase).

The type of carrier utilized in the present invention depends on the type of product form desired for the composition. The carrier can be solid, semi-solid or liquid. Suitable carriers include liquids, semi-solids (e.g., creams, lotions, gels, sticks, ointments, and pastes), sprays and mousses. Preferably the carrier is in the form of a lotion, cream or a gel, more preferably one which has a sufficient thickness or yield point to prevent the particles from sedimenting. In some embodiments, the carrier is inert, while in other embodiments it provides dermatological benefits. In some embodiments, the carrier is applied directly to the skin and/or hair, while in other embodiments, it is applied via a woven or non-woven wipe or cloth. In yet other embodiments, it is in the form of a patch, mask or wrap. In still further embodiments, it is aerosolized or otherwise sprayed or pumped onto the skin and/or hair. The carrier chosen is physically and chemically compatible with the essential components described herein, and should not unduly impair stability, efficacy or other use benefits associated with the compositions of the present invention.

Preferred carriers contain a dermatologically acceptable, hydrophilic diluent. Suitable hydrophilic diluents include water, organic hydrophilic diluents such as C₂ – C₁₀, preferably C₂ – C₆, preferably, C₁ – C₆ monohydric alcohols and low molecular weight glycols and polyols, including propylene glycol, polyethylene glycol polypropylene glycol, glycerol, butylene glycol, 1,2,4-butanetriol, sorbitol esters, 1,2,6-hexametriol, pentyline glycol, hexylene glycol, sorbitol esters, ethoxylated ethers, propoxylated ethers, and combinations thereof. The diluent is preferably liquid. Water is a preferred diluent. The composition preferably comprises at least about 20% of the hydrophilic diluent.

In some embodiments, suitable carriers also comprise an emulsion comprising a hydrophilic phase, especially an aqueous phase, and a hydrophobic phase (e.g., a lipid, oil or oily material). As well known to those skilled in the art, the hydrophilic phase is dispersed in the hydrophobic phase, or vice versa, to form respectively hydrophilic or hydrophobic dispersed and continuous phases, depending on the composition of ingredients. The term “dispersed phase” is a term well-known to one skilled in the art of emulsion technology, used in reference to the phase which 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 emulsion may be or comprise (e.g., in a triple or other multi-phase emulsion) an oil-in-water emulsion or a water-in-oil emulsion such as a water-in-silicone emulsion. Oil-in-water emulsions typically comprise from about 1% to about 60% (preferably about 1% to about 30%) of the dispersed hydrophobic phase and from about 1% to about 99% (preferably from about 10% to about 90%) of the continuous hydrophilic phase, while water-in-oil emulsions typically comprise from about 1%
to about 98% (preferably from about 40% to about 90%) of the dispersed hydrophilic phase and
from about 1% to about 50% (preferably about 1% to about 30%) of the continuous hydrophobic
phase.

In further embodiments, the carrier also includes one or more components that facilitate
penetration through the upper stratum corneum barrier to the lower levels of the skin. Examples of
penetration enhancers include, but are not limited to, propylene glycol, azone, ethoxydiglycol,
dimethyl isosorbide, urea, ethanol and dimethyl sulfoxide, as well as microemulsions, liposomes
and nanoemulsions.

In some additional embodiments, the compositions of the present invention comprise
humectants which are preferably present at a level of from about 0.01% to about 20%, preferably
from about 0.1% to about 15% and preferably from about 0.5% to about 10%. Preferred
humectants include, but are not limited to, compounds selected from polyhydric alcohols, sorbitol,
glycerol, urea, betaine, D-pantenol, DL-pantenol, calcium pantothenate, royal jelly, panthetine,
pantotheine, panthenyl ethyl ether, panganic acid, pyridoxin, pantoyl lactose Vitamin B complex,
sodium pyrrolidone carboxylic acid, hexane - 1, 2, 6, - triol, guanidine or its derivatives, and
mixtures thereof.

Suitable polyhydric alcohols for use herein include, but are not limited to polyalkylene
glycols and preferably alkylene polyols and their derivatives, including propylene glycol,
dipropylene glycol, polypropylene glycol, polyethylene glycol and derivatives thereof, sorbitol,
hydroxypropyl sorbitol, erythritol, treitol, pentaerythritol, xylitol, glucitol, mannitol, pentylene
glycol, hexylene glycol, butylene glycol (e.g., 1,3-butylene glycol), hexane triol (e.g., 1,2,6-
hexanetriol), trimethylol propane, neopentyl glycol, glycerine, ethoxylated glycerine, propane-1,3
diol, propoxylated glycerine and mixtures thereof. The alkoxylated derivatives of any of the above
polyhydric alcohols are also suitable for use herein. Preferred polyhydric alcohols of the present
invention are selected from glycerine, butylene glycol, propylene glycol, pentylene glycol,
hexylene glycol, dipropylene glycol, polyethylene glycol, hexane triol, ethoxylated glycerine and
propoxylated glycerine and mixtures thereof.

Suitable humectants useful herein are sodium 2-pyrrolidone-5-carboxylate (NaPCA),
guanidine; glycolic acid and glycolate salts (e.g., ammonium and quaternary alkyl ammonium);
lactic acid and lactate salts (e.g., ammonium and quaternary alkyl ammonium); aloe vera in any of
its variety of forms (e.g., aloe vera gel); hyaluronic acid and derivatives thereof (e.g., salt
derivatives such as sodium hyaluronate); lactamide monoethanolamine; acetamide
monoethanolamine; urea; betaine, panthenol and derivatives thereof; and mixtures thereof.

In some embodiments, at least part (up to about 5% by weight of composition) of a
humectant is incorporated into the compositions of the present invention in the form of an
admixture with a particulate cross-linked hydrophobic acrylate or methacrylate copolymer, itself preferably present in an amount of from about 0.1% to about 10%, which can be added either to the aqueous or disperse phase. This copolymer is particularly valuable for reducing shine and controlling oil while helping to provide effective moisturization benefits and is described in further detail in WO96/03964, incorporated herein by reference.

In some embodiments, the oil-in-water and water-in-oil compositions of the present invention comprise from about 0.05% to about 20%, preferably from about 1% to about 15%, preferably from about 2% to about 10%, preferably from about 2% to about 5% of a dermatologically acceptable emollient. Emollients tend to lubricate the skin, increase the smoothness and suppleness of the skin, prevent or relieve dryness of the skin and/or protect the skin. Emollients are typically water-immiscible, oily or waxy materials and emollients can confer aesthetic properties to a topical composition. A wide variety of suitable emollients are known (See e.g., Sagarin, Cosmetics. Science and Technology, 2nd Edition, Vol. 1, pp. 32-43 [1972]; and WO 00/24372), and find use herein, contains numerous examples of materials suitable as emollients.

Additional emollients include, but are not limited to the following:

i) Straight and branched chain hydrocarbons having from about 7 to about 40 carbon atoms, such as mineral oils, dodecane, squalane, cholesterol, hydrogenated polyisobutylene, isohexadecane, isoeicosane, isoctahexacontane, isohexapentacontahectane, and the C7-C40 isoparaffins, which are C7-C40 branched hydrocarbons. Suitable branched chain hydrocarbons for use herein are selected from isopentacontacontane, petrolatum and mixtures thereof;

ii) C1-C30 fatty acid esters of C1-C30 carboxylic acids, C12-15 alkyl benzoates and of C2-C30 dicarboxylic acids, e.g. isononyl isononanoate, isostearyl neopentanoate, isodecyl octanoate, isodecyl isononanoate, tridecyl isononanoate, myristyl octanoate, octyl pelargonate, octyl isononanoate, myristyl myristate, myristyl neopentanoate, myristyl octanoate, isopropyl myristate, myristyl propionate, isopropyl stearate, isopropyl isostearate, methyl isostearate, behenyl behenate, dioctyl maleate, diisopropyl adipate, and diisopropyl dilinoleate and mixtures thereof also find use in the present invention;

iii) C1-C30 mono- and poly- esters of sugars and related materials. These esters are derived from a sugar or polyol moiety and one or more carboxylic acid moieties. Depending on the constituent acid and sugar, these esters can be in either liquid or solid form at room temperature. Examples include: glucose tetraoleate, the galactose tetraesters of oleic acid, the sorbitol tetraoleate, sucrose tetraoleate, sucrose pentaoleate, sucrose hexaoleate, sucrose heptaoleate, sucrose octaoleate, sorbitol hexaester. Other materials include cottonseed oil or soybean oil fatty acid esters of sucrose. Other examples of such materials are described in WO 96/16636, incorporated by reference herein;
iv) Vegetable oils and hydrogenated vegetable oils. Examples of vegetable oils and hydrogenated vegetable oils include safflower oil, grapeseed oil, coconut oil, cottonseed oil, menhaden oil, palm kernel oil, palm oil, peanut oil, soybean oil, rapeseed oil, linseed oil, rice bran oil, pine oil, nut oil, sesame oil, sunflower seed oil, partially and fully hydrogenated oils from the foregoing sources and mixtures thereof;

v) Soluble or collooidally-soluble moisturizing agents. Examples include hyaluronic acid and chondroitin sulfate.

In some embodiments, the compositions of the present invention contain an emulsifier and/or surfactant, generally to help disperse and suspend the disperse phase within the continuous aqueous phase. A surfactant may also be useful if the product is intended for skin or hair cleansing. For convenience hereinafter, “emulsifiers” are encompassed by the term “surfactants.” Thus, as used herein, the term “surfactant(s)” refers to surface active agents, whether used as emulsifiers or for other surfactant purposes such as skin cleansing. Known, including conventional surfactants find use in the present invention, provided that the selected agent is chemically and physically compatible with essential components of the composition and provides the desired characteristics (See e.g., WO 00/24372). Suitable surfactants include non-silicone derived materials, silicone-derived materials, and mixtures thereof.

In further embodiments, the compositions of the present invention comprise preferably from about 0.05% to about 30%, more preferably from about 0.5% to 15%, and most preferably from about 1% to 10% of a surfactant or mixture of surfactants. The exact surfactant or surfactant mixture chosen depends upon the pH of the composition, the other components present and the desired final product aesthetics.

Among the nonionic surfactants that are useful herein are those that can be broadly defined as condensation products of long chain alcohols (e.g., C₈₋₃₀ alcohols), with sugar or starch polymers (e.g., glycosides). Other useful nonionic surfactants include the condensation products of alkylene oxides with fatty acids (i.e., alkylene oxide esters of fatty acids). These materials have the general formula RCO(X)ₙOH wherein R is a C₁₀₋₃₀ alkyl group, X is -OCH₂CH₂- (i.e., derived from ethylene glycol or oxide) or -OCH₂CH₂(OH)₂CH₃- (i.e., derived from propylene glycol or oxide) and n is an integer from about 6 to about 200. Other nonionic surfactants are the condensation products of alkylene oxides with 2 moles of fatty acids (i.e., alkylene oxide diesters of fatty acids). These materials have the general formula RCO(X)ₙOOCR wherein R is a C₁₀₋₃₀ alkyl group, X is -OCH₂CH₂-(i.e., derived from ethylene glycol or oxide) or -OCH₂CH₂CH₃-(i.e., derived from propylene glycol or oxide) and n is an integer from about 6 to about 100. In some embodiments, an emulsifier for use herein is preferably a fatty acid ester blend based on a mixture
of sorbitan fatty acid ester and sucrose fatty acid ester, especially a blend of sorbitan stearate and sucrose cocomate. Further suitable examples include a mixture of cetearyl alcohols and cetearyl glucosides. However, it is not intended that the present invention be limited to any particular emulsifier, as various suitable emulsifiers are known in the art.

In additional embodiments, the hydrophilic surfactants useful herein alternatively or additionally include any of a wide variety of cationic, anionic, zwitterionic, and amphoteric surfactants such as are known in the art (See, e.g., McCutcheon’s, Emulsifiers and Detergents, North American and International Editions, MC Publishing Co. [2003]; U.S. Patent No. 5,011,681 U.S. Patent No. 4,421,769; and U.S. Patent No. 3,755,560).

A variety of anionic surfactants are also useful herein (See e.g., U.S. Patent No. 3,929,678). Exemplary anionic surfactants include, but are not limited to alkoyl isethionates (e.g., C_{12} - C_{30}), alkyl and alkyl ether sulfates and salts thereof, alkyl and alkyl ether phosphates and salts thereof, alkyl methyl taurates (e.g., C_{12} - C_{30}), and soaps (e.g., substituted alkylamine and alkali metal salts, e.g., sodium or potassium salts) of fatty acids.

Amphoteric and zwitterionic surfactants are also useful herein. Examples of preferred amphoteric and zwitterionic surfactants which find use in the compositions of the present invention are those which are broadly described as derivatives of aliphatic secondary and tertiary amines in which the aliphatic radical can be straight or branched chain and wherein one of the aliphatic substituents contains from about 8 to about 22 carbon atoms (preferably C_{8} - C_{18}) and one contains an anionic water solubilizing group (e.g., carboxy, sulfonate, sulfate, phosphate, or phosphonate). Examples, include but are not limited to alkyl imino acetates and iminodialkanoates and aminoalkanoates, imidazolinium and ammonium derivatives. Other suitable amphoteric and zwitterionic surfactants are those selected from the group consisting of betaines, sultaines, hydroxysultaines, and branched and unbranched alkanoyl sarcosinates, and mixtures thereof.

In further embodiments, some emulsions of the present invention include a silicone containing emulsifier or surfactant. A wide variety of silicone emulsifiers find use herein. These silicone emulsifiers are typically organically modified organopolysiloxanes, also known to those skilled in the art as silicone surfactants. Useful silicone emulsifiers include, but are not limited to dimethicone copolymers. These materials are polydimethyl siloxanes which have been modified to include polyether side chains such as polyethylene oxide chains, polypropylene oxide chains, mixtures of these chains and polyether chains containing moieties derived from both ethylene oxide and propylene oxide. Other examples include alkyl-modified dimethicone copolymers (i.e., compounds which contain C_{2}-C_{30} pendant side chains). Still other useful dimethicone copolymers include materials having various cationic, anionic, amphoteric, and zwitterionic pendant moieties.
In some embodiments, the compositions of the present invention comprise at least one polymeric thickening agent. The polymeric thickening agents useful herein preferably have a number average molecular weight of greater than about 20,000, more preferably greater than about 50,000, and most preferably greater than about 100,000. In some embodiments, the compositions of the present invention comprise from about 0.01% to about 10%, preferably from about 0.1% to about 8% and more preferably from about 0.2% to about 5% by weight of the composition of the polymeric thickening agent or mixtures thereof.

Preferred polymer thickening agents for use herein include, but are not limited to non-ionic thickening agents and anionic thickening agents or mixtures thereof. Suitable non-ionic thickening agents include, but are not limited to polyacrylamide polymers, crosslinked poly(N-vinylpyrrolidones), polysaccharides, natural or synthetic gums, polyvinylpyrrolidone and polyvinylalcohol. Suitable anionic thickening agents include, but are not limited to acrylic acid/ethyl acrylate copolymers, carboxyvinyl polymers and crosslinked copolymers of alkyl vinyl ethers and maleic anhydride. Commercially available thickeners (e.g., Carbopol; Noveon) find use in some embodiments of the present invention. Suitable Carbopol resins may be hydrophobically modified, and other suitable resins are described in WO98/22085, or mixtures thereof.

In some embodiments, the present compositions comprise at least one silicone oil phase. Silicone oil phase(s) generally comprises from about 0.1% to about 20%, preferably from about 0.5% to about 10%, and more preferably from about 0.5% to about 5%, of the composition. The silicone oil phase preferably comprises one or more silicone components.

In some embodiments, silicone components are fluids, including straight chain, branched and cyclic silicones. Suitable silicone fluids useful herein include silicones inclusive of polyalkyl siloxane fluids, polyary siloxane fluids, cyclic and linear polyalkylsiloxanes, polyalkoxylated silicones, amino and quaternary ammonium modified silicones, polyalkylaryl siloxanes or a polyether siloxane copolymer and mixtures thereof. Volatile, as well as non-volatile silicone fluids find use herein. Silicone fluids generally have an average molecular weight of less than about 200,000. In preferred embodiments, suitable silicone fluids have a molecular weight of about 100,000 or less, preferably about 50,000 or less, and more preferably about 10,000 or less. Preferably the silicone fluid is selected from silicone fluids having a weight average molecular weight in the range from about 100 to about 50,000 and preferably from about 200 to about 40,000.

Typically, silicone fluids have a viscosity ranging from about 0.65 to about 600,000 mm²s⁻¹, preferably from about 0.65 to about 10,000 mm²s⁻¹ at 25°C. The viscosity can be measured by means of a glass capillary viscometer as set forth in Dow Corning Corporate Test Method CTM0004, July 29, 1970. Suitable polydimethyl siloxanes that can be used herein include commercially available compounds (e.g., from the General Electric Company and Dow Corning).
Also useful are essentially non-volatile polyalkylaryl siloxanes, for example, polymethylphenylsiloxanes, having viscosities of about 0.65 to 30,000 mm²s⁻¹ at 25°C (General Electric Company or from Dow Corning). Cyclic polydimethylsiloxanes suitable for use herein are those having a ring structure incorporating from about 3 to about 7 (CH₃)₂SiO moieties, preferably about 5 or more.

In additional embodiments, silicone gums find use herein. In some preferred embodiments, a silicone oil phase comprises a silicone gum or a mixture of silicones including the silicone gum. Typically, silicone gums have a viscosity at 25°C in excess of about 1,000,000 mm²s⁻¹. The silicone gums include dimethicones as known in the art (See e.g., U.S. Pat. No. 4,152,416; and Noll, Chemistry and Technology of Silicones, Academic Press, New York [1968]). Silicone gums such as those described in General Electric Silicone Rubber Product Data Sheets SE 30, SE 33, SE 54 and SE 76, also find use in the present invention. Specific examples of silicone gums include polydimethylsiloxane, (polydimethylsiloxane)(methylvinylsiloxane) copolymer, poly(dimethylsiloxane)(diphenyl)(methylvinylsiloxane) copolymer and mixtures thereof. Preferred silicone gums for use herein are silicone gums having a molecular weight of from about 200,000 to about 4,000,000 selected from dimethiconol, dimethicone copolyol, dimethicone and mixtures thereof.

In some embodiments, a silicone phase herein preferably comprises a silicone gum incorporated into the composition as part of a silicone gum-fluid blend. When the silicone gum is incorporated as part of a silicone gum-fluid blend, the silicone gum preferably constitutes from about 5% to about 40%, especially from about 10% to 20% by weight of the silicone gum-fluid blend. Suitable silicone gum-fluid blends herein are mixtures consisting essentially of:

(i) a silicone having a molecular weight of from about 200,000 to about 4,000,000 selected from dimethiconol, fluoro silicone and dimethicone and mixtures thereof; and

(ii) a carrier which is a silicone fluid, the carrier having a viscosity from about 0.65 mm²s⁻¹ to about 100 mm²s⁻¹,

wherein the ratio of i) to ii) is from about 10:90 to about 20:80 and wherein said silicone gum-based component has a final viscosity of from about 100 mm²s⁻¹ to about 100,000 mm²s⁻¹, preferably from 500 mm²s⁻¹ to about 10,000 mm²s⁻¹.

Further silicone components suitable for use in a silicone oil phase herein include crosslinked polyorganosiloxane polymers, optionally dispersed in a fluid carrier. In general, when present the crosslinked polyorganosiloxane polymers, together with its carrier (if present) comprises from about 0.1% to about 20%, preferably from about 0.5% to about 10%, and more preferably from about 0.5% to about 5% of the composition. Such polymers comprise
polyorganosiloxane polymers crosslinked by a crosslinking agent (See e.g., WO98/22085). Examples of suitable polyorganosiloxane polymers for use herein include, but are not limited to methyl vinyl dimethicone, methyl vinyl diphenyl dimethicone and methyl vinyl phenyl methyl diphenyl dimethicone.

Another class of silicone components suitable for use in a silicone oil phase herein includes polydiorganosiloxane-polyoxyalkylene copolymers containing at least one polydiorganosiloxane segment and at least one polyoxyalkylene segment (See e.g., WO98/22085). Suitable polydiorganosiloxane-polyalkylene copolymers are available commercially under the tradenames BELSIL® from Wacker-Chemie GmbH. A particularly preferred copolymer fluid blend for use herein includes Dow Corning DC3225C which has the CTFA designation Dimethicone/Dimethicone copolyol.

In further embodiments, compositions of the present invention comprise an organic sunscreen. In some embodiments, suitable sunscreens have UVA absorbing properties, while others have UVB absorbing properties, and still others comprise a mixture thereof. The exact amount of the sunscreen active varies, depending upon the desired Sun Protection Factor (i.e., the “SPF”) of the composition, as well as the desired level of UV protection. SPF is a commonly used measure of photoprotection of a sunscreen against erythema. The SPF is defined as a ratio of the ultraviolet energy required to produce minimal erythema on protected skin to that required to produce the same minimal erythema on unprotected skin in the same individual. Amounts of the sunscreen used are preferably from about 2% to about 20%, and more preferably from about 4% to about 14%. Suitable sunscreens include, but are not limited to those approved for use in the United States, Japan, Europe and Australia. The compositions of the present invention preferably comprise an SPF of about 2 to about 30, preferably about 4 about 30, and more preferably about 4 to about 15.

In some embodiments, the compositions of the present invention may one or more UVA absorbing sunscreen actives that absorb UV radiation having a wavelength of from about 320nm to about 400nm. Suitable UVA absorbing sunscreen actives include, but are not limited to dibenzoylmethane (See e.g., Lowe and Shaath (eds.), Sunscreens: Development, Evaluation, and Regulatory Aspects, Marcel Dekker, Inc.) derivatives, anthranilate derivatives such as methylanthraniolate and homomethyl, 1-N-acetyl anthranilate, and mixtures thereof. The UVA absorbing sunscreen active is preferably present in an amount sufficient to provide broad spectrum UVA protection either independently, or in combination with, other UV protective actives which may be present in the composition.

Suitable UVA sunscreen actives include dibenzoylmethane sunscreen actives and their derivatives. They include, but are not limited to, those selected from 2-methyl dibenzoylmethane,
4-methyldibenzoylmethane, 4-isopropylidibenzoylmethane, 4-tert-butyldibenzoylmethane, 2, 4-
dimethyldibenzoylmethane, 2, 5-dimethyldibenzoylmethane, 4, 4'-diisopropylbenzoylmethane, 4-
(1, 1-dimethylethyl)-4'-methoxydibenzoylmethane, 2-methyl-5-isopropyl-4'
-methoxydibenzoylmethane, 2-methyl-5-tert-butyl-4'-methoxy-dibenzoylmethane, 2, 4-dimethyl-4'
-methoxydibenzoylmethane, 2, 6-dimethyl-4'-tert-butyl-4’methoxydibenzoylmethane, and mixtures
thereof. Preferred dibenzoyl sunscreen actives include those selected from 4-(1, 1-dimethylethyl)-
4'-methoxydibenzoylmethane, 4-isopropylidibenzoylmethane, and mixtures thereof. A preferred
sunscreen active is 4-(1, 1-dimethylethyl)-4’-methoxydibenzoylmethane.

The sunscreen active 4-(1, 1-dimethylethyl)-4’-methoxydibenzoylmethane, which is also
known as butyl methoxydibenzoylmethane or “avobenzone,” is commercially available under the
names of Parsol® 1789 from Givaudan Roure (International) S. A., and Eusolex® 9020 from
Merck & Co., Inc. The sunscreen 4-isopropylidibenzoylmethane, which is also known as
isopropylidibenzoylmethane, is commercially available from Merck under the name of Eusolex®
8020.

In some embodiments, the compositions of the present invention further include one or
more UVB sunscreen actives that absorb(s) UV radiation having a wavelength of about 290nm to
about 320nm. The compositions comprise an amount of the UVB sunscreen active that is safe and
effective in providing UVB protection either independently, or in combination with, other UV
protective actives which may be present in the compositions. The compositions comprise from
about 0.1% to about 20%, preferably from about 0.1% to about 12%, and more preferably from
about 0.5% to about 8% by weight, of each UVB absorbing organic sunscreen, or as mandated by
the relevant regulatory authority(s).

A variety of UVB sunscreen actives are suitable for use herein (See e.g., U.S. Pat No.
5,087,372; U.S. Pat.No. 5,073,371; U.S. Pat. No. 5,073,372; U.S. Pat. No. 4,937,370; and U.S.
Pat. No. 4,999,186). Preferred UVB sunscreen actives are selected from 2-ethylhexyl-2-cyano-3,
2-ethylhexyl N,N-dimethyl-p-aminobenzoate, p-aminobenzoic acid, oxybenzone, homomenthyl
salicylate, octyl salicylate, 4,4’-methoxy-t-butyldibenzoylmethane, 4-isopropyl dibenzoylmethane,
3-benzylidene camphor, 3-(4-methylbenzylidene) camphor, 3-diphenylacrylate, 2-phenyl-
benzimidazole-5-sulphonic acid (PBSA), cinnamate esters and their derivatives such as 2-
ethylhexyl-p-methoxycinnamate, salicylate esters and their derivatives such as triethanolamine
salicylate, ethylhexyl salicylate, octyldimethyl para-aminobenzoic acid, camphor derivatives and
their derivatives, and mixtures thereof. Preferred organic sunscreen actives include 2-ethylhexyl-2-
cyano-3, 3-diphenylacrylate, 2-phenyl-benzimidazole-5-sulphonic acid (PBSA), octyl-p-
methoxycinnamate, and mixtures thereof. Salt and acid neutralized forms of the acidic sunscreens
are also useful herein.
In some embodiments, at least one agent is added to any of the compositions useful in the present invention to stabilize the UVA sunscreen to prevent it from photo-degrading on exposure to UV radiation and thereby maintaining its UVA protection efficacy. A wide range of compounds are reported to have these stabilizing properties and should be chosen to complement both the UVA sunscreen and the composition as a whole (See e.g., U.S. Pat. Nos 5,972,316; 5,968,485; 5,935,556; 5,827,508; and WO 00/06110). Preferred examples of stabilizing agents for use in the present invention include 2-ethylhexyl-2-cyano-3, 3-diphenylacrylate, ethyl-2-cyano-3, 3-diphenylacrylate, 2-ethylhexyl-3, 3-diphenylacrylate, ethyl-3, 3-bis(4-methoxyphenyl)acrylate, diethylhexyl 2,6 naphthalate and mixtures thereof (Symrise Chemical Company).

In some embodiments, at least one agent is added to any of the compositions useful in the present invention to improve the skin substantivity of those compositions, particularly to enhance their resistance to being washed off by water or rubbed off. Examples include, but are not limited to, acrylates/C_{12-18} alkylmethacrylate copolymer, acrylate/acylate copolymer, dimethicone, dimethiconol, graft-copoly (dimethylsiloxane/butyl methacrylate), lauryl dimethicone, PVP/Hexadecane copolymer, PVP/Eicosene copolymer, tricontanyl PVP and trimethoxysiloxy silicate.

In addition to organic sunscreens, in some embodiments, the compositions of the present invention additionally comprise inorganic physical sunblocks (See e.g., TFA International Cosmetic Ingredient Dictionary, 6th Edition, pp. 1026-28 and 1103 [1995]; Sayre et al., J. Soc. Cosmet. Chem., 41:103-109 [1990]; and Lowe et al., supra). Preferred inorganic physical sunblocks include zinc oxide and titanium dioxide and mixtures thereof.

When used in preferred embodiments, the physical sunblocks are present in an amount such that the present compositions are transparent on the skin (i.e., non-whitening), preferably from about 0.5% to about 20%, preferably from about 0.5% to about 10%, and more preferably from about 0.5% to 5% by weight. When titanium dioxide is used, it can have an anatase, rutile or amorphous structure. Manufacturers of micronized grade titanium dioxide and zinc oxide for sunscreen use include, but are not limited to Tayca Corporation, Uniqema, Shinetsu Chemical Corporation, Kerr-McGee, Nanophase, Nanosource, Sachtleben, Elementis, and BASF Corporation, as well as their distribution agents and those companies that further process the material for sunscreen use. Physical sunblock particles (e.g., titanium dioxide and zinc oxide) can be uncoated or coated with a variety of materials including but not limited to amino acids, aluminum compounds such as alumina, aluminum stearate, aluminum laurate, and the like; carboxylic acids and their salts (e.g., stearic acid and its salts); phospholipids, such as lecithin; organic silicone compounds; inorganic silicone compounds such as silica and silicates and mixtures thereof. In some preferred embodiments, the compositions of the present invention
comprise from about 0.1% to about 15%, preferably from about 0.1% to about 7%, and more preferably from about 0.5% to about 5%, by weight, of inorganic sunscreen.

In some preferred embodiments, the composition of the present invention also include preservatives. Such preservatives include, but are not limited to pentylene glycol, ethylene diamine tetra acetate (EDTA) and their salts, chlorhexidine (and its diacetate, dihydrochloride, digluconate derivatives), 1,1,1-trichloro-2-methyl-2-propanol, parachloro metaxylenol, polyhexamethylenebiguanide hydrochloride, dehydroacetic acid, diazolidinyl urea, 2,4-dichlorobenzyl alcohol, 4,4-dimethyl-1,3-oxazolidine, formaldehyde (e.g., 37% aqueous solution, with 10-15% methanol to avoid polymerization), glutaraldehyde, dimethylidantoin, imidazolidinyl urea, 5-Chloro-2-methyl-4-isothiazolin-3-one, ortho-phenylphenol, 4-hydroxybenzoic acid esters (e.g., "paraben") and its methyl-, ethyl-, propyl-, isopropyl-, butyl-, and isobutyl-esters, trichlosan, 2-phenoxethanol, phenyl mercuric acetate, borate, nitrate, quaternium-15, salicylate, salicylic acid and its salts, calcium, calcium sorbate, sorbic acid and its salts, iodopropynyl butylcarbamate zinc pyrithione, benzyl alcohol, 5-bromo-5-nitro-1,3-dioxane, 2-bromo-2-nitropropane-1,3-diol, benzoic acid and its salts, sulfites, bisulfites, phenoxyethanol, chloroxylenol, diazolidinyl urea, methylparabens, propylparabens, isopropylparabens, isobutylparabens, butylparabens, ethylparaben, phenoxyethanol PG, and benzalkonium chloride.

A variety of optional ingredients such as neutralizing agents, perfumes and perfume solubilizing agents, and coloring agents, also find use in some of the compositions herein. It is preferred that any additional ingredients enhance the skin softness/smoothness benefits of the product. In addition it is preferred that any such ingredients do not negatively impact the aesthetic properties of the product.

Other optional materials include keratolytic agents, as well as water-soluble and/or solubilizable preservatives preferably at a level of from about 0.1% to about 5% (e.g., Germall 115, methyl, ethyl, propyl and butyl esters of hydroxybenzoic acid, benzyl alcohol, DMDM hydantoin iodopropynyl butylcarbamate available under the trade name Glydant Plus from Lonza; EDTA, EUXYL® K400, Bromopol (2-bromo-2-nitropropane-1,3-diol) and phenoxypropanol); antibacterials (e.g., IRGASAN®) and phenoxyethanol (preferably at levels of from about 0.1% to about 5%; as well as soluble or colloidally-soluble moisturizing agents such as hyaluronic acid, chondroitin sulfate, and starch-grafted sodium polyacrylates (e.g., SANWET® IM-1000, IM-1500 and IM-2500, available from Celanese Superabsorbent Materials, Portsmouth, VA; See e.g., U.S. Pat. No. 4,076,663; vitamins such as vitamin A, vitamin C, vitamin E and derivatives thereof and building blocks thereof such as phytantriol, and vitamin K and components thereof such as the fatty alcohol dodecatrienol; alpha and beta hydroxyacids; aloe vera; sphingosines and phytosphingosines, cholesterol; skin whitening agents; N-acetyl cysteine; colouring agents;
antibacterial agents such as TCC/TCS, also known as triclosan and trichlorocarbon; perfumes and perfume solubilizers. Examples of alpha hydroxy acids include glycolic acid, lactic acid, malic acid, citric acid, glycolic acid in conjunction with ammonium glycolate, alpha-hydroxy ethanoic acid, alpha-hydroxyoctanoic acid, alpha-hydroxycaprylic acid, hydroxycaprylic acid, mixed fruit acid, tri-alpha hydroxy fruit acids, triple fruit acid, sugar cane extract, alpha hydroxy and botanicals, l-alpha hydroxy acid and glycomer in crosslinked fatty acids (e.g., alpha nutrium). Preferred examples of alpha hydroxy acids are glycolic acid and lactic acid. It is preferred that alpha hydroxy acids are used in levels of up to about 10%. It is not intended that the present invention be limited to any particular compound derived from any particular source, as any suitable additive compound, whether obtained from natural sources or through synthesis in the laboratory find use in the present invention.

Other optional materials include water-soluble or solubilizable preservatives preferably at a level of from about 0.1% to about 5% each, such as Germall 115, methyl, ethyl, propyl and butyl esters of hydroxybenzoic acid, benzyl alcohol, DMDM hydantoin iodopropynyl butylcarbanate available under the trade name Glydant Plus from Lonza, EDTA, Euxyl (RTM) K400, Bromopol (2-bromo-2-nitropropane-1,3-diol), pentylene glycol and phenoxypropanol; anti-bacterials such as Irgasan (RTM) and phenoxethanol (preferably at levels of from 0.1% to about 5%). Antibacterial agents such as TCC/TCS, also known as triclosan and trichlorocarbon are also useful in compositions of the present invention.

Neutralizing agents suitable for use in neutralizing acidic group containing hydrophilic gelling agents herein include sodium hydroxide, potassium hydroxide, ammonium hydroxide, monoethanolamine, diethanolamine, amino methyl propanol, tris-buffer and triethanolamine.

Other optional materials that find use in the present invention include any of the numerous functional and/or active ingredients known to those skilled in the art (See e.g., McCutcheon’s Functional Materials, North American and International Editions, MC Publishing Co. [2003]) As indicated above, non-limiting examples include keratolytic agents; soluble or colloidally-soluble moisturizing agents such as hyaluronic acid and chondroitin sulfate; vitamins such as vitamin A, vitamin C, vitamin E, vitamin K and derivatives thereof and building blocks thereof; phytantriol; fatty alcohols such as dodecatrienol; alpha and beta hydroxyacids; aloe vera; sphingosines and phytosphingosines, cholesterol; skin whitening agents; N-acetyl cysteine; coloring agents;

Examples of alpha hydroxy acids include glycolic acid, lactic acid, malic acid, and citric acid (whether derived synthetically or from natural sources and whether used alone or in combination) and their esters or relevant buffered combinations. Other examples of alpha-hydroxy acids include: alpha-hydroxy ethanoic acid, alpha-hydroxyoctanoic acid, alpha-hydroxycaprylic acid, and
hydroxycaprylic acid. Preferred examples of alpha hydroxy acids include glycolic acid and lactic acid. It is preferred that alpha hydroxy acids are used in levels of up to about 10%.

Optional materials include pigments that, where water-insoluble, contribute to and are included in the total level of oil phase ingredients. Pigments suitable for use in the compositions of the present invention can be organic and/or inorganic. Also included within the term “pigment” are materials having a low color or luster, such as matte finishing agents, light scattering agents, and formulation aids such as micas, sericites, and carbonate salts. Further examples of suitable pigments include titanium dioxide, iron oxides, glutamate iron oxides, zinc oxide, bismuth oxychloride, ultramarine blue, (all of which may be either pre-dispersed and/or pre-coated or not) D&C dyes and lakes, FD&C colors, natural color additives such as carmine, and mixtures thereof. Depending upon the type of composition, a mixture of pigments is usually used in preferred embodiments of the present invention. Preferred pigments for use herein from the viewpoint of moisturization, skin feel, skin appearance and emulsion compatibility are treated pigments. In some embodiments, the pigments are treated with compounds, including but not limited to amino acids, silicones, lecithin and ester oils.

In preferred embodiments, the pH of the compositions herein is in the range from about 3.5 to about 10, preferably from about 4 to about 8, and more preferably from about 5 to about 7, wherein the pH of the final composition is adjusted by addition of acidic, basic or buffer salts as necessary, depending upon the composition of the forms and the pH-requirements of the compounds.

The compositions of the present invention are prepared by standard techniques well known to those skilled in the art. In general the aqueous phase and/or the oil phase are prepared separately, with materials of similar phase partitioning being added in any order. If the final product is an emulsion, the two phases are then combined with vigorous stirring and/or homogenization as necessary, to reduce the size of the internal phase droplets. Any ingredients in the formulation with high volatility, or which are susceptible to hydrolysis or decomposition at high temperatures, are added with gentle stirring towards the end of the process, post emulsification if applicable. Dosage frequency and amount will depend upon the desired performance criteria.

In some embodiments of the present invention, method of decreasing VEGF activity are provided. In these embodiments, the methods comprise applying to an organism in need thereof an effective amount of any one of the compounds set forth herein. In additional preferred embodiments, the present invention provides compounds for treatment of an organism in need thereof, including humans and other animals.
Experimental

The following Examples serve to illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

In the experimental disclosure which follows, the following abbreviations apply: PI (proteinase inhibitor), BBI (Bowman-Birk inhibitor), STI (Soybean Trypsin inhibitor); ppm (parts per million); VEGF and VegF (vascular endothelial growth factor); M (molar); mM (millimolar); μM (micromolar); nM (nanomolar); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); gm (grams); mg (milligrams); μg (micrograms); pg (picograms); L (liters); ml and mL (milliliters); μl and μL (microliters); cm (centimeters); mm (millimeters); μm (micrometers); nm (nanometers); U (units); V (volts); MW (molecular weight); sec (seconds); min(s) (minute/minutes); hr(s) (hour/hours); °C (degrees Centigrade); QS (quantity sufficient); ND (not done); NA (not applicable); rpm (revolutions per minute); H2O (water); dH2O (deionized water); HCl (hydrochloric acid); aa (amino acid); bp (base pair); kb (kilobase pair); kD (kilodaltons); cDNA (copy or complimentary DNA); DNA (deoxyribonucleic acid); ssDNA (single stranded DNA); dsDNA (double stranded DNA); dNTP (deoxyribonucleotide triphosphate); RNA (ribonucleic acid); MgCl2 (magnesium chloride); NaCl (sodium chloride); w/v (weight to volume); v/v (volume to volume); g (gravity); OD (optical density); A405 (absorbance at 405 nm); V max (the maximum initial velocity of an enzyme catalyzed reaction); FGFrI(IIIc) (FGF-5 receptor); Dulbecco’s phosphate buffered solution (DPBS); SOC (2% Bacto-Tryptone, 0.5% Bacto Yeast Extract, 10 mM NaCl, 2.5 mM KCl); Terrific Broth (TB; 12 g/l Bacto Tryptone, 24 g/l glycerol, 2.31 g/l KH2PO4, and 12.54 g/l K2HPO4); OD280 (optical density at 280 nm); OD600 (optical density at 600 nm); PAGE (polyacrylamide gel electrophoresis); PBS (phosphate buffered saline [150 mM NaCl, 10 mM sodium phosphate buffer, pH 7.2]); PBST (PBS+0.25% Tween® 20); PEG (polyethylene glycol); PCR (polymerase chain reaction); RT-PCR (reverse transcription PCR); SDS (sodium dodecyl sulfate); bME, BME and βME (beta-mercaptoethanol or 2-mercaptoethanol); Tris-HCl (tris[hydroxymethyl]ammonomethane-hydrochloride); Tricine (N-[tris-(hydroxymethyl)-methyl]-glycine); CHES (2-(N-cyclo-hexylamino) ethane-sulfonic acid); TAPS (3-[[tris-(hydroxymethyl)-methyl]-amino]-propanesulfonic acid); CAPS (3-(cyclo-hexylamino)-propanesulfonic acid); DMSO (dimethyl sulfoxide); DTT (1,4-dithio-DL-threitol); Glut and GSH (reduced glutathione); GSSG (oxidized glutathione); TCEP (Tris[2-carboxyethyl] phosphate); Tris (tris(hydroxymethyl)ammonomethane); HEPES (N-[2-Hydroxyethyl]piperazine-N-[2-ethanesulfonic acid]); HBS (HEPES buffered saline); SDS (sodium dodecylsulfate); Tris-HCl (tris[hydroxymethyl]ammonomethane-hydrochloride); Ci (Curies) mCi (milliCuries); μCi (microCuries); TLC (thin layer chromatography); Ts (tosyl); Bn (benzyl); Ph (phenyl); Ms (mesyl); Et (ethyl), Me (methyl); Klenow (DNA polymerase I large (Klenow) fragment); rpm
(revolutions per minute); EGTA (ethylene glycol-bis(β-aminoethyl ether) N, N', N', N'-tetraacetic acid); EDTA (ethylenediaminetetraacetic acid); bla (β-lactamase or ampicillin-resistance gene); PDS (plasma-derived bovine serum that has been dialyzed to remove growth factors; dialysis of defibrinated bovine plasma is performed against DMEM for about 6 hours at 4°C, with stirring, the media is changed and dialysis is continued overnight; the dialyzed PDS is collected after 24 hours, and sterile filtered twice through a 0.2 μm filter); FCS and FBS (fetal calf serum); GE Healthcare (GE Healthcare, Chalfont St. Giles, United Kingdom); DNA2.0 (DNA2.0, Menlo Park, CA); OXOID (Oxoid, Basingstoke, Hampshire, UK); Megazyme (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co., Wicklow, Ireland); Corning (Corning Life Sciences, Corning, NY); (NEN (NEN Life Science Products, Boston, MA); Pharma AS (Pharma AS, Oslo, Norway); Dynal (Dynal, Oslo, Norway); Bio-Synthesis (Bio-Synthesis, Lewisville, TX); ATCC (American Type Culture Collection, Rockville, MD); Gibco/BRL (Gibco/BRL, Grand Island, NY); Sigma (Sigma Chemical Co., St. Louis, MO); Pharmacia (Pharmacia Biotech, Piscataway, NJ); NCBI (National Center for Biotechnology Information); Applied Biosystems (Applied Biosystems, Foster City, CA); Clontech (CLONTECH Laboratories, Palo Alto, CA); Difco (Difco Laboratories, Detroit, MI); Oxoid (Oxoid Inc., Ogdensburg, NY); GIBCO BRL or Gibco BRL (Life Technologies, Inc., Gaithersburg, MD); Millipore (Millipore, Billerica, MA); Bio-Rad (Bio-Rad, Hercules, CA); Invitrogen (Invitrogen Corp., San Diego, CA); NEB (New England Biolabs, Beverly, MA); Cambrex (Cambrex Bioproducts, East Rutherford, NJ); Sigma (Sigma Chemical Co., St. Louis, MO); Pierce (Pierce Biotechnology, Rockford, IL); Takara (Takara Bio Inc. Otsu, Japan); Roche (Hoffmann-La Roche, Basel, Switzerland); EM Science (EM Science, Gibbstown, NJ); Qiagen (Qiagen, Inc., Valencia, CA); Biodesign (Biodesign Intl., Saco, Maine); Biosource (Biosource, Intl., Camarillo, CA); Aptagen (Aptagen, Inc., Herndon, VA); Molecular Devices (Molecular Devices, Corp., Sunnyvale, CA); R&D Systems (R&D Systems, Minneapolis, MN); Stratagene (Stratagene Cloning Systems, La Jolla, CA); Marsh (Marsh Biosciences, Rochester, NY); Bio-Tek (Bio-Tek Instruments, Winooski, VT); Biacore (Biacore, Inc., Piscataway, NJ); PeproTech (PeproTech, Rocky Hill, NJ); SynPep (SynPep, Dublin, CA); Chemicon (CHEMICON, Temecula, CA); Clinical Research Laboratories, (Clinical Research Laboratories, Inc., Piscataway, NJ); and Microsoft (Microsoft, Inc., Redmond, WA).

EXAMPLE 1

Dermatological Compositions

In this Example, various dermatological compositions comprising any of the compounds of the present invention are provided as follows.
MOISTURIZING BODYWASH (pH 7)

<table>
<thead>
<tr>
<th>RAW MATERIAL</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>QS</td>
</tr>
<tr>
<td>Glycerin</td>
<td>4.0</td>
</tr>
<tr>
<td>PEG-6 Caprylic/Capric Glycerides</td>
<td>4.0</td>
</tr>
<tr>
<td>Palm Kernel Fatty acids</td>
<td>3.0</td>
</tr>
<tr>
<td>Sodium Laureth-3 Sulphate</td>
<td>45.0</td>
</tr>
<tr>
<td>Cocamide MEA</td>
<td>3.0</td>
</tr>
<tr>
<td>Sodium Lauroamphoacetate</td>
<td>25.0</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>10.0</td>
</tr>
<tr>
<td>Polyquaternium-10</td>
<td>0.70</td>
</tr>
<tr>
<td>Preservative, fragrance, color</td>
<td>QS</td>
</tr>
<tr>
<td>Compound</td>
<td>1000ppm</td>
</tr>
</tbody>
</table>

BODY WASH

<table>
<thead>
<tr>
<th>RAW MATERIAL</th>
<th>pH 8</th>
<th>pH 6.5</th>
<th>pH 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>(INCI Designation)</td>
<td>Amount</td>
<td>Amount</td>
<td>Amount</td>
</tr>
<tr>
<td>Deionized water</td>
<td>QS</td>
<td>QS</td>
<td>QS</td>
</tr>
<tr>
<td>Sodium Laureth Sulphate</td>
<td>12</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Cocamidopropyl Betaine</td>
<td>8</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Decyl Glucoside</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Polyquaternium-10</td>
<td>0.25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Polyquaternium-7</td>
<td>0</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>Preservative, fragrance, color</td>
<td>QS</td>
<td>QS</td>
<td>QS</td>
</tr>
<tr>
<td>Compound</td>
<td>250ppm</td>
<td>500ppm</td>
<td>1000ppm</td>
</tr>
</tbody>
</table>
### BODY LOTION

<table>
<thead>
<tr>
<th>RAW MATERIAL</th>
<th>pH 7</th>
<th>pH 7</th>
<th>pH 7.5</th>
<th>pH 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>(INCI Designation)</td>
<td>Amount</td>
<td>Amount</td>
<td>Amount</td>
<td>Amount</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>QS</td>
<td>QS</td>
<td>QS</td>
<td>QS</td>
</tr>
<tr>
<td>Glycerine</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Isohexadecane</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Niacinamide</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Isopropyl Isostearate</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Polyacrylamide (and) Isoparaffin (and) Laureth-7</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Petrolatum</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Nylon 12</td>
<td>2</td>
<td>2</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Dimethicone</td>
<td>2</td>
<td>2</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Sucrose Polycottonseed Oil</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Stearyl Alcohol 97%</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D Panthenol</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>DL-alpha Tocopherol Acetate</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cetyl Alcohol 95%</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Behenyl Alcohol</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Cetearyl Alcohol (and) Cetearyl Glucoside</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Compounds</td>
<td>250 ppm</td>
<td>500 ppm</td>
<td>750 ppm</td>
<td>1000 ppm</td>
</tr>
</tbody>
</table>

### ULTRA-HIGH MOISTURIZING EMULSION

<table>
<thead>
<tr>
<th>RAW MATERIAL</th>
<th>pH 7</th>
<th>pH 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>(INCI Designation)</td>
<td>Amount</td>
<td>Amount</td>
</tr>
<tr>
<td>Deionized water</td>
<td>QS</td>
<td>QS</td>
</tr>
<tr>
<td>Glycerin</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>PEG 400</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Niacinamide</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Isohexadecane</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Dimethicone</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Polyacrylamide (and) Isoparaffin (and) Laureth-7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Isopropyl Isostearate</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Polymethylsilsesquioxane</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cetyl Alcohol 95%</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sucrose Polycottonseed Oil</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D-Panthenol</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tocopherol Acetate</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Stearyl Alcohol 95%</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Cetearyl Glucoside</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>PEG-100-Stearate</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Preservative, fragrance, color</td>
<td>QS</td>
<td>QS</td>
</tr>
<tr>
<td>Compound</td>
<td>250 ppm</td>
<td>100 ppm</td>
</tr>
</tbody>
</table>
### MOISTURIZING CREAM

<table>
<thead>
<tr>
<th>RAW MATERIAL (INCI Designation)</th>
<th>pH 7</th>
<th>pH 7</th>
<th>pH 7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized water</td>
<td>QS</td>
<td>QS</td>
<td>QS</td>
</tr>
<tr>
<td>Glycerine</td>
<td>3</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Petrolatum</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Cetyl Alcohol 95%</td>
<td>1.5</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>Dimethicone Copolyol</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Isopropyl Palmitate</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Carbopol 954 (Noveon)</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Dimethicone (350cs)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Stearyl Alcohol 97%</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Peg-100-stearate</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Titanium Dioxide</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Preservative, color, fragrance</td>
<td>QS</td>
<td>QS</td>
<td>QS</td>
</tr>
<tr>
<td>Compound</td>
<td>50ppm</td>
<td>250ppm</td>
<td>1000ppm</td>
</tr>
</tbody>
</table>

### LEAVE-ON HAIR CONDITIONER

<table>
<thead>
<tr>
<th>RAW MATERIAL (INCI Designation)</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>QS</td>
</tr>
<tr>
<td>Isostearimidopropyl Morpholine Lactate</td>
<td>6.0</td>
</tr>
<tr>
<td>Hydroxyethylcellulose</td>
<td>1.0</td>
</tr>
<tr>
<td>Preservative, fragrance, color</td>
<td>QS</td>
</tr>
<tr>
<td>Compound</td>
<td>1000ppm</td>
</tr>
</tbody>
</table>
CREAM RINSE (pH 4)

<table>
<thead>
<tr>
<th>RAW MATERIAL</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>(INCI Designation)</td>
<td></td>
</tr>
<tr>
<td>Deionized Water</td>
<td>QS</td>
</tr>
<tr>
<td>Behentrimonium Chloride</td>
<td>2.0</td>
</tr>
<tr>
<td>Trilaureth-4 Phosphate</td>
<td>1.5</td>
</tr>
<tr>
<td>Cetyl alcohol</td>
<td>2.0</td>
</tr>
<tr>
<td>Citric acid</td>
<td>QS</td>
</tr>
<tr>
<td>Preservative, fragrance, color</td>
<td>QS</td>
</tr>
<tr>
<td>Compound</td>
<td>1000ppm</td>
</tr>
</tbody>
</table>

NOURISHING HAIR CONDITIONER/TREATMENT (pH 6)

<table>
<thead>
<tr>
<th>RAW MATERIAL</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>(INCI Designation)</td>
<td></td>
</tr>
<tr>
<td>Deionized Water</td>
<td>QS</td>
</tr>
<tr>
<td>Behentrimonium Methosulfate (and) Cetyl Alcohol</td>
<td>4.0</td>
</tr>
<tr>
<td>Wheat germ oil</td>
<td>1.0</td>
</tr>
<tr>
<td>Cetyl alcohol</td>
<td>0.5</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>5.0</td>
</tr>
<tr>
<td>PEG-60 Lanolin</td>
<td>1.0</td>
</tr>
<tr>
<td>Panthenol</td>
<td>2.0</td>
</tr>
<tr>
<td>Lupin amino acids</td>
<td>1.0</td>
</tr>
<tr>
<td>Cocodimonium Hydroxypropyl Hydrolyzed Wheat Protein</td>
<td>1.0</td>
</tr>
<tr>
<td>Fragrance, preservative, color</td>
<td>QS</td>
</tr>
<tr>
<td>Compound</td>
<td>1000ppm</td>
</tr>
</tbody>
</table>

CONDITIONING SHAMPOO

<table>
<thead>
<tr>
<th>RAW MATERIAL</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>(INCI Designation)</td>
<td></td>
</tr>
<tr>
<td>Deionized Water</td>
<td>QS</td>
</tr>
<tr>
<td>Sodium Laureth Sulfate 30%</td>
<td>27.0</td>
</tr>
<tr>
<td>Cocamidopropyl Betaine</td>
<td>3.7</td>
</tr>
<tr>
<td>Coco-Glucoside (and) Glyceryl Oleate</td>
<td>5.0</td>
</tr>
<tr>
<td>Coco-Glucoside (and) Glycol Distearate (and) Glycerine</td>
<td>3.0</td>
</tr>
<tr>
<td>Guar Hydroxypropyl Trimonium Chloride</td>
<td>0.1</td>
</tr>
<tr>
<td>Laureth-2</td>
<td>1.55</td>
</tr>
<tr>
<td>Fragrance, preservative, color</td>
<td>QS</td>
</tr>
<tr>
<td>Compound</td>
<td>1000ppm</td>
</tr>
</tbody>
</table>
ANTI-DANDRUFF SHAMPOO

<table>
<thead>
<tr>
<th>RAW MATERIAL</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>QS</td>
</tr>
<tr>
<td>Magnesium Aluminum Silicate</td>
<td>1.0</td>
</tr>
<tr>
<td>Hydroxypropyl Methylcellulose</td>
<td>0.8</td>
</tr>
<tr>
<td>Sodium Olefin Sulfate 40%</td>
<td>35.0</td>
</tr>
<tr>
<td>Lauramide DEA</td>
<td>4.0</td>
</tr>
<tr>
<td>Soymamide DEA</td>
<td>1.0</td>
</tr>
<tr>
<td>Quaternium-70 Hydrolyzed Collagen</td>
<td>2.0</td>
</tr>
<tr>
<td>Zinc Pyrithione 40%</td>
<td>4.0</td>
</tr>
<tr>
<td>Fragrance, preservative, color</td>
<td>QS</td>
</tr>
<tr>
<td>Compound</td>
<td>1000ppm</td>
</tr>
</tbody>
</table>

EXAMPLE 2

Panning of a Phage Displayed Peptide Library

In this Example, experiments conducted to pan a phage displayed library are described. A commercially available phage peptide library PhD C7C (NEB) was panned against hVEGF_{165} (R&D systems) for 3 rounds according to the manufacturer’s instructions. This procedure yielded the sequence profiles summarized in Figure 1. Individual clones were confirmed using phage ELISA according to the manufacturers instructions (See, Figure 2).

EXAMPLE 3

BIAcore™ Binding Analysis of Anti-TGFβ-1 Peptides

In this Example, experiments conducted to assess the affinities of the peptides for VEGF are described. Affinities of the peptides for VEGF were measured using a BIAcore™-3000 surface plasmon resonance system (Biacore). A CM5 sensor chip was conditioned with 50 mM NaOH, 0.1% HCl, 0.1% SDS, and 0.08% H_{3}PO_{4} and activated for covalent coupling of FGF-5 using N-ethyl-N′-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier’s instructions (Biacore). Human VEGF_{165} (Biosource) was diluted to 5 μg/ml in 20 mM sodium acetate, pH 4.8 and injected at a flow rate of 2 μl/min to achieve approximately 1000 to 6000 response units (RU) of coupled protein. TNFα (human TNFα, Biosource, Int., Camarillo, CA) was similarly coupled to the CM5 sensor chip to approximately 850 to 3500 RU in the reference lane. A solution of 1 M ethanolamine was injected as a blocking agent. In some experiments, an additional solution of EDC and NHS were injected to improve baseline stability and a solution of 1 M ethanolamine injected as a blocking agent. The reference lane is activated with EDC and NHS and blocked with ethanolamine.
Peptides were synthesized using standard FMOC chemistry, purified by reverse phase HPLC to >95% purity (SynPep), and stored at 10 mg/mL in DMSO. For kinetic measurements, twofold serial diluted peptides in HBS-EP buffer, 0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20 (Biacore), were injected at 25°C at a flow rate of 20 μL/min. Twofold serial diluted DMSO samples and buffer samples were also injected for background subtraction. Kinetic parameters were calculated using BIAevaluation 3.1 software.

EXAMPLE 4

Construction of Peptide-BLA Scaffolds

In this Example, methods used in the construction of anti-VEGF-BBI constructs are described. Plasmid pCM01 (5.1kb) encodes a 15-amino acid peptide sequence CK37281 fused to the N-terminus of Enterobacter cloacae β-lactamase (BLA) with a pIII signal sequence and C-terminal 6XHis tag, (See, Figure 4). The plasmid also carries a chloramphenicol resistance gene (CAT) as a selectable marker and expression is driven by a lac promoter (Plac). Plasmid pCM01 was constructed using a Bbs1 vector, pME30 constructed from pCB04. pCB04 was digested with DraIII and Spe I (NEB), resulting in 2.8 kb and 2.1kb fragments. To make the inserts, the oligo pairs NtermStf2-F and NtermStf2-R (5 μM) were combined in 50 μl total volume in water, the mixture was heated at 95°C in heat block for 5 minutes, and the block was allowed to cool to room temperature.

Oligos: NtermStf2-F and NtermStf2-R for stuffer vector insert:
NtermStf2-F
5’ [Phos]  
CTAGTGTCTTCCGATCAAGTCGACAACAGCCTGTCTGCAGATCTGCAGAAGACTTGGCGGAG  
GTGCTCGCGAATACGATTACCCTCCGCTGATGAAAGCACAGA 3’ (SEQ ID NO:26)

NtermStf2-R
5’ [Phos]  
GTGCTTTTACATCGCGGGTAAATCGATATTCCGACCACCTCCGAGCTTCCAGAATCTG  
CAGACAGGGCTTGTGTCGAGCTTGAAGACA 3’ (SEQ ID NO:27)

The 2.8kb fragment, 2.1kb fragment, and stuffer insert (100bp) were ligated overnight at 16°C in a 1:1:5 molar ratio respectively using 10 μl of the DNA mix and 10 μl of Takara solution I ligase. Ligations were purified using Zymo Research DNA clean kit and eluted in 2x 8 μl of water. Then, 5 μl of ligation mix was transformed into 50 μl Top 10 electrocompetent cells (Invitrogen),
250 μl SOC was added and the cells grown for 1 hr at 37°C. The transformation mix was diluted 1/10 and plated on both LA + 5 ppm CMP and LA + 5 ppm CMP + 0.1 ppm CTX plates, followed by incubation overnight at 37 °C. 12 colonies were picked from CMP plates, grown in LB + 5 ppm CMP, DNA was isolated and digested with BbsI enzyme (2 sites in stuffer plasmid). pCB04 (WT) was also digested as control. One clone had the correct sequence and was designated pME22.

The VEGF peptide-BLA expression plasmid pCM01 was constructed from pME22 using the following primers for the BBs1 insert (See, Figure 5); Oligos VegF-F, VegF-5R, VegF-3RP for peptide insert.

**VegF-F**

5'ACTAGTCGTTCTTTCTATTTCTCAGCTCTGCTTACCCCTGTGGCCGACTTTCTGTCGCGTGGAGGTTCCAGCCAGTTGCTCAAGAAAAACAGCTG 3' (SEQ ID NO:28)

**VegF-5R**

5' AGCAGAGTGAGATAGAAGGAAACGC 3' (SEQ ID NO:29)

**VegF-3RP**

5' [Phos]CGCCCAGCTTTTTCTGACACTGG 3' (SEQ ID NO:30)

BLA-peptide fusion proteins pCM01 and pCB04 (WT) and a biased library pCM04 were expressed in *E. coli* (TOP10; Invitrogen) in 1-L shake flasks in the presence of 5 ppm CMP and 0.1 ppm cefotaxime antibiotic at 25°C for 40 hrs. Cell pastes were harvested from the 200 ml cell cultures by centrifugation at 3,000xg for 10 min. The pastes were then treated with 25 ml of B-PER reagent (Pierce) for 40 min with slow mixing. The extract was separated by centrifugation at 20,000xg for 20 min. BLA activity of all liquid fractions was assayed using nitrocefin and the concentration of fusion proteins in each fraction was calculated assuming the same specific activity as the WT enzyme. Fusion proteins were purified by IMAC chromatography. The imidazole-eluted BLA-active fractions were pooled and the purity was found better than 95% as checked by SDS-PAGE (See, Figure 6).
EXAMPLE 5
Screening a Peptide-BLA Scaffold Library

In this Example, experiments to screen a peptide-BLA scaffold library are described.

COSTAR plates (96-well) were coated with 0.5 μg (100 μL of 5 μg/mL) hVEGF_{165} (Preprotech) with gentle rocking at 4 °C overnight, followed by blocking with Superblock blocking buffer (Pierce) for several hours at room temperature. His-tag purified samples of pCM01 and pCM04 were diluted serially into BLA assay buffer and 100 μl portions were transferred to VEGF coated wells. After one hour, plates were washed six times with PBS, 0.05% TWEEN®-20 and 200 μL of nitrocefin assay buffer containing 0.1 mg/ml nitrocefin (Oxoid) was added to measure residual bound beta-lactamase activity at Abs_{469}/min. Control wells contained pCB04 beta-lactamase as a control (See, Figure 7).

EXAMPLE 6
Inhibition of HUVE Cell Proliferation by aVEGF Peptides.

In this Example, experiments conducted to determine the effects of aVEGF peptides on HUVE cells are described. HUVE cells (human umbilical vein cells; Cambrex) were passaged 1-5 times and maintained according to manufacturer’s instructions. HUVE cell growth was stimulated by 0.03 to 20 ng/ml VEGF with the highest proliferation at 10 ng/ml VEGF_{165}. This concentration was also used in subsequent experiments. A series of a VEGF peptides from 0.5 nM to 25 μM (and an anti-VEGF monoclonal antibody control (R&D Systems)) were mixed with 10 ng/mL VEGF prior to addition to HUVE cells seeded in triplicate in 96-well plates. Cell proliferation was measured by ^{3}H-thymidine incorporation (See, Figure 8). Significant inhibition was observed down to 0.4 μM anti-VEGF.

EXAMPLE 7
Inhibition of Blood Vessel Tube Formation by VEGF Peptide Conjugates

In this Example, experiments conducted to assess blood vessel tube formation are described. This in vitro angiogenesis assay was obtained as a kit from Chemicon and used according to the manufacturer’s instructions.

This assay provides a simple model of angiogenesis in which the induction or inhibition of tube formation by exogenous signals can be monitored. An endothelial cell suspension of low passage HUVE cells was mixed with different concentrations of the inhibitor in the presence of 10 ng/mL VEGF, before adding the cells to "ECMatrix" (i.e., a solution that is polymerized in situ and provides a solid gel of basement proteins prepared so that endothelial cells align and form hollow
tube-like structures). Tube formation is a multi-step process involving cell adhesion, migration, differentiation and growth. The resulting tube formation was measured under an inverted light microscope at 20X-100X magnification. Significant inhibition of tubule formation was observed at concentrations above 1 μM peptide.

EXAMPLE 8

Construction of Phage-Displayed VEGF-Biased Peptide Libraries

In this Example, experiments conducted to constructed phage-display libraries are described. The affinity maturation libraries used for panning VEGF were constructed using the C7C gene III phage-display system known in the art (See, Noren and Noren [2001]). Oligonucleotides were synthesized and phosphorylated as known in the art. The oligonucleotides used to construct the libraries employ NNK (where N = G, A, T, C and K = G or T) codons. The NNK cloning scheme eliminates the potential for two stop codons and still encodes all twenty amino acids. The random peptide library displayed 9 random amino acids with two cysteines fixed at positions 2 and 9 (XCX7CGGGS; SEQ ID NO:31; X represents any amino acid). Seven CK37282 biased peptide libraries were created using the same methods as for the random library.

EXAMPLE 9

Construction of anVEGF Bowman Birk Inhibitor (BBIVENGF)

In this Example, construction of an anti-VEGF BBI construct is described. A synthetic gene coding for Bowman Birk Inhibitor (See, Figure 9) with appropriate restriction sites for introducing small peptide coding sequences into the trypsin loop (SacI-EcoRI) and/or chymotrypsin loop (EcoRI-SalI) was cloned into pET-22b (Novagen) using Ndel/XhoI cloning sites according to standard procedures known in the art. The resulting vector, pET BBI, was used as a template to insert the sequences CK37281, CK37282 into BBI loops as double-stranded oligonucleotide cassettes (Operon). Constructs were transformed into BL-21(DE3) E.coli, and plated on medium containing 50 μg/mL ampicillin. Plasmid DNA from individual clones was isolated using methods known in the art (Qiagen) and the correct inserts confirmed by DNA sequencing. Additional peptides of interest include PS-AV1 (1KSAIC-KYYLYWW-CF1V; SEQ ID NO:16) and PS-AV2 (1KSAIC-TLWKSYYW-CF1V; SEQ ID NO:17).

Fusion proteins and wild-type BBI were expressed in 14-L fermentors. Cell pastes were harvested and protein isolated from inclusion bodies using a modification of the FoldIt screening procedure (Hampton) (See, Figure 10).
EXAMPLE 10
BIACore™ Binding Analysis of BBI-VEGF

In this Example, experiments conducted to determine the binding affinity of constructs produced as indicated in Example 9 are described. Affinities of BBI-VEGF constructs for VEGF were measured using BIACore-3000 surface plasmon resonance (Biacore). A CM5 sensor chip was conditioned with 50 mM NaOH and activated for covalent coupling of VEGF using N-ethyl-N’-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier’s instructions (Biacore). VEGF (human VEGF165, Biosource) was diluted to 5µg/mL in 20mM sodium acetate, pH 4.8, and injected at a flow rate of 2 µL/min to achieve approximately 1000 to 6000 response units (RU) of coupled protein. Trypsin and chymotrypsin were similarly coupled to the CM5 sensor chip to approximately 850 to 3500 RU in remaining lanes. A solution of 1M ethanolamine was injected as a blocking agent. Selective binding affinity to VEGF of refolded BBI-VEGF is shown in Figure 11.

EXAMPLE 11
In vitro Cell Proliferation Assay to Test the Activity of hVEGF Inhibitory Peptides

In this Example, experiments conducted to determine the anti-proliferative activity of anti-VEG peptides are described. The antiproliferative activity of VEGF inhibitory peptides was determined using human umbilical vein endothelial cells (HUVEC) as follows. An early passage (less than six) of HUVEC was seeded in 96-well plates at 5000 cells per well and starved for 18 hrs in 200 µl EBM medium (Cambrex) without growth factors and supplemented with 0.5% FBS, at 37 °C with 5% CO₂. The medium was replaced with 180 µl of growth medium containing EBM medium with 5% fetal bovine serum and 1% DMSO. Then, 20 µl of VEGF preincubated for one hour with varying peptide concentrations (the final DMSO concentration of all the wells was 1%) were added to the wells for a final VEGF concentration of 10 ng/ml. Human VEGF antibody (R & D Systems) was used as a positive control. Cells with 0.31 to 20 ng/ml concentrations of VEGF alone in the growth medium were used to construct a standard growth curve. The cells were further incubated for 48 hrs, and the cell proliferation was measured using an MTS assay (CellTiter 96 Aqueous One Solution Cell Proliferation Assay Kit; Promega). Then, 40 µl of the MTS tetrazolium solution was added to each well and after 3 and 4 hours incubation, the plates were read at 490 nM. The absorption of media alone was subtracted from all data points. The results indicated that the VEGF inhibitory peptides CK37281 and CK37283 have IC50 in the micromolar range.
EXAMPLE 12

Repeat Insult Patch Testing (RIPT) of an Anti-VEGF Peptide on Human Skin

In this Example, experiments conducted to determine the patch test result for anti-VEGF peptides are described. Samples of the aVEGF peptide CK37281 dosed at 0.5% (w/v) were formulated in a base formulation containing deionized water/butylene glycol. Approximately 0.2 mL of the formulation was applied to 200 human volunteers in a repeated insult patch test according to procedures designed by Clinical Research Laboratories, Inc. (Piscataway, NJ). The results indicated that there was no dermal irritation or sensitization on the skin of these volunteers.

Having described the preferred embodiments of the present invention, it will appear to those ordinarily skilled in the art that various modifications may be made to the disclosed embodiments, and that such modifications are intended to be within the scope of the present invention.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The molecular complexes and the methods, procedures, treatments, molecules, specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.
The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.
CLAIMS

1. A composition comprising at least one peptide selected from the group consisting of SEQ ID NO:S:1-14, 16 and 18, wherein said peptide binds to a vascular endothelial growth factor.

2. The composition of Claim 1, wherein said peptide is expressed in a protease resistant scaffold.

3. The composition of Claim 2, wherein said scaffold is a protease inhibitor.

4. The composition of Claim 3, wherein said protease inhibitor is selected from the group consisting of Bowman-Birk Inhibitor, soybean trypsin inhibitor, and Eglin chymotrypsin inhibitor.

5. The composition of Claim 4, wherein said scaffold is Bowman-Birk inhibitor.

6. The composition of Claim 3, wherein said protease resistant scaffold and said peptide comprise a fusion protein.

7. The composition of Claim 6, wherein said composition comprises an amino acid sequence selected from the group consisting of SEQ ID NO:S:22-24.

8. The composition of Claim 6, wherein said scaffold comprises the amino acid sequence set forth in SEQ ID NO:19.

9. The composition of Claim 8, wherein said scaffold comprises the amino acid sequences set forth in SEQ ID NO:S:20 and 21.

10. The composition of Claim 9, wherein said amino acid sequences set forth in SEQ ID NO:S:20 and 21 are replaced by at least one peptide having an amino acid sequence selected from the group consisting of SEQ ID NO:S:1-8, 16 and 17.
11. A cosmetic or pharmaceutical composition comprising said at least one peptide of Claim 1.

12. The composition of Claim 11, wherein said composition is capable of modulating angiogenesis.

13. The composition of Claim 11, wherein said composition further comprises a scaffold comprising a protease inhibitor.

14. The composition of Claim 13, wherein said protease inhibitor is selected from the group consisting of Bowman-Birk Inhibitor, soybean trypsin inhibitor, and Eglin chymotrypsin inhibitor.

15. The composition of Claim 14, wherein said scaffold is Bowman-Birk inhibitor.

16. The composition of Claim 15, wherein said scaffold comprises the amino acid sequence set forth in SEQ ID NO:19.

17. The composition of Claim 15, wherein said scaffold comprises the amino acid sequences set forth in SEQ ID NOS:20 and 21.

18. The composition of Claim 17, wherein said amino acid sequences set forth in SEQ ID NOS:20 and 21 are replaced by at least one peptide having an amino acid sequence selected from the group consisting of SEQ ID NOS:1-8, 16 and 17.

19. A method for modulating angiogenesis comprising:
   i) providing a composition comprising a peptide contained within a scaffold;
   ii) providing a subject to be treated; and
   ii) applying said composition to said subject in an area in which angiogenesis modulation is desired.

20. The method of Claim 19, wherein said peptide binds to a vascular endothelial growth factor (VEGF).
21. The method of Claim 20, wherein said vascular endothelial growth factor (VEGF) is VEGF-A.

22. The method of Claim 19, wherein said scaffold is selected from the group consisting of Bowman-Birk inhibitor, soybean trypsin inhibitor, and Eglin chymotrypsin inhibitor.

23. The method of Claim 22, wherein said scaffold is Bowman-Birk inhibitor.

24. The method of Claim 22, wherein said scaffold comprises the amino acid sequence set forth in SEQ ID NO:19.


26. The method of Claim 24, wherein said amino acid sequences set forth in SEQ ID NOS:20 and 21 are replaced by at least one peptide having an amino acid sequence selected from the group consisting of SEQ ID NOS:1-8, 16 and 17.

27. The method of Claim 26, wherein said scaffold and said peptide are encoded by an amino acid sequence selected from the group consisting of SEQ ID NOS:22-24.

28. A method for decreasing the activity of a vascular endothelial growth factor comprising the steps of:
   i) providing a subject; and
   ii) administering the composition comprising at least one peptide that binds to said vascular endothelial growth factor to said subject, under conditions such that the activity of said vascular endothelial growth factor is decreased.

29. The method of Claim 28, wherein said vascular endothelial growth factor (VEGF) is VEGF-A.

30. The method of Claim 28, wherein said composition comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:22-24.
VEGF
3rd Round with C7C Library Acid Eluted

CK37281  YNLYGWT-  (SEQ. ID NO:1)
CK37282  -TLWPTFW  (SEQ. ID NO:2)
CK37283  -NLWPHFW  (SEQ. ID NO:3)
CK37284  -SLWPAFW  (SEQ. ID NO:4)
CK37286  -APWNSHI  (SEQ. ID NO:5)
CK37287  -APWNLHI  (SEQ. ID NO:6)
CK37289  -TLWPSYW  (SEQ. ID NO:7)
Consensus LWP  W

YNLYGWT(5) CK37281 (SEQ. ID NO:8)
APWNSHI(2) CK37286 (SEQ. ID NO:9)
APWNLHI(2) CK37287 (SEQ. ID NO:10)

TLWPTFW CK37282 (SEQ. ID NO:11)
TLWPSYW(5) CK37289 (SEQ. ID NO:12)
NLWPHFW CK37283 (SEQ. ID NO:13)
SLWPAFW(2) CK37284 (SEQ. ID NO:14)

FIG. 1

SUBSTITUTE SHEET (RULE 26)
FIG. 4A

FIG. 4B

SUBSTITUTE SHEET (RULE 26)
FIG. 4C

pCM 01
5112 bp

CK37281-Lys
pIII Signal
Sequence

BLA

Amber TAG Stop
His Tag
Linker

gIII d2

Linker
**FIG. 5**
FIG._6

FIG._10

SUBSTITUTE SHEET (RULE 26)