PEPTIDES, COMPOSITIONS AND METHODS OF USE

Field

The present disclosure relates to novel peptides useful in the treatment and/or prevention of skin conditions, such as skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone. The present disclosure also relates to compositions comprising the peptides, and to methods and uses involving the peptides and compositions.

Background

Aging is a natural biological process marked by the gradual deterioration of an organism's cells, tissues, and systems over time, leading to an accumulation of a wide variety of molecular and cellular damage over time which leads to increased senescence. Even though facial wrinkles are often the first indicator of aging, the manifestation of aging extends beyond facial features. In fact, aging is a systemic condition that affects the whole body.

In the field of geroscience, which studies the molecular relationship between aging and age-related diseases, they noticed that both processes have many similarities, which they termed as the 7 pillars of aging, namely adaptation to stress, macromolecular damage, proteostasis, aberrant metabolism, epigenetics, stem cell regeneration and inflammation. These 7 pillars are interconnected and modulating each other; interestingly, they all converge on inflammation, which means impairment in any one of the pillar fuels inflammation, and subsequently affecting the rest of the pillars. This shows that inflammation is playing a pivotal role in driving the development of aging and age-related diseases (Franceschi et al., 2018). The inflammation that drives the progression of aging is termed as inflammaging.

There remains a need for effective treatments for conditions associated with inflammaging and/or skin aging, such as wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone.

Summary

In one aspect there is provided a peptide comprising an amino acid sequence having the formula (I):

Xaa1-Xaa2-Xaa3 (I)

wherein:

Xaa1 is an amino acid residue selected from the group consisting of Trp, Phe, Tyr, Arg, His, Met, Lys and Asn;

Xaa2 is an amino acid residue selected from the group consisting of Trp, Phe, Tyr, Arg, Asp, Gln, Thr, Pro and Val; and

Xaa3 is an amino acid residue selected from the group consisting of Trp, Phe, Tyr, Arg, Leu, Val, Ile, His, Ala, Gln, Lys, Asp and Thr; and

wherein either:

(i) the peptide is a tripeptide;

Xaa1 is an N-terminal residue which is optionally derivatised; and

Xaa3 is a C-terminal residue which is optionally derivatised;

or (ii) Xaa1 and Xaa3 are cyclised to form a cyclic peptide;

or a salt thereof.

In some embodiments, Xaa1 is a Trp, Tyr, Arg or Phe residue, which residue is optionally derivatised. In some embodiment, Xaa1 is derivatised by being acylated, alkylated, biotinylated, PEGylated or phosphorylated. In some embodiments, Xaa1 is acylated at the N-terminus, optionally wherein Xaa1 is acylated at the N-terminus with an acyl group selected from acetyl, palmitoyl and myristoyl.

In some embodiments, Xaa2 is a Trp residue.

In some embodiments, Xaa3 is a Trp, Phe, Tyr, Arg, Leu or Val residue, which residue is optionally derivatised. In some embodiments, Xaa3 is derivatised by being esterified, amidated, alkylated, biotinylated, PEGylated or phosphorylated. In some other embodiments, Xaa3 is not derivatised.

In some embodiments, the peptide is selected from the group consisting of:

Trp-Trp-Trp;

Trp-Trp-Phe;

Trp-Trp-Tyr;

Trp-Trp-Arg;

Tyr-Trp-Trp;

Arg-Trp-Tyr;

Phe-Trp-Tyr;

Phe-Trp-Phe;

Trp-Trp-Leu; and

Trp-Trp-Val;

wherein the N-terminal residue Xaa1 is optionally acylated;

or a salt thereof. In some embodiments, the N-terminal residue is palmitoylated.

In some embodiments, the peptide is

N-palmitoyl-Trp-Trp-Phe; or

N-palmitoyl-Trp-Trp-Trp.

In another aspect, there is provided a pharmaceutical or cosmetic composition comprising i) a peptide as defined herein, or a salt thereof; and ii) a pharmaceutically and/or cosmetically acceptable excipient.

In some embodiments, the composition is formulated for topical administration.

In some embodiments, the composition comprises a further compound which inhibits bone morphogenetic protein (BMP) activity, optionally wherein the further compound is selected from the group consisting of a peptide which exhibits noggin-like activity, a peptide which inhibits a BMP, a Wnt promoter, and an anti-inflammatory agent (e.g. niacinamide, a curcuminoid derivative such as tetrahydroferuloylmethane, baicalin, nordihydroguiaretic acid, resveratrol, epigallocatechin gallate, and/or ascorbic acid or a derivative thereof).

In some embodiments, the composition comprises one or more of the following: a further anti-aging agent, a further anti-inflammatory agent, a sunscreen, an antioxidant, and a moisturizing agent.

In some embodiments, the composition comprises one or more of the following: an extracellular matrix enhancer, an autophagy enhancer, a stem cell enhancer, a skin exfoliator, a senolytic, a senomorphic, a DNA repair enhancer, an agent to reduce telomere attrition or promote telomere elongation, and an agent which promotes cellular adaptation to stress.

In some embodiments, the peptide of formula (I) or salt thereof is present in the composition in an amount of from 0.00001 to 1 wt. %, optionally from 0.0001 to 0.1 wt.%.

In some embodiments, the composition is in the form of a cream, lotion, paste, wax, liquid, semisolid composition or a sprayable composition.

In another aspect there is provided a method of inhibiting bone morphogenetic protein 2 (BMP-2) in a subject, comprising administering an effective amount of a

peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, to the subject. There is also provided use of a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for the manufacture of a medicament, for inhibiting bone morphogenetic protein 2 (BMP-2). There is also provided a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for use in inhibiting BMP-2. There is also provided use of a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for inhibiting BMP-2.

In another aspect, there is provided a method of treating or preventing a disease or disorder associated with bone morphogenetic protein 2 (BMP-2) expression in a subject, comprising administering an effective amount of a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, to the subject. There is also provided use of a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for the manufacture of a medicament, for treating or preventing a disease or disorder associated with BMP-2 expression. There is also provided a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for use in treating or preventing a disease or disorder associated with BMP-2 expression.

In another aspect, there is provided a method of treating and/or preventing one or more of skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone in a subject, comprising administering an effective amount of a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, to a subject in need thereof, optionally wherein the method is for treating and/or preventing one or more of cosmetic skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone in a subject. There is also provided use of a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for the manufacture of a medicament for therapeutic treatment and/or prevention of one or more of skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone. There is also provided a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for use in the therapeutic

treatment and/or prevention of one or more of skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone. There is also provided use of a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for treatment and/or prevention of one or more of cosmetic skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone.

In some embodiments, the composition is applied topically to the skin of the subject.

Brief Description of the Drawings

Figure 1 is a schematic diagram of a 3D inflammatory skin model.

Figure 2 shows the positive correlation of BMP-2 protein secretion versus a skin donor's age. Correlation of data is performed using Pearson's analysis.

Detailed Description

Definitions

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art.

The present disclosure may refer to the contents of certain documents being incorporated herein by reference. In the event of any inconsistent teaching between the teaching of the present disclosure and the contents of those documents, the teaching of the present disclosure takes precedence.

It is to be understood that if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art.

As used herein, the word "comprise" and other forms of the word, such as "comprising" and "comprises," means including but not limited to, and is not intended to exclude, for example, other additives, components, integers, or steps.

Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

As used herein, the term “and/or”, e.g., “X and/or Y” shall be understood to mean either "X and Y" or "X or Y" and shall be taken to provide explicit support for both meanings or for either meaning.

As used herein, the term about, unless stated to the contrary, refers to +/- 10%, of the designated value.

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or groups of compositions of matter. Thus, as used herein, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. For example, reference to "a" includes a single as well as two or more; reference to "an" includes a single as well as two or more; reference to "the" includes a single as well as two or more and so forth.

Unless otherwise indicated, terms such as "first," "second," etc. are used herein merely as labels, and are not intended to impose ordinal, positional, or hierarchical requirements on the items to which these terms refer. Moreover, reference to a “second” item does not require or preclude the existence of lower-numbered item (e.g., a “first” item) and/or a higher-numbered item (e.g., a “third” item).

As used herein, the phrase “at least one of”, when used with a list of items, means different combinations of one or more of the listed items may be used and only one of the items in the list may be needed. The item may be a particular object, thing, or category. In other words, “at least one of” means any combination of items or number of items may be used from the list, but not all of the items in the list may be required. For example, “at least one of item A, item B, and item C” may mean item A; item A and item B; item B; item A, item B, and item C; or item B and item C. In some cases, “at least one of item A, item B, and item C” may mean, for example and without limitation, two of item A, one of item B, and ten of item C; four of item B and seven of item C; or some other suitable combination.

Each embodiment of the present disclosure described herein is to be applied *mutatis mutandis* to each and every other embodiment unless specifically stated otherwise or required otherwise by context.

Peptides

The peptides of the present disclosure are useful in reducing the visible signs of aging, such as reducing wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone. Without wishing to be bound by theory, it is believed that the signs of aging are reduced by inhibition of bone morphogenetic protein 2 (BMP-2). The peptides described herein are tripeptides, and comprise combinations of three specific amino acids as described below.

In one aspect, there is provided a peptide comprising an amino acid sequence having the formula (I):

Xaa1-Xaa2-Xaa3 (I)

wherein:

Xaa1 is an amino acid residue selected from the group consisting of Trp, Phe, Tyr, Arg, His, Met, Lys and Asn;

Xaa2 is an amino acid residue selected from the group consisting of Trp, Phe, Tyr, Arg, Asp, Gln, Thr, Pro and Val; and

Xaa3 is an amino acid residue selected from the group consisting of Trp, Phe, Tyr, Arg, Leu, Val, Ile, His, Ala, Gln, Lys, Asp and Thr; and

wherein either:

(i) the peptide is a tripeptide;

Xaa1 is an N-terminal residue which is optionally derivatised; and

Xaa3 is a C-terminal residue which is optionally derivatised;

or (ii) Xaa1 and Xaa3 are cyclised to form a cyclic tripeptide;

or a salt thereof.

The amino acid sequences as represented here have the N-terminal residue on the left and the C-terminal residue on the right. Unless expressly indicated otherwise (e.g. D-Trp), the amino acid residues in the sequence disclosed herein are L-amino acid residues.

Throughout this specification, three-letter abbreviations for amino acids are used, as well as one-letter abbreviations, as will be known in the art. For example, tryptophan is Trp or W, phenylalanine is Phe or F, etc.

The N-terminus of a peptide of the disclosure may be an unfunctionalized amine, in salt form, or derivatized as described herein. The C-terminus of a peptide of the disclosure may be an unfunctionalised acid group, i.e. CO_2H, or may be in salt form or

derivatised as described herein. For amino acid side chain groups which are charged at physiological pH, those may be present in neutral form (e.g. as the free acid or amine), or in the form of a salt.

Salts

The peptide of the present disclosure may be provided in any suitable form, including charged, neutral and zwitterionic. In some embodiments, the peptide of the present disclosure is provided in salt form. Suitable salts include those formed with organic or inorganic acids or bases. Exemplary acid addition salts include, but are not limited to, sulfate, citrate, acetate, oxalate, fluoride, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Exemplary base addition salts include, but are not limited to, ammonium salts, alkali metal salts, for example those of potassium and sodium, alkaline earth metal salts, for example those of calcium and magnesium, and salts with organic bases, for example dicyclohexylamine, N-methyl-D-glucamine (meglumine), morpholine, thiomorpholine, piperidine, pyrrolidine, a mono-, di- or tri-lower alkylamine, for example ethyl-, *tert*-butyl-, diethyl-, diisopropyl-, triethyl-, tributyl- or dimethyl-propylamine, or a mono-, di- or trihydroxy lower alkylamine, for example mono-, di- or triethanolamine.

Xaa1

The peptide and/or salt thereof comprises an amino acid sequence having the formula (I), wherein Xaa1 is an N-terminal amino acid residue which is optionally derivatized, and Xaa1 is selected from the group consisting of Trp, Phe, Tyr, Arg, His, Met, Lys and Asn.

In some embodiments, Xaa1 is selected from Trp, Tyr, Arg and Phe, which residue is optionally derivatised. In some embodiments Xaa1 is Trp, which is optionally derivatized at the N-terminal. In some embodiments Xaa1 is Tyr, which is optionally derivatized at the N-terminal. In some embodiments Xaa1 is Arg, which is optionally derivatized at the N-terminal. In some embodiments Xaa1 is Phe, which is optionally derivatized at the N-terminal.

In some embodiments, Xaa1 is an underivatised amino acid residue. In some other embodiments, Xaa1 is derivatized, e.g. via the N-terminal amine.

Xaa1 Derivatives

Any suitable derivative of Xaa1 may be used. Examples of suitable derivatives include acyl groups, alkyl groups, biotinylation, PEGylation and phosphorylation.

The derivatization is typically *via* the N-terminus of Xaa1 (i.e. the amino group). Alternatively, or in addition, Xaa1 may be derivatized at another suitable position such as the indole of tryptophan, the aromatic -OH of tyrosine, a nitrogen of the guanidino group of arginine, or the phenyl group of phenylalanine.

In some embodiments, Xaa1 is acylated at the N-terminus (i.e. *via* the N-terminal amine group of Xaa1).

An acyl derivative is a compound having a -C(=O)-Y group. Y may for example contain from 1 to 34 carbon atoms. Acylated derivatives may for example in some embodiments provide one or more of an improved solubility profile of the tripeptide, improved processability or stability, or longer circulating time in the area being treated. Examples of acyl groups include C_2–C_{35} alkanoyl, C_2–C_{35} alkenoyl, C_2–C_{35} alkynoyl, C_3–C_{15} cycloalkanoyl, C_3–C_{15} heterocycloalkanoyl, C_3–C_{15} aroyl, or C_3–C_{15} heteroaroyl, where C_n represents the number of carbon atoms, n, in the acyl group (including the carbonyl carbon). For C_2–C_{35} alkenoyl there may be one or more alkene groups present, which may be *cis*, *trans*, or a mixture of *cis* and *trans*. For C_2–C_{35} alkynoyl there may be one or more alkyne groups present. Specific examples of acylated derivatives include acetyl (C_2 alkanoyl), propionyl (C_3 alkanoyl), propenoyl (C_3 alkenoyl), propynoyl (C_3 alkynoyl), butyryl (C_4 alkanoyl), crotonoyl (C_4 alkenoyl), benzoyl (C_7 aroyl), capryloyl (C_8 alkanoyl), caproyl (C_{10} alkanoyl), lauroyl (C_{12} alkanoyl), myristoyl (C_{14} alkanoyl), myristoleoyl (C_{14} alkenoyl), palmitoyl (C_{16} alkanoyl), palmitoleoyl (C_{16} alkenoyl), stearoyl (C_{18} alkanoyl), oleoyl (C_{18} alkenoyl), elaidoyl (C_{18} alkenoyl), linoleoyl (C_{18} alkenoyl), linolenoyl (C_{18} alkenoyl), stearidonoyl (C_{18} alkenoyl), arachidoyl (C_{20} alkanoyl), gadoleoyl (C_{20} alkenoyl), eicosenoyl (C_{20} alkenoyl) and behenoyl (C_{22} alkanoyl), amongst others.

In some embodiments, the acyl group is selected from C_2–C_{35} alkanoyl, C_5–C_{30} alkanoyl, C_{10}–C_{25} alkanoyl, and C_{15}–C_{20} alkanoyl. In some embodiments, the acylated derivative is selected from C_2 alkanoyl, C_3 alkanoyl, C_4 alkanoyl, C_5 alkanoyl, C_6

alkanoyl, C_7 alkanoyl, C_8 alkanoyl, C_9 alkanoyl, C_{10} alkanoyl, C_{11} alkanoyl, C_{12} alkanoyl, C_{13} alkanoyl, C_{14} alkanoyl, C_{15} alkanoyl, C_{16} alkanoyl, C_{17} alkanoyl, C_{18} alkanoyl, C_{19} alkanoyl and C_{20} alkanoyl.

In some embodiments, Xaa1 is acylated at the N-terminus with an acyl group selected from acetyl, palmitoyl and myristoyl.

In some embodiments, Xaa1 is acylated at the N-terminus with a palmitoyl group.

An alkyl derivative is one having an -R group, wherein R is a straight chain or branched C_1–C_{35} alkyl group, where C_n represents the total number of carbon atoms, n, in the straight or branched alkyl chain. Examples of alkyl groups include C_2–C_{35} alkyl, C_5–C_{30} alkyl, C_{10}–C_{25} alkyl, and C_{15}–C_{20} alkyl, which may be straight or branched. In some embodiments, the alkylated derivative is selected from ethyl, C_3 alkyl, C_4 alkyl, C_5 alkyl, C_6 alkyl, C_7 alkyl, C_8 alkyl, C_9 alkyl, C_{10} alkyl, C_{11} alkyl, C_{12} alkyl, C_{13} alkyl, C_{14} alkyl, C_{15} alkyl, C_{16} alkyl, C_{17} alkyl, C_{18} alkyl, C_{19} alkyl and C_{20} alkyl, which may be straight chain or branched. Examples of suitable straight and branched C_1–C_{35} alkyl substituents include methyl, ethyl, n-propyl, 2-propyl, n-butyl, sec-butyl, t-butyl, hexyl, 2-ethylhexyl, 2-propylheptyl, decyl, hexadecyl, and the like.

A biotinylated derivative is one having a biotin group. Typically, the -COOH group of biotin is attached, either directly or indirectly (via a linking group) to the amino acid (e.g. to the N-terminal nitrogen).

A PEGylated derivative is one containing poly(ethylene glycol) group. PEGylated derivatives may for example provide one or more advantages such as increased solubility (e.g. water solubility), processability, stability, circulating time, or decreased immunogenicity.

In some embodiments, the PEG group has an average molecular weight in the range of from 300 to 100,000 Da, or from 300 to 10,000 Da, or from 300 to 2,500 Da. In some embodiments, the PEG group is selected from PEG 300, PEG 400, PEG 500, PEG 600, PEG 700, PEG 800, PEG 900, PEG 1000, PEG 1500, PEG 2000, PEG 2500, PEG 3000, PEG 3500, PEG 4000, PEG 4500, PEG 5000, PEG 10000, PEG 15000, PEG 20000, PEG 25000, PEG 30000, PEG 35000, PEG 40000, PEG 45000, PEG 50000, PEG 60000, PEG 70000, PEG 80000, PEG 90000 and PEG 100000, where the number indicates the average molecular weight of the PEG group. Average molecular weight

may for example refer to number average molecular weight or weight average molecular weight.

A phosphorylated derivative is one containing a phosphate group. Typically, the phosphate group is attached to the amino acid *via* a heteroatom of the amino acid. Where the phosphate group is attached to the amino acid via an -OH group of the amino acid, the resulting derivative may be referred to as a phosphoester. Where the phosphate group is attached to the amino acid via an -NH group of the amino acid, the resulting derivative may be referred to as a phosphoramidate. This may be achieved in a number of ways, as discussed below. Phosphorylated derivatives of the invention may in some embodiments provide one or more advantages, such as improved solubility, processability, stability, or circulating time, or improved selectivity of the target protein.

In some embodiments, Xaa1 is an amino acid residue selected from Trp, Tyr, Arg and Phe, which is N-acylated. In some embodiments, Xaa1 is N-acyl-Trp. In some embodiments, Xaa1 is N-palmitoyl-Trp.

Xaa2

The peptide and/or salt form thereof comprises an amino acid sequence having the formula (I), wherein Xaa2 is selected from the group consisting of Trp, Phe, Try, Arg, Asp, Gln, Thr, Pro and Val.

In some embodiments, Xaa2 is a Trp residue.

Xaa3

The peptide and/or salt form thereof comprises an amino acid sequence having the formula (I), wherein Xaa3 is a C-terminal residue which is optionally derivatised and Xaa3 is selected from the group consisting of Trp, Phe, Tyr, Arg, Leu, Val, Ile, His, Ala, Gln, Lys, Asp and Thr.

In some embodiments, Xaa3 is a Trp, Phe, Tyr, Arg, Leu or Val residue, which residue is optionally derivatised.

In some embodiments Xaa3 is Trp, which is optionally derivatized at the C-terminal. In some embodiments Xaa3 is Phe, which is optionally derivatized at the C-terminal. In some embodiments Xaa3 is Tyr, which is optionally derivatized at the C-terminal. In some embodiments Xaa3 is Arg, which is optionally derivatized at the C-terminal. In some embodiments Xaa3 is Leu, which is optionally derivatized at the C-

terminal. In some embodiments Xaa3 is Val, which is optionally derivatized at the C-terminal.

In some embodiments, Xaa3 is an underivatised amino acid residue (i.e. it is not derivatized). This may be designated by Xaa3 or Xaa3-OH, where the OH represents the C-terminal carboxylic acid group of the amino acid. In some other embodiments, Xaa3 is derivatized, e.g. via the C-terminal carboxylic acid group.

Xaa3 Derivatives

Any suitable derivative of Xaa3 may be used. Example of suitable derivatives include esters, amides, alkyl groups, biotinylation, PEGylation, and phosphorylation.

The derivatization is typically *via* the C-terminus of Xaa3 (i.e. the -COOH group). Alternatively or in addition Xaa3 may be derivatized at another suitable position such as the indole of tryptophan, the aromatic -OH of tyrosine, a nitrogen of the guanidino group of arginine, or the phenyl group of phenylalanine.

In some embodiments, the derivative is attached to the C-terminus of Xaa3, that is, the -COOH group of that amino acid.

An ester derivative is one containing a -C(=O)-OR group, e.g. attached to Xaa3 via the carbonyl group, in which R may for example be an alkyl group as described herein.

An amide derivative is one containing a -C(=O)-NHR group, e.g. attached to Xaa3 via the carbonyl group, in which R may for example be an alkyl group as described herein.

Biotinylated derivatives, PEGylated derivatives and phosphorylated derivatives are as discussed above.

In some embodiments, Xaa1 is derivatised and Xaa3 is not derivatised. In some embodiments Xaa1 is not derivatised and Xaa3 is derivatised. In some embodiments, Xaa1 and Xaa3 are not derivatised. In some embodiments, Xaa1 and Xaa3 are both derivatised.

Peptides

In some embodiments, the peptides and/or salt forms thereof are selected from the group consisting of: Tyr-Trp-Trp; Tyr-Trp-Phe; Tyr-Trp-Tyr; Tyr-Trp-Arg; Tyr-Trp-Leu; Tyr-Trp-Val; Arg-Trp-Trp; Arg-Trp-Phe; Arg-Trp-Tyr; Arg-Trp-Arg; Arg-

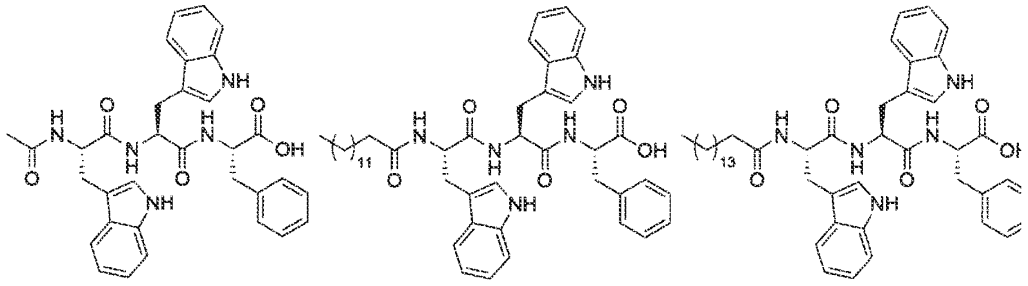
Trp-Leu; Arg-Trp-Val; Phe-Trp-Trp; Phe-Trp-Phe; Phe-Trp-Tyr; Phe-Trp-Arg; Phe-Trp-Leu; and Phe-Trp-Val, wherein the first amino acid (Xaa1) is an N-terminal residue which is optionally derivatised, and/or the third amino acid (Xaa3) is a C-terminal residue which is optionally derivatised. In some embodiments, the N-terminal residue is acylated, preferably acetylated, palmitoylated or myristoylated.

In some embodiments, the peptide and/or salt form thereof is selected from the group consisting of: Trp-Trp-Trp; Trp-Trp-Phe; Trp-Trp-Tyr; Trp-Trp-Arg; Tyr-Trp-Trp; Arg-Trp-Tyr; Phe-Trp-Tyr; Phe-Trp-Phe; Trp-Trp-Leu; and Trp-Trp-Val, wherein the first amino acid (Xaa1) is an N-terminal residue which is optionally derivatised, and/or the third amino acid (Xaa3) is a C-terminal residue which is optionally derivatised. In some embodiments, the N-terminal residue is acylated, preferably acetylated, palmitoylated or myristoylated.

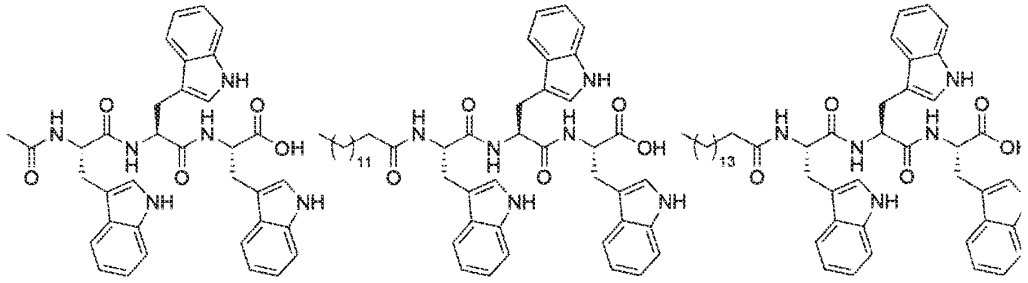
In some embodiments, the peptide and/or salt form thereof is Trp-Trp-Phe, wherein the first amino acid (Trp) is an N-terminal residue which is optionally derivatised, and/or the third amino acid (Phe) is a C-terminal residue which is optionally derivatised. In some embodiments, the N-terminal Trp residue is acylated, preferably acetylated, palmitoylated or myristoylated. In some embodiments, the peptide is N-acetyl-Trp-Trp-Phe, N-palmitoyl-Trp-Trp-Phe or N-myristoyl-Trp-Trp-Phe, preferably N-palmitoyl-Trp-Trp-Phe.

In some embodiments, the peptide and/or salt form thereof is Trp-Trp-Trp, wherein the first amino acid (Trp) is an N-terminal residue which is optionally derivatised, and/or the third amino acid (Trp) is a C-terminal residue which is optionally derivatised. In some embodiments, the N-terminal Trp residue is acylated, for example acetylated, palmitoylated or myristoylated. In some embodiments, the peptide is N-acetyl-Trp-Trp-Trp, N-palmitoyl-Trp-Trp-Trp or N-myristoyl-Trp-Trp-Trp, preferably N-palmitoyl-Trp-Trp-Trp.

The structures of N-acetyl-Trp-Trp-Phe, N-palmitoyl-Trp-Trp-Phe, N-myristoyl-Trp-Trp-Phe, N-palmitoyl-Trp-Trp-Phe, N-acetyl-Trp-Trp-Trp, N-palmitoyl-Trp-Trp-Trp, N-myristoyl-Trp-Trp-Trp, and N-palmitoyl-Trp-Trp-Trp are shown below:



N-acetyl-Trp-Trp-Phe N-myristoyl-Trp-Trp-Phe N-palmitoyl-Trp-Trp-Phe



N-acetyl-Trp-Trp-Trp N-myristoyl-Trp-Trp-Trp N-palmitoyl-Trp-Trp-Trp

In some embodiments, Xaa1 and Xaa3 are cyclised to form a cyclic tripeptide. Any suitable means may be used to form the cyclic peptide. Xaa1 and Xaa3 may for example be directly linked, e.g. *via* amide bond formation between the amino group of Xaa1 and the carboxyl group of Xaa3. Alternatively, if desired, Xaa1 and Xaa3 may be indirectly linked, e.g. *via* use of a linking group.

In some embodiments, the peptide is any of the example peptides described herein, or a salt thereof.

Preparation of Peptides

The peptides and/or salts thereof encompassed by the present disclosure may be prepared by any suitable means. For example, they may be chemically synthesized, as will be known to persons skilled in the arts of synthetic organic chemistry and/or medicinal chemistry.

Amino acid building blocks, protecting groups, derivatizing groups and other chemicals used to synthesise the peptides may be purchased from commercial sources such as Sigma Aldrich/Merck. Synthetic methods, such as those described in *March's Advanced Organic Chemistry*, 8th Edition (Michael B. Smith), *Comprehensive Organic*

Synthesis, 2nd Edition (Paul Knochel and Gary A Molander) or *Bioconjugate Techniques*, 3rd Edition (Greg T. Hermanson), may be used for example. Peptide synthesizers are also commercially available, such as from Biotage (https://www.biotage.com/peptide-synthesis), and may be utilised.

For example, protecting groups may be used. In the case of amine groups, carbamate protecting groups such as Boc groups or Z (also known as Cbz) groups may be used as protecting groups. In the case of acid groups, ester protecting groups such as methyl ester, ethyl ester, benzyl ester and t-butyl ester groups may be used. Such groups can readily be introduced under mild conditions, or suitably protected amino acids may be available commercially. Such groups can be removed selectively under mild conditions, and by selection of a suitable protecting group strategy, the peptides can be assembled, and desired functional groups unveiled at the appropriate stage of the synthesis.

Peptide bonds may be formed between amino acids using amidation chemistry known in the art, for example using peptide coupling agents such as EDCI, DCC, BOP, PyBOP, HATU, TBTU, FDPP and the like, optionally in the presence of additives such as HOBt, and typically in the presence of a suitable base, e.g. an amine base such as NMM.

Derivatives such as acyl derivatives can be prepared by chemistry known in the art, for example using amidation chemistry to introduce an N-acyl group. In some embodiments, N-acylated amino acids may be available commercially.

Biotinylated derivatives can be prepared in a number of ways, such as by reaction of a selective, activated biotin reagent with a functional group on the amino acid, such as a free -NH_2, -SH, -COOH or -CHO, or via non-selective biotinylation reagents. Further details can be found for example in *Bioconjugate Techniques*, 3rd Edition (2013), Academic Press (Greg T. Hermanson).

PEGylated derivatives can be prepared in a number of ways, such as by reaction of a selective, activated PEG reagent with a functional group on the amino acid, such as a free -NH_2, -SH, -COOH or -CHO, or via non-selective PEG reagents. Further details can be found for example in "Protein PEGylation: An overview of chemistry and process considerations", *European Pharmaceutical Review* **2010**, *15* (1), 18–26 (Fee *et al.*).

Phosphorylated derivatives can be prepared in a number of ways, such as detailed in *Protein Phosphorylation: Mechanisms, Types, and Research Techniques* (https://www.creative-proteomics.com/).

Compositions

While it is possible for the tripeptide or cyclic tripeptide compound of the present disclosure to be administered alone, it is preferable for it to be present in a pharmaceutical or cosmetic composition. The composition may be a pharmaceutical composition, or it may be a cosmetic composition. i.e. useful for non-therapeutic applications, such as cosmetic improvement and/or enhancement of the appearance of skin.

Accordingly, in one aspect, the invention provides a pharmaceutical or cosmetic composition comprising i) a peptide of the present disclosure as described above, or a salt thereof, and ii) a pharmaceutically and/or cosmetically acceptable excipient. According to certain preferred embodiments the pharmaceutical or cosmetic composition is formulated for topical administration. Advantageously, the compositions are useful in improving and/or enhancing the appearance of skin, such as reducing the visible signs of aging, including wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone. It is believed that inhibition of bone morphogenetic protein-2 (BMP-2), promoting maintenance of epidermal stemness, reduced epidermal senescence and/or increased protein expression in one or more of collagen IV, collagen VII and pro-collagen 1A1, is responsible for the improved appearance of skin.

In some embodiments, the pharmaceutical or cosmetic composition of the present disclosure comprises the peptide of formula (I) or a salt thereof and/or a derivative thereof, in an amount of from 0.00001 wt.% to 1 wt.%, preferably from 0.0001 wt.% to 0.1 wt.%. In some embodiments, the peptide of formula (I) or salt thereof is present in the composition in an amount in the range of from 0.0005 wt.% to 0.0010 wt.%, or from 0.0009 wt.% to 0.0014 wt.%, or from 0.0012 wt.% to 0.0050 wt.%, or from 0.0040 wt.% to 0.0060 wt.%, or from 0.0060 wt.% to 0.0110 wt.%, or from 0.010 wt.% to 0.015 wt.%, or from 0.014 wt.% to 0.022 wt.%, or from 0.020 wt.% to 0.045 wt.%, or from 0.040 wt.% to 0.080 wt.%, or from 0.070 wt.% to 0.110 wt.%, or from 0.11 wt.% to 0.22 wt.%, or from 0.20 wt.% to 0.45 wt.%, or from 0.40 wt.% to 0.80 wt.%, or from 0.70 wt.% to 1.00 wt.%. In one embodiment, the peptide of formula (I)

or salt thereof is present in the composition in an amount of about 0.001 wt.%. In another embodiment, the peptide of formula (I) or salt thereof is present in the composition in an amount of about 0.005 wt.%. In another embodiment, the peptide of formula (I) or salt thereof is present in the composition in an amount of about 0.010 wt.%. In some embodiments, the peptide of formula (I) is Trp-Trp-Phe or a derivative thereof, or Trp-Trp-Trp or a derivative thereof, for example wherein the derivative is a palmitoyl derivative, e.g. wherein the N-terminal residue is palmitoylated.

In some embodiments, the pharmaceutical or cosmetic composition of the present disclosure comprises a further compound which inhibits bone morphogenetic protein (BMP) activity. Bone morphogenetic proteins have the ability to induce the formation of bone and cartilage, but may also have other roles in the appearance of skin. Examples of compounds which inhibit bone morphogenetic protein (BMP) activity include noggin and peptides which exhibit noggin-like activity, peptides which inhibit a BMP, Wnt promoters, and anti-inflammatory agents (e.g. niacinamide, curcuminoid derivatives such as tetrahydroferuloylmethane, baicalin, nordihydroguiaretic acid, resveratrol, epigallocatechin gallate, and/or ascorbic acid or derivatives thereof). Examples of noggin and peptides which exhibit noggin-like activity are described in WO 2021/186243 A1. An example of a peptide which inhibits BMP is BMPR1A-ECD, as described in *BMB Reports* **2013**, *46*(9), 465–470 (Yoon *et al.*). Examples of anti-inflammatory agents are described in EP 2383283 A2 and JP 2021-167305 A.

In some embodiments, the pharmaceutical or cosmetic composition of the present disclosure comprises one or more of the following: a further anti-aging agent, a further anti-inflammatory agent, a sunscreen, an antioxidant, and a moisturizing agent. It is possible that some of these agents may have multiple effects, such as being both an anti-aging agent and an antioxidant.

Further anti-aging agents include those described in the art, and can include as an active ingredient a compound selected from the group consisting of: alpha hydroxy acids, biotin, caffeine, ceramides, coenzyme Q10, collagen, epidermal growth factor, ferulic acid, glycolic acid, green tea extract, hyaluronic acid, hydroquinone, jojoba oil, kojic acid, niacinamide, retinoids, retinol, vitamin C and its derivatives and vitamin E and its derivatives.

Further anti-inflammatory agents include those that are described in the art, and can include as an active ingredient a compound selected from the group consisting of:

aloe vera extract, azelaic acid, bisabolol, calendula, centella asiatica extract, ceramides, colloidal oatmeal, cucumber extract, curcumin, German chamomile extract, green tea extract, licorice extract, niacinamide, sea buckthorn oil, vitamin C and its derivatives, vitamin E and its derivatives, witch-hazel extract and zinc.

Sunscreens are known in the art, and can include as an active ingredient a compound selected from the group consisting of: zinc oxide, titanium oxide, oxybenzone (benzophenone-3), 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid (benzophenone-4), p- dioxybenzone (benzophenone-8), sodium dihydroxy dimethoxy disulfobenzophenone (benzophenone-9), aminobenzoic acid, avobenzone, bemotrizinol, bisoctrizole, diethylamino hydroxybenzoyl hexyl benzoate, diethylhexyl butamido triazone (iscotrizinol), dimethico-diethylbenzalmalonate, drometrizole trisiloxane, ecamsule, 2-ethoxyethyl p-methoxycinnamate (cinoxate), 2-ethylhexyl 4-dimethylaminobenzoate (padimate O), ethylhexyl methoxycinnamate, 2-ethylhexyl salicylate (octyl salicylate), ethylhexyl triazone, homomethyl salicylate (homosalate), isopentyl-4-methoxycinnamate (amiloxate), menthyl anthranilate (meradimate), 4-methylbenzylidene camphor (enzacamene), octinoxate, octisalate, octocrylene, phenylbenzimidazole sulfonic acid, phenylene bis-diphenyltriazine, tris-biphenyl triazine, and trolamine salicylate.

Antioxidants are known in the art, and can include as an active ingredient a compound selected from the group consisting of: albizia flower extract, allantoin, ashwagandha extract, BHT, bisabolol, carrot oil, beta-carotene, chaparral extract, citric acid, coenzyme Q10, cucumber fruit extract, edelweiss extract, ferulic acid, gingko biloba leaf extract, goji berry extract, goldenseal extract, green tea extract, Kakadu plum extract, mallow extract, mulberry root extract, resveratrol, sea buckthorn extract, soapwort extract, vitamin A and its derivatives, vitamin C and its derivatives, vitamin E and its derivatives, watermelon extract, and white tea extract.

Moisturizing agents are known in the art, and can include as an active ingredient a compound selected from the group consisting of: alpha-hydroxy acids, argan oil, ceramides, cholesterol, cocoa butter, dimethicone, evening primrose oil, fatty acids, glycerin, glyceryl stearate, grape seed oil, hyaluronic acid, macadamia oil, non-comedogenic fatty acids (e.g. stearic acid), petrolatum, shea butter, squalene and sunflower oil.

In some embodiments, the pharmaceutical or cosmetic composition of the present disclosure comprises one or more of the following: an extracellular matrix enhancer, an autophagy enhancer, a stem cell enhancer, a skin exfoliator, a senolytic, a senomorphic, a DNA repair enhancer, an agent to reduce telomere attrition or promote telomere elongation, and an agent which promotes cellular adaptation to stress.

An extracellular matrix (ECM) enhancer is a compound that assists in maintenance of the ECM in skin, which provides mechanical support to tissues, and are described in *Frontiers in Cell and Developmental Biology* **2021**, *9*, 682414 (Pfisterer *et al.*). An autophagy enhancer is a compound that modulates autophagy, which has been linked to a number of skin disorders, and are described in *Frontiers of Pharmacology* **2022**, *13*, 844756 (Klapan *et al.*). A stem cell enhancer is a compound that promotes the production of stem cells in the skin to promote production of healthy skin. An exfoliator is a composition which assists in cleaning and removing dead skin cells. A senolytic is a compound that assists in removal of senescent cells, which are thought to accumulate in the skin and are no longer able to produce collagen and elastin. A senomorphic agent is a compound that can halt senescent cells or reverse their activity in skin. Senescence and compounds that target senescent cells are described in *Aging Cell* **2023**, *22*, e13845 (Danczak-Pazdrowska *et al.*). A DNA repair enhancer is a compound that assists with DNA repair, which may be of benefit with UV-induced DNA damage, as described in *Proc. Natl. Acad. Sci. USA* **1997**, 94(23), 12627-12632 (Eller *et al.*). An agent to reduce telomere attrition or promote telomere elongation is a compound that may assist in prevention of telomere shortening, as described in *Exp. Dermatol.* **2011**, 20(4), 297–302 (Buckingham *et al.*). An agent which promotes cellular adaptation to stress is a compound which may ease stress on, for example skin immune cells, which may assist in regulation of tissue inflammation, and are described in *Clinical Therapeutics* **2020**, *42*, 757-770 (Pondeljak *et al.*). The above components are commercially available, such as from https://www.ulprospector.com/en/na/PersonalCare and https://cosmetics.specialchem.com/.

In some embodiments, the pharmaceutical or cosmetic composition of the present disclosure comprises one or more of the following: a peptide of formula (I) or a salt thereof, as described herein; water; a C_{12-15} alkyl benzoate; PPG-3 myristyl

ether; hydroxyethyl acrylate; sodium acrylolydimethyl taurate copolymer; squalane; polysorbate 60; ethylhexylglycerin; and phenoxyethanol.

A C_{12-15} alkyl benzoate may for example be used as a solvent in the preparation of skin compositions, and may for example be present in the composition in an amount of from 0.001 wt.% to 20 wt.%, for example from 1 wt.% to 10 wt.%. In some embodiments, the C_{12-15} alkyl benzoate is present in the composition in an amount in the range of from 0.001 wt.% to 1.0 wt.%, or from 1.0 wt.% to 3.0 wt.%, or from 2.0 wt.% to 6.0 wt.%, or from 3.0 wt.% to 7.0 wt.%, or from 4.0 wt.% to 10.0 wt.%, or from 5.0 wt.% to 15.0 wt.%, or from 6.0 wt.% to 20 wt.%. In some embodiments, the C_{12-15} alkyl benzoate is present in the composition in an amount of about 5.0 wt.%. In these embodiments, the peptide of formula (I) may for example be Trp-Trp-Phe or a derivative thereof, or Trp-Trp-Trp or a derivative thereof, for example wherein the derivative is a palmitoyl derivative, for example wherein the N-terminal residue is palmitoylated.

PPG-3 myristyl ether (polypropylene glycol ether of myristyl alcohol may be present in the composition in an amount of from 0.001 wt.% to 20 wt.%, preferably from 1 wt.% to 10 wt.%. In some embodiments, the PPG-3 myristyl ether is present in the composition in an amount in the range of from 0.001 wt.% to 1.10 wt.%, or from 1.0 wt.% to 3.0 wt.%, or from 2.0 wt.% to 6.0 wt.%, or from 3.0 wt.% to 7.0 wt.%, or from 4.0 wt.% to 10.0 wt.%, or from 5.0 wt.% to 15.0 wt.%, or from 6.0 wt.% to 20 wt.%. In some embodiments, the PPG-3 myristyl ether is present in the composition in an amount of about 5.0 wt.%. In these embodiments, the peptide of formula (I) may for example be Trp-Trp-Phe or a derivative thereof, or Trp-Trp-Trp or a derivative thereof, for example wherein the derivative is a palmitoyl derivative, for example wherein the N-terminal residue is palmitoylated.

Hydroxyethyl acrylate is a stabilizer for oil emulsions in water-based formulations, and may be present in the composition in an amount of from 0.001 wt.% to 10 wt.%, preferably from 1 wt.% to 8 wt.%. In some embodiments, the hydroxyethyl acrylate is present in the composition in an amount in the range of from 0.001 wt.% to 1.10 wt.%, or from 0.01 wt.% to 1.2 wt.%, or from 0.02 wt.% to 1.5 wt.%, or from 0.03 wt.% to 2.0 wt.%, or from 0.04 wt.% to 2.5 wt.%, or from 0.05 wt.% to 3.0 wt.%, or from 1.0 wt.% to 8.0 wt.%. In these embodiments, the peptide of formula (I) may for example be Trp-Trp-Phe or a derivative thereof, or Trp-Trp-Trp or a derivative thereof,

for example wherein the derivative is a palmitoyl derivative, for example wherein the N-terminal residue is palmitoylated.

Sodium acrylolydimethyl taurate copolymer is used in skin compositions to improve texture and viscosity, and may be present in the composition in an amount of from 0.001 wt.% to 10 wt.%, preferably from 1 wt.% to 8 wt.%. In some embodiments, the sodium acrylolydimethyl taurate copolymer is present in the composition in an amount in the range of from 0.001 wt.% to 1.10 wt.%, or from 0.01 wt.% to 1.2 wt.%, or from 0.02 wt.% to 1.5 wt.%, or from 0.03 wt.% to 2.0 wt.%, or from 0.04 wt.% to 2.5 wt.%, or from 0.05 wt.% to 3.0 wt.%, or from 1.0 wt.% to 8.0 wt.%. In these embodiments, the peptide of formula (I) may for example be Trp-Trp-Phe or a derivative thereof, or Trp-Trp-Trp or a derivative thereof, for example wherein the derivative is a palmitoyl derivative, for example wherein the N-terminal residue is palmitoylated.

Squalane is used in skincare products for its hydrating and moisturizing benefits, and has emollient properties that can help smooth and soften skin, and may be present in the composition in an amount of from 0.001 wt.% to 10 wt.%, preferably from 1 wt.% to 8 wt.%. In some embodiments, the squalane is present in the composition in an amount in the range of from 0.001 wt.% to 1.10 wt.%, or from 0.01 wt.% to 1.2 wt.%, or from 0.02 wt.% to 1.5 wt.%, or from 0.03 wt.% to 2.0 wt.%, or from 0.04 wt.% to 2.5 wt.%, or from 0.05 wt.% to 3.0 wt.%, or from 1.0 wt.% to 8.0 wt.%. In these embodiments, the peptide of formula (I) may for example be Trp-Trp-Phe or a derivative thereof, or Trp-Trp-Trp or a derivative thereof, for example wherein the derivative is a palmitoyl derivative, for example wherein the N-terminal residue is palmitoylated.

Polysorbate 60 (polyoxyethylene (20) sorbitan monostearate) is an emulsifier which is used in topical compositions, and may be present in the composition in an amount of from 0.001 wt.% to 10 wt.%, preferably from 1 wt.% to 8 wt.%. In some embodiments, the polysorbate 60 is present in the composition in an amount in the range of from 0.001 wt.% to 1.10 wt.%, or from 0.01 wt.% to 1.2 wt.%, or from 0.02 wt.% to 1.5 wt.%, or from 0.03 wt.% to 2.0 wt.%, or from 0.04 wt.% to 2.5 wt.%, or from 0.05 wt.% to 3.0 wt.%, or from 1.0 wt.% to 8.0 wt.%. In these embodiments, the peptide of formula (I) may for example be Trp-Trp-Phe or a derivative thereof, or Trp-Trp-Trp or a derivative thereof, for example wherein the derivative is a palmitoyl derivative, for example wherein the N-terminal residue is palmitoylated.

Ethylhexylglycerin is a skin-softening agent, and can act as a preservative and carrier/suspending agent, with mild humectant properties. The ethylhexylglycerin may be present in the composition in an amount of from 0.001 wt.% to 2 wt.%, preferably from 0.01 wt.% to 1.0 wt.%. In some embodiments, the ethylhexylglycerin is present in the composition in an amount in the range of from 0.001 wt.% to 0.5 wt.%, or from 0.002 wt.% to 0.6 wt.%, or from 0.005 wt.% to 0.7 wt.%, or from 0.10 wt.% to 0.8 wt.%, or from 0.20 wt.% to 0.9 wt.%, or from 0.30 wt.% to 1.0 wt.%. In some embodiments, the ethylhexylglycerin is present in the composition in an amount of about 0.30 wt.%. In these embodiments, the peptide of formula (I) may for example be Trp-Trp-Phe or a derivative thereof, or Trp-Trp-Trp or a derivative thereof, for example wherein the derivative is a palmitoyl derivative, for example wherein the N-terminal residue is palmitoylated.

Phenoxyethanol is a preservative, and may be present in the composition in an amount of from 0.001 wt.% to 2 wt.%, preferably from 0.01 wt.% to 1.0 wt.%. In some embodiments, the phenoxyethanol is present in the composition in an amount in the range of from 0.001 wt.% to 0.5 wt.%, or from 0.002 wt.% to 0.6 wt.%, or from 0.005 wt.% to 0.7 wt.%, or from 0.10 wt.% to 0.8 wt.%, or from 0.20 wt.% to 0.9 wt.%, or from 0.30 wt.% to 1.0 wt.%, or from 0.40 wt.% to 1.5 wt.%. In some embodiments, the phenoxyethanol is present in the composition in an amount of about 0.40 wt.%. In these embodiments, the peptide of formula (I) may for example be Trp-Trp-Phe or a derivative thereof, or Trp-Trp-Trp or a derivative thereof, for example wherein the derivative is a palmitoyl derivative, for example wherein the N-terminal residue is palmitoylated.

A solvent, such as water, may be present in the composition.

In some embodiments, the composition comprises one or more of the following: a peptide of formula (I) or a salt thereof as defined herein; water; a C_{12-15} alkyl benzoate; PPG-3 myristyl ether; hydroxyethyl acrylate; sodium acrylolydimethyl taurate copolymer; squalene; polysorbate 60; ethylhexylglycerin; and phenoxyethanol.

In some embodiments, the composition comprises the following:
a peptide of formula (I) or a salt thereof as defined herein; water; a C_{12-15} alkyl benzoate; PPG-3 myristyl ether; hydroxyethyl acrylate; sodium acrylolydimethyl taurate copolymer; squalene; polysorbate 60; ethylhexylglycerin; and phenoxyethanol.

The compositions of the present disclosure additionally may also contain other additives. For example, the compositions of the present disclosure may include known additional cosmetic or pharmaceutical agents.

In some embodiments, the composition consists of or consists essentially of the above components and one or more carriers and/or excipients, and optionally one or more additional cosmetic or pharmaceutical agents which do not inhibit BMP activity, or which are not a further anti-aging agent, a further anti-inflammatory agent, a sunscreen, an antioxidant, a moisturizing agent, an extracellular matrix enhancer, an autophagy enhancer, a stem cell enhancer, a skin exfoliator, a senolytic, a senomorphic, a DNA repair enhancer, an agent to reduce telomere attrition or promote telomere elongation, or an agent which promotes cellular adaptation to stress.

The CTFA Cosmetic Ingredient Handbook, Second Edition, 1992 describes a wide variety of non-limiting cosmetic and pharmaceutical ingredients commonly used in the skin care industry, which may be suitable for use in the compositions of the present disclosure. Examples of suitable formulation ingredients include: antioxidants, binders, biological additives, buffering agents, thickeners, pearlescent agents, polymers, astringents, silicones, odor absorbers, gelling agents (e.g. hydrophilic or lipophilic gelling agents), fragrance, humectants, fillers, opacifying agents, skin-lightening agents, skin tanning agents, perfumes, conditioners, exfoliating agents, solvents/solubilisers (e.g. methylpropanediol), vitamins, colouring agents, pH adjusters (e.g. sodium hydroxide), preservatives (e.g. ethyhexylglycerin, phenoxyethanol, or hydroxyacetophenone), rheological modifiers, foaming agents, surfactants, emollients, natural extracts, essential oils, skin sensates, scalp soothing agents, and scalp healing agents.

The composition of the present disclosure may also comprise other skin conditioning agents, including, but not limited to, vitamins or vitamin derivatives and macro or micro capsules containing vitamins or their derivatives, plant extracts, polyphenol compounds, sunscreen additives, sunscreen agents, sensorial additives or texturisers, natural extracts and essential oils.

The compositions of the present disclosure may be formulated as appropriate for any desired method of administration. For example, the composition of the present disclosure may be formulated as a topical composition, suitable to be applied topically to the skin.

In some embodiments, the composition of the present disclosure may be in the form of a cream, lotion, paste, wax, liquid, semisolid composition or a sprayable composition.

In some embodiments, the composition comprises a pharmaceutically or cosmetically acceptable carrier or vehicle, and/or comprises one or more excipients.

The pharmaceutically or cosmetically acceptable vehicle may be any pharmaceutically or cosmetically acceptable vehicle, including pharmaceutically and/or dermatologically acceptable carriers, including surfactants. Pharmaceutically and/or dermatologically acceptable carriers are preferably compatible with skin, nails, mucous membranes, tissues and/or hair, and may include any conventionally used pharmaceutical or dermatological carriers meeting these requirements. The carriers and/or vehicles may aid in formulating the ascorbic acid or a derivative and/or a salt thereof, or anti-hyperpigmentation agent, into suitable pharmaceutical or cosmetic formulations. Such carriers may be readily selected by one of ordinary skill in the art. Carriers include, but are not limited to, emollients, emulsifiers, and rheological modifiers.

Emollients suitable for use in the vehicle or carrier for the compositions of the disclosure include, for example, C_{12-15} alkyl benzoates, PPG-3 myristyl ether, paraffinum liquidum and dimethicone. Emulsifiers suitable for use in the vehicle or carrier for the compositions of the disclosure include, for example, hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer, ceteareth-20 (cetostearyl alcohol, which is polyethylene glycol ether of cetearyl alcohol). Rheological modifiers suitable for use in the vehicle or carrier for the compositions of the disclosure include, for example, carbomer, glyceryl polyacrylate, and acrylates/C_{10-30} alkyl acrylate crosspolymer. Other suitable carriers include, but are not limited to, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene and/or polyoxypropylene compounds, emulsifying wax, sorbitan monostearate, polysorbate 60 (polyoxyethylene (20) sorbitan monostearate), cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol, sodium hyaluronate, hyaluronic acid, and water.

For topical administration, the compositions of the present disclosure may be provided in any suitable form, for example the compositions may be formulated as ointments, liquids, lotions, pastes, foams, sprays, serums, masks, gels, hydrogels or transdermal patches, leave-on and rinse-off lotions or toners, skin ampoules, skin

cleansers, facial and eye make-up removers, shower gels/creams, toilet bars, depilatories, all colour cosmetics including, but not limited to, lipsticks and foundations, sunless tanners or sunscreen lotions, mousses, gels, liposomes, or other topically suitable forms.

Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening, emulsifying and/or gelling agents (e.g. emulsion of oil in water, emulsion of water in oil or emulsion of water in silicone). Lotions may be formulated with an aqueous or oily base and generally also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents, thickening agents, colouring agents or preservatives (e.g., methyl paraben, propyl paraben, phenoxyethanol). Ointments, creams, lotions and the like may also be formulated to comprise C_{1-3}-alkoxylated oils and waxes (e.g., ethoxylated vegetable oils, ethoxylated jojoba oil/wax, ethoxylated lanolin oil, ethoxylated coconut oil, ethoxylated cocoa butter).

Any cosmetic product that contains water (i.e., creams, gels, lotions, etc) has the potential for bacteria, yeast, and mould to grow, and may in some embodiments require a preservative. Exemplary preservatives for the compositions of the present disclosure include ethylhexylglycerine, phenoxyethanol, chlorphensin and sodium metabisulfite. Exemplary emollients include C_{12-15} alkyl benzoate, PPG-3 myristyl ether, isopropyl lauroyl sarcosinate, glycerin and bis-PEG-18 methyl ether dimethyl silane. An exemplary humectant is 1,2-hexane diol or betaine. An exemplary rheological modifier includes hydroxyethyl acrylate, sodium acryloyldimethyl taurate copolymer, or mixtures thereof.

The topical use could for example be by way of incorporating the components in a leave-on or in a rinse off product that is applied to the human body primarily for improving skin appearance and general aesthetic benefit. "Skin" as used herein, is meant to include the external surface of mammals, especially humans and includes skin of the body, face and the scalp. The use of the compositions of the present disclosure may for example be by way of incorporation in a leave-on composition.

The compositions can be in the form of a liquid, lotion, cream, foam, scrub, gel, soap bar, toner, or applied with an implement or via a face mask, pad or patch in any form of materials. Non-limiting examples of such compositions include leave-on skin lotions and creams in the forms of oil/silicone in water emulsion, water in oil/silicone

emulsion and multiple emulsions, serums, shampoos, conditioners, shower gels, toilet bars, antiperspirants, deodorants, depilatories, lipsticks, foundations, mascara, sunscreen lotions, ointments, sprays, pastes, mousses, foams, gels, liposomes, or other topically suitable forms.

In some embodiments, the compositions of the present disclosure are in the form of a cream, lotion, paste, wax, liquid, semisolid composition or a sprayable composition.

In the case where the compositions are in the form of a spray, the carrier is suitably water, and the compositions may further comprise a preservative such as benzyl alcohol (for example, in an amount of from 0.1 – 5 wt%), co-solvents such as propylene glycol (for example, in an amount of from 1 – 20 wt%), and surfactants such as PEG-40 hydrogenated castor oil (for example, in an amount of from 1 – 20 wt%).

In the case where the compositions are in the form of a lotion, they may for example comprise water in the presence of a suitable emulsifying agent. Examples of suitable emulsifying agents are ceteareth-20, PEG-100 stearate, glyceryl stearate, sodium cetearyl sulfate. The compositions may further comprise emollients in the form of esters and/or hydrocarbons and/or silicones and/or plant oils and fragrances, preservatives (such as benzyl alcohol at levels of, for instance, 0.1 – 10%) and humectant and/or excipients (such as glycerol, for instance at levels of 0.5 – 10% and cyclomethicone at levels of between 0.1 – 20%).

When the compositions are in the form of an ointment, they may for example comprise a conventional ointment base to which the active ingredient is added. The ointment base may be a paraffin, such as soft paraffin, or a combination of soft and liquid paraffin. Other ointment bases may also be used such as polyalkylene glycol (such as polyethylene or polypropylene glycol) base. Other possible components of an ointment composition include emulsifying wax (for example, in an amount of between 1 – 40%, preferably 5 – 40%), and optionally one or more preservatives.

When the compositions are in the form of a liquid soap, any known liquid soap may be used. A preferred liquid soap with key composition saponified fatty acids derived from palm kernel oil or coconut oil. The pH of the liquid soap base may be adjusted to that which is suitable for topical use.

When the compositions are an anhydrous cream, any suitable cream base may be used. A preferred cream base is petrolatum cream and/or natural plant oils and waxes with introduction of consistency factors.

Advantageously, the components of the present disclosure can be prepared in a stabilised topical composition and applied on the skin.

Accordingly, the components of the present disclosure may for example be provided in an encapsulated format within the compositions. Advantageously, the encapsulation technology may be used to protect and enhance transdermal delivery. Suitable encapsulation technologies may be selected from the group consisting of liposomes, niosomes, nano-emulsions, and cyclodextrin inclusion technologies. Suitable encapsulation technologies may be produced by any means known in the art and are not limited to the present disclosure.

An encapsulation material may for example comprise an emulsifier and a co-emulsifier. The emulsifier may be selected from glyceryl esters of different fatty chains, including, but not limited to, glyceryl citrate, glyceryl lactate, glyceryl myristate, glyceryl laurate, glyceryl linoleate, glyceryl oleate or any combination thereof or any mixed glyceryl esters of fatty acids including citrate, lactate, myristate, laurate, linoleate and/or oleate.

Where used, the stabiliser can for example be selected from organic substances of any kind that may solubilise the emulsifiers. Suitable examples include alcohols or glycols, including, but not limited to, ethanol, isopropanol, propylene glycol, pentylene glycol, caprylyl glycol, especially pentylene glycol.

Preparation of Compositions

The compositions of the present disclosure may be prepared by any suitable method known to a person skilled in the art.

In some embodiments, the compositions are prepared as compositions suitable for topical use.

In some embodiments, the compositions are prepared as emulsions. For example, the compositions of the present disclosure may be prepared by homogenisation of (A) water, (B) an organic phase and (C) a phase containing preservatives. The organic phase (B) may comprise, for example, the peptide of formula (I) or a salt thereof and/or a derivative thereof, as described herein, a C_{12-15} alkyl benzoate, PPG-3 myristyl ether, hydroxyethyl acrylate, sodium acrylolydimethyl taurate copolymer, squalane and polysorbate 60. The phase containing preservatives (C) may comprise, for example, ethylhexylglycerin and phenoxyethanol. Accordingly, the composition may be prepared

by adding phase (B) into phase (A), followed by homogenisation (for example at about 2500 rpm, for a period of about 5 min), then phase (C) can be added to the resulting composition and the composition stirred until homogenous (for example at about 100-200 rpm, for a period of about 30 min). Alternatively, phase (A) may be added into phase (B), followed by homogenisation (for example at about 2500 rpm, for a period of about 5 min), then phase (C) can be added to the resulting composition and the composition stirred until homogenous (for example at about 100-200 rpm, for a period of about 30 min).

The compositions may comprise suitable solvents or agents which act to enhance the absorption of other ingredients into the skin, humectants and anti-irritants, gums for producing a gel base that may have skin smoothing and soothing properties, emulsion stabilising and viscosity controlling agents, agents which act to prevent deterioration of the formulation, moisture attracting agents, emollients, hair/skin-conditioning agents, agents which help improve the overall texture and finish of skin care formulae, gelling agents, preservatives, agents which reduce the appearance of large pores, age spots and hyperpigmentation, including lactic acids, and subjecting this mixture to homogenisation.

In some embodiments, the compositions are prepared as compositions suitable for topical use, by combining suitable solvents which may enhance the absorption of other ingredients into skin, such as methylpropanediol, humectants and anti-irritants, such as betaine, natural gums such as sclerotium gum, emulsion stabilizing and viscosity agents such as sodium polyacryloyldimethyl taurate, anti-deterioration, agents such as disodium EDTA, moisture attractants, such as sodium hyaluronate, water-dispersible silicones, such as bis-PEG-18 methyl ether dimethyl silane, which may act as emollients and with added foam-boosting properties, hair/skin conditioning agents, such as PEG-12 dimethicone, other suitable emollients selected from C12-15 alkyl benzoate, PPG-3 myristyl ether and glycerin, gelling agents selected from hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer, agents which reduce the appearance of large pores, age spots and hyperpigmentation, including lactic acids, preservatives selected from ethylhexylglycerine and phenoxyethanol, antioxidants including sodium metabisulfite, biocides including chlorphensin with deionised water, and subjecting this mixture to homogenisation.

Uses of Peptides

The present disclosure further relates to therapeutic and cosmetic uses of the peptides and compositions. For example, the disclosure relates to methods and uses for inhibiting a bone morphogenetic protein (BMP) in a subject (for example bone morphogenetic protein-2 (BMP-2); treating or preventing a disease or disorder associated with bone morphogenetic protein 2 (BMP-2) expression in a subject; treating and/or preventing one or more of skin aging, wrinkles, fine lines sagged skin, hyperpigmented spots and uneven skin tone in a subject; and treating and/or preventing one or more of cosmetic skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone in a subject; using the peptide of formula (I) or a salt thereof, or compositions described herein.

The peptide of formula (I) or a salt thereof, or composition described herein may be administered by any suitable means, for example it may be applied topically to the skin of the subject. The compositions may be used to treat, control, prevent and ameliorate the visible signs of skin aging in a safe and non-invasive way. As discussed above, it has now been discovered that bone morphogenetic protein 2 (BMP-2) in the dermal layer of skin correlates directly with the apparent age of a subject's skin, and it may be involved in inflammation, thus contributing to visibly aged skin. Advantageously, the novel findings by the present inventors have emerged as a strategy for treating the visible signs of aging caused by inflammation by inhibiting bone morphogenetic protein 2 (BMP-2). Accordingly, it has been identified that a peptide of formula (I) or a salt thereof and/or a derivative thereof provides superior anti-aging properties, providing superior results in abrogating the effects of BMP-2 induced skin inflammaging by promoting epidermal stemness, reducing the senescent cells, increasing the pro-collagen 1A1 protein expression, and enhancing the collagen IV, collagen VII and pro-collagen 1A1 protein expression in skin. For example, N-palmitoyl-L-tryptophan-L-tryptophan-L-phenylalanine has been shown to synergistically inhibit BMP-2 in the epidermis compared to control compositions with no tripeptide, in a human skin model.

The present inventors have thus determined that inhibiting the activity of a BMP such as BMP-2 in skin has emerged as a new strategy for treating the visible signs of aging in skin.

Advantageously, application of the composition of the present disclosure in a method as defined herein demonstrates minimal negative effects on tissue viability of skin.

Accordingly, in one aspect, there is provided a method of inhibiting BMP-2 in a subject, comprising administering an effective amount of a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, to the subject.

There is also provided a method of method of treating or preventing a disease or disorder associated with BMP-2 expression in a subject, comprising administering an effective amount of a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, to the subject.

There is also provided a method of treating and/or preventing one or more of skin aging, wrinkles, fine lines sagged skin, hyperpigmented spots and uneven skin tone in a subject, comprising administering an effective amount of a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, to a subject in need thereof, optionally wherein the method is for treating and/or preventing one or more of cosmetic skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone in a subject.

In another aspect, there is provided a use of a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for the manufacture of a medicament, for inhibiting BMP-2.

There is also provided a use of a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for the manufacture of a medicament, for treating or preventing a disease or disorder associated with BMP-2 expression.

There is also provided a use of a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for the manufacture of a medicament for therapeutic treatment and/or prevention of one or more of skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone.

There is also provided a use of a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for inhibiting BMP-2.

There is also provided a use of a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for treatment and/or prevention of one or more of cosmetic skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone.

In another aspect, there is provided a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for use in inhibiting BMP-2.

There is also provided a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for use in treating or preventing a disease or disorder associated with BMP-2 expression.

There is also provided a peptide of formula (I) or a salt thereof as defined herein, or of a pharmaceutical or cosmetic composition as defined herein, for use in the therapeutic treatment and/or prevention of one or more of skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone.

In some embodiments, the composition is effective in abrogating the effects of BMP-2 induced skin inflammaging by enhancing the keratinocyte colony forming efficiency (epidermal stemness) and/or collagen XVII in skin. In some embodiments, the composition is effective in abrogating the effects of BMP-2 induced skin inflammaging by reducing the senescent cells and/or increasing the pro-collagen 1A1 protein expression in skin. In some embodiments, the composition is effective in abrogating the effect of BMP-2 induced skin inflammaging by enhancing the collagen IV, collagen VII and/or pro-collagen 1A1 protein expression in skin.

In some embodiments, the composition is applied topically, to the skin of a subject.

In some embodiments, the subject is a human subject. In some embodiments the subject is an adult subject. In some embodiments the subject is a human subject of at least 20 years age, at least 25 years age, at least 30 years age, at least 35 years age, at least 40 years age, at least 45 years age, at least 50 years age, at least 55 years age, or at least 60 years age. In some embodiments, the subject is male or identifies as male. In some embodiments, the subject is female or identifies as female.

The present disclosure also relates to non-therapeutic methods for reducing the visible signs of aging of skin, for example, the composition of the present disclosure

may be applied in cosmetic uses to reduce skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone in a subject.

Accordingly, in one aspect, there is provided a non-therapeutic method of skin lightening of a subject, comprising administering an effective amount of a peptide or composition as defined herein to the subject.

In some embodiments, the peptide or composition is applied topically to the skin of the subject.

The compositions of the present disclosure may for example be a topical composition that is to be applied on an external surface of a subject. In particular embodiments, the external surface may be one or more of the scalp, face, arms, legs or torso of a subject. Advantageously, the compositions of the present disclosure demonstrate effective skin bioavailability which allows skin penetration to treat the visible signs of aging, which has been demonstrated in the efficacy studies described herein.

It should be appreciated that the frequency with which the compositions of the present disclosure should be applied will vary depending on the desired effect. In some embodiments, the degree of therapeutic and/or cosmetic enhancement will vary and may be directly proportional with the total amount of compositions used. Topical application of the compositions may be administered in any amount and at any frequency sufficient to bring about the desired level of improvement in appearance.

The peptides and compositions may be administered according to any suitable dosing or administration regime. In some embodiments, the composition of the present disclosure is administered once daily, or twice daily, or three times daily, or four times daily, for example for a period of time sufficient to bring about a desired level of improvement in skin aging. Alternatively, the compositions may be used less frequently if that will provide the required effects. For example, it may be applied once every other day or once, twice, three or four times a week, or on an ad hoc or as needed basis.

For example, a user may topically administer a composition of the present disclosure directly to the skin where more youthful looking skin is desired by gently massaging the composition of the present disclosure into the desired area.

Topical application of the compositions of the present disclosure may continue for any suitable period of time. For example, within a few days to a few weeks, a user may notice a reduction in the visible signs of aging. Advantageously, topical treatment

with the composition of the present disclosure may demonstrate anti-aging effects by significantly improving the skin tone in, for example, as short as 4-weeks of product application.

The peptides and compositions may be administered in any suitable amount. For example, the compositions may be applied in amounts ranging from about 1 ppm to about 10,000 ppm of peptide, or from about 2 ppm to about 2,000 ppm, or from about 3 ppm to about 1,000 ppm, or from about 4 ppm to about 500 ppm, or from about 5 ppm to about 100 ppm, or from about 5 ppm to about 50 ppm, or from about 10 ppm to about 25 ppm, or from about 10 ppm to about 20 ppm, or from about 10 ppm to about 200 ppm, or from about 20 ppm to about 150 ppm, or from about 50 ppm to about 125 ppm of peptide.

When topically applied, the peptide or composition of the present disclosure may be applied to the area to be treated, for example, the face, by any suitable technique, such as spraying, dabbing, swabbing, rubbing or massaging. For example, in one embodiment, a composition of the present disclosure may be provided in a liquid, lotion, cream, foam, serum, ointment, foundation, scrub, gel, soap bar, toner, or applied with an implement or via a face mask, pad or patch in any form of materials or other leave-on product formulation, which preferably may be applied on at least a daily basis.

Those skilled in the art will appreciate that the disclosure herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications.

Examples

The present disclosure is further illustrated by the following non-limiting examples.

Example 1 – Establishment of a 3D inflammatory skin model

The objective of this experiment was to establish a biomimetic 3D inflammatory skin model that allows the assessment of topical applications.

Methodology

Establishment of the novel 3D inflammatory skin model

A human monocytic U937 cell line (ATCC, Manassas, VA, USA) was grown in a complete medium consisting of Roswell Park Memorial Institute 1640 medium (RPMI 1640) supplemented with 10% fetal bovine serum (FBS; HyClone, Australia) in a CO_2 humid incubation chamber at 37°C. Three million cells were then treated with 10 nM phorbol myristate acetate (PMA; Nacalai Tesque, Japan) for 24 h to induce differentiation into the macrophage phenotype. Epidermal Full-thickness Human skin models (EFT-400) were purchased from MatTek (Ashland, MA). Before co-culturing with differentiated U937 cells, EFT-400 tissues were first equilibrated in EFT assay media (MatTek, MA) for 24 h at 37°C in a 5% CO_2 incubator.

Differentiated U937 cells, which were characterized by attached cells, were trypsinized and seeded onto a 6-well plate at a density of 100,000 cells/mL per well in RPMI 1640 supplemented with 1% FBS. The cells were then incubated at 37°C in a 5% CO_2 incubator for 2 h for the cells to attach. 1 mL of co-culture media was prepared by mixing RPMI 1640 with 1% FBS/EFT assay media at a 6:4 ratio and added to each well. Equilibrated EFT-400 skin tissues were then added onto the wells containing the differentiated U937 cells at the bottom of the well. Lipopolysaccharide (LPS; Nacalai Tesque, Japan) at 5 µg/mL was added into the culture media to induce an inflammatory response. As a positive control, 50 µL of 100 µM dexamethasone (Nacalai Tesque, Japan) was applied topically onto the skin tissue. After treatment, the co-culture model was incubated at 37°C for 24 h. The tissues were also kept at -80°C for RNA extraction for qPCR analysis.

Cytokine profiling by using Bio-Rad Bio-Plex Pro Human Cytokine 27-Plex Assay and ELISA

Cytokine profiling was performed using Bio-Rad Bio-Plex Pro Human Cytokine 27-Plex Assay (Bio-Rad, Hercules, CA) and two of the significantly regulated cytokines, TNF-α and IL-1β, were validated using ELISA Max Deluxe kit (Biolegend, San Diego, CA). Both assays were performed according to the manufacturer's instructions.

Results and Discussion

In this study, a novel and robust 3D inflammatory skin model has been developed (Fig. 1). The 3D inflammatory skin model involves the co-culture of lipopolysaccharide-induced U937 macrophage cells and a 3D reconstituted full thickness skin tissue in a

trans well. In an inflammation event, monocytes are one of the first immune cells to be recruited to the injured site. Upon migration from the systemic circulation to the injured site, monocytes will differentiate to become fully functional macrophages. The macrophages will phagocytize dead or damaged cells and secrete inflammatory cytokines to further initiate the inflammation process. In this study, U937 cells, a monocyte cell line, are differentiated into macrophages before co-culturing them with skin tissue. Lipopolysaccharide (LPS) was then added to the culture media to induce inflammation for 24 h and the culture supernatant was collected for cytokine profiling.

To further understand the cytokine secretion profile in the 3D inflammatory skin model, a Bio-Rad Bio-Plex Pro Human Cytokine 27-Plex Assay was conducted to profile 27 common inflammatory cytokines. The quantification data are shown in Table 1, below. Based on the data, 6 cytokines, interleukin-9 (IL-9), interleukin-17A (IL-17A), macrophage inflammatory protein-1α (MIP-1α), macrophage inflammatory protein-1β (MIP-1β), interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α), were significantly induced in the presence of 5 μg/mL LPS and were suppressed with the addition of 100 μM dexamethasone (anti-inflammatory steroid drug). Therefore, these pro-inflammatory cytokines were suitable endpoint inflammation markers for this model, and this also validates the inflammatory status of the skin model.

Table 1: Differentially expressed cytokines in the culture supernatant of a 3D inflammatory skin model

Cytokines	Treatments (% vs Untreated Control)		
	Untreated Control	LPS	LPS + Dexamethasone
TNF-α	100	191.72 ± 9.09**	97.26 ± 15.55
IL-1β	100	224.37 ± 77.88*	138.54 ± 10.74
IL-9	100	404.97 ± 90.40****	221.32 ± 50.84**
IL-17a	100	220.72 ± 12.38****	138.98 ± 2.17**
MIP-1α	100	1782.81 ± 307.49***	424.88 ± 20.20
MIP-1β	100	1499.03 ± 380.56***	447.19 ± 122.11*

Six cytokines (IL-9, IL-17a, MIP-1α, MIP-1β, IL-1β and TNF-α) were significantly expressed when compared to the inflamed skin induced with LPS, indicating that these

cytokines can be used as inflammatory markers for this model. Data are expressed in mean ± S.E.M. of two independent experiments (n=2). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant difference when compared to LPS using one-way ANOVA analysis.

Conclusion

A biomimetic 3D inflammatory skin model was developed that allows the assessment of topical compositions.

Example 2 – Establishment of the role of BMP-2 in inflammation and skin aging

The objectives of this experiment were to validate the role of BMP-2 in inflammation, and to investigate the correlation of BMP-2 with skin aging.

Methodology

Determination of BMP-2 gene expression in the in vitro 3D inflammatory skin model

An in vitro 3D inflammatory skin model was cultured according to Example 1. The skin tissues were then harvested for RNA extraction. Total RNA was extracted from the skin tissues using NucleoSpin RNA kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. cDNAs were synthesized by using the iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad, Hercules, CA). qPCR was performed using Bio-Rad PrimePCR with customized 16 genes with CFX96 Touch Real-Time PCR Detection System (Bio-Rad) according to the manufacturer's instructions.

Correlation of BMP-2 with age

Ex vivo skin explants from donors from different ages were obtained from Genoskin® for this study. The skins were equilibrated in the provided tissue culture media for one hour prior to experiment. After equilibration, culture medium was harvested for BMP-2 protein quantification. The culture media was then concentrated using a 3 kDa spin column at 14,000 X g for 20 min at 4°C. 50 µL of concentrated culture supernatant was used to quantify for BMP-2 protein expression using Human BMP2 ELISA Kit (Abcam, Cambridge, MA, USA) according to manufacturer's instructions.

Determination of the effect of BMP-2 on keratinocyte stemness using clonogenic assay

The human primary keratinocytes were cultured in complete F media at 37°C with 5% CO_2 in a humidified incubator. The complete F media consisted of one-part F12 medium and three parts DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% bovine calf serum, 1% antibiotic-antimycotic solution, 25 ng/mL hydrocortisone, 5 µg/mL insulin, 0.125 ng/mL EGF, and cholera toxin 0.1 nM. The murine fibroblasts, 3T3-J2 cells were first cultured in complete DMEM supplemented with 10% BCS and 1% AA at 37°C in a humidified 5% CO_2 atmosphere. In order to establish the feeder cells, 3T3-J2 cells were treated with mitomycin C (4 µg/mL) for 2 h at 37°C. The cells can then be trypsinized and directly seeded in a 6-well plate (2×10^5 cells/well) for clonogenic assay or cryopreserved in a freezing medium (1×10^6 cells/vial) at liquid nitrogen for future usage.

The feeder cells were seeded in 6-well plates (200,000 cells/well/2 mL) and incubated overnight at 37°C in a humidified 5% CO_2 atmosphere. After the attachment of the feeder cells to each well surface, primary keratinocytes were seeded on the feeder cells layer at low density (800 cells/well/2 mL) to allow colony formation. The co-culture models were then treated with complete F media supplemented with BMP2 (100 ng/mL), BMP2+Noggin (100 ng/mL) or ROCK inhibitor (10 µM) on the second day of primary keratinocyte seeding. EGF is excluded from the complete F media as it inhibits the activity of BMP-2. The treatments were repeated every two days and the formation of the well-defined colonies was observed using an inverted light microscope (40× magnification). After 14 days of culture, the co-culture models were fixed with 4% paraformaldehyde and stained with crystal violet. The images of the 6-well plate with colonies were acquired using Bio-Rad imaging system and each well's total colony surface area were quantified using the NIS-Elements software (Nikon, Japan).

Validation of the effect of BMP-2 on skin stemness in ex vivo human skin explant

Human skin explants obtained from Genoskin® were equilibrated for at least 1 h. On the same day after equilibration, the culture media was changed, and 100 ng/mL BMP-2 recombinant proteins (R&D Systems, Minneapolis, USA) with or without 100 ng/mL noggin recombinant proteins (R&D Systems, Minneapolis, USA) were treated systemically in the culture media for 24 h. The next day, the culture media was changed, and the same treatment was repeated for another 2 d, for a total of 3 d. On day 4, the

skin tissues were fixed in 4% paraformaldehyde and processed for cryosectioning and immunofluorescent staining on collagen XVII. The protein expression of collagen XVII was quantified using NIS software (Nikon, Japan).

Results and Discussion

BMP-2 gene is up-regulated in the 3D inflamed skin

Inflammaging describes the phenomena of the presence of chronic, low-grade inflammation that occurs with aging. Even though inflammaging is quite well researched in the medical field, the precise mechanism of how inflammation leads to skin aging remains to be fully understood. To answer this, DNA microarray analyses were performed on the 3D inflamed skin. Results show that the modulated genes were significantly enriched in pathways that are involved in reduced cell proliferation and increased skin differentiation (data not shown). Based on this, a panel of 16 genes were shortlisted and they were validated using qPCR gene expression analyses. Table 2 shows the gene expression results of all 16 genes, whereby BMP2 gene which was up-regulated by 4.03-fold in the inflamed skin showed the highest gene expression changes.

Table 2: qPCR gene expression results for the inflamed skin vs normal skin treatment group

Genes	qPCR	
	Fold change in inflamed skin	p-value
AKT3	1.14	0.37
ATM	1.21	0.24
BMP2	**4.03**	0.001
DEFB113	1.77	0.65
GDF9	1.43	0.42
MORC3	1.37	0.024
PAIP2	1.18	0.030
PDE5A	-1.07	0.92
PIGA	1.61	0.00057
PIK3CA	-1.08	0.71
PLCG1	1.21	0.35
RASA2	1.13	0.19
RSAD2	1.86	0.05

TERF2IP	1.04	0.68
TIMP3	-1.04	0.88
WEE1	1.60	0.05

Positive correlation of BMP-2 protein secretion with age

To understand if BMP-2 is correlated with aging, the BMP-2 protein expression was quantified in skin explants obtained from donors from different ages. Fig. 2 depicts that there is a significant positive correlation between BMP-2 protein secretion with skin donor's age.

BMP-2 reduced epidermal stemness in primary keratinocytes and in human skin explants

In order to study the effect of BMP-2 in affecting the epidermal stemness, a clonogenic assay was conducted on primary keratinocytes treated with BMP-2. Table 3 shows that the colony surface area of primary keratinocytes treated with recombinant human BMP-2 proteins was significantly smaller by 24.5%, compared to the untreated cells, indicating that BMP-2 reduces the proliferative capability of the primary keratinocytes, which in turn reduces the epidermal stemness. The combination of BMP-2 and its antagonist, noggin, abrogates the effect on BMP-2's impact on keratinocytes, thus validating the BMP-2 induced reduction of epidermal stemness.

The role of BMP-2 in reducing epidermal stemness was also validated in the human skin explants. Collagen XVII is well-known for its pivotal role in maintaining the epidermal stem cell homeostasis, and the loss of collagen XVII expression in the basal keratinocyte leads to asymmetric division and the gradual loss of proliferative stem cells in the epidermis, leading to skin aging. Table 3 depicts the collagen XVII protein expression after treatment with BMP-2 with and without its inhibitor, noggin, on human skin explants. BMP-2 treatment reduces collagen XVII protein expression by 14% and in the presence of noggin, the BMP-2's activity is abrogated.

Table 3: Colony forming efficiency of primary keratinocytes treated with BMP-2 recombinant proteins and effect of BMP-2 on collagen XVII protein expression in human skin explants

Treatment	Colony forming efficiency (% compared to Untreated Control)	Collagen XVII protein expression (% compared to Untreated Control)
Untreated control	100 ± 9.30	100 ± 16.35
rhBMP2	74.65 ± 10.15****	85.74 ± 20.57*
rhBMP2 + Noggin	102.57 ± 11.06####	116.77 ± 38.21**

All values are expressed in mean ± S.E.M. *p<0.05, **p<0.01, ***p<0.001, and **** p<0.0001 significant difference using two-way ANOVA; ####p<0.0001 significant difference when compared to rhBMP2 using Student's T-test (n=5).

Conclusion

From these results it can be seen that skin inflammation causes up-regulation of BMP2 gene; BMP-2 is positively correlated with skin age; and BMP-2 leads to skin aging through reduced epidermal stemness.

Example 3 – Validation of BMP-2 for skin aging (senescent cells and collagen-1)

The objective of this experiment was to investigate the effects of BMP-2 in causing skin aging.

Methodology

Validation of the effect of BMP-2 on senescent cells and Collagen I in ex vivo human skin explant

Human skin explants obtained from Genoskin® were equilibrated for at least 1 h. On the same day after equilibration, the culture media was changed, and 100 ng/mL BMP-2 recombinant proteins (R&D Systems, Minneapolis, USA) with or without 100 ng/mL noggin recombinant proteins (R&D Systems, Minneapolis, USA) were treated systemically in the culture media for 24 h. The next day, the culture media was changed, and the same treatment was repeated for another 2 d, for a total of 3 d. On day 4, the skin tissues were fixed in 4% paraformaldehyde and processed for cryosectioning and immunofluorescent staining on collagen I and senescent cells (Senescence-associated beta-galactosidase and p21Cip1/Waf1 positive). The protein expression of collagen I was quantified using NIS software (Nikon, Japan). The number of senescent cells were counted manually using a cell counter.

Results and Discussion

Cellular senescence is a hallmark of aging defined by stable exit from the cell cycle in response to cellular damage and stress. Senescent cells can develop a characteristic pathogenic senescence-associated secretory phenotype (SASP) that drives secondary senescence and disrupts tissue homeostasis, resulting in loss of tissue repair and regeneration. Table 4 shows that BMP-2 treatment significantly increased senescent cells in the human skin explant by 421%. Concomitant reduction of collagen I was also observed in human skin explant treated with recombinant human BMP-2. This validates that BMP-2 plays a role in contributing to skin aging.

Table 4: Effect of BMP-2 on senescent cells in human skin explants

Treatment	Number of senescent cells (% compared to Untreated Control)	Pro-collagen I protein expression (% compared to Untreated Control)
Untreated control	100 ± 92.03	100 ± 24.95
rhBMP2	521.21 ± 264.26***	61.30 ± 25.59****
rhBMP2 + Noggin	352.02 ± 206.36	91.04 ± 32.90***

Data are expressed in mean ± S.E.M of two independent experiments (n=2). ***p<0.001, ****p<0.0001 significant difference when compared to untreated control using Two-Way ANOVA.

Conclusion

From these results it can be seen that BMP-2 treatment leads to increased senescent cells and reduction of collagen I in human skin explant, which leads to skin aging.

Example 4 – *In silico* design of anti-BMP2 peptides

The objective of this experiment was to design a tripeptide to inhibit dimerization of BMP-2 *in silico*.

Methodology

Preparation of Macromolecule

A 3-dimensional (3D) crystal structure of the BMP-2 protein was downloaded from the Protein Data Bank (PDB). Tripeptides (3 amino acids) were generated for virtual screening. The drug targets were then optimized for docking using AutoDockTools (ADT) 1.5.6 software by removing the water molecules, adding polar hydrogen atoms, and merging the non-polar hydrogen atoms. ADT was used as well to convert the structure into .pdbqt file for subsequent virtual screening.

Preparation of Peptides with 3 Amino Acids & Standard Ligands

Pymol 1.7 software was used to build and optimize 3D models of all peptides. Raccoon 1.0 software was used to prepare the .pdbqt files for all the required peptides. The top 100 peptides ranked based on binding energy were subjected for further elongation with other top ranked peptides into longer peptides. A fragment derived from amino acid residues from BMP-2 dimer binding site - IPLACCVP was prepared using Discovery Studio Visualizer 4.5 (DSV) as a standard ligand (BIOVIA, 2015; San Diego: Dassault Systèmes). The strategy was to design the peptide binding in the middle of the BMP-2 dimer to prevent its dimerization and subsequent activation.

Virtual Screening and Analysis

Blind docking was employed for all the virtual screening process via Autodock Vina 1.1.2 software because it covers the whole surface of the target protein. The standard ligand was first used to go through blind redocking using Autodock Vina 1.1.2 software on a Linux workstation to help in the optimization of docking process, followed by the generated peptides. DSV were then used to run the interactions analysis of virtual screening result based on binding energies and visual inspection of binding interactions between ligands and protein as reported previously. Peptides that showed better or similar binding energy and binding interactions when compared to the standard ligand, were chosen as potential peptide inhibitors.

Results and Discussion

A total of 8000 tripeptide combinations were virtually screened. Table 5 shows the top 10 tripeptides that have the highest binding affinity to the BMP-2 dimer binding site. All 10 tripeptides have higher binding affinity than the native ligand, which is the standard used in this study, indicating a high potential to prevent the dimerization of

BMP-2 which is essential for its activation. As observed in all the tripeptides, the middle amino acid consists of tryptophan (W), and the first and third amino acid are mainly aromatic amino acids, either tryptophan (W), tyrosine (Y) or phenylalanine (F). Some peptides also consist of arginine (R), leucine (L) and valine (V). Two exemplary tripeptides (WWW and WWF) were investigated for further validation on their effects in inhibiting BMP-2 induced skin inflammaging.

Table 5: Exemplary tripeptides designed to bind to the BMP-2 dimer binding site to prevent dimerization; data are sorted according to the binding affinity

No.	Tripeptides	Max Binding Affinity	Min Binding Affinity	Percentage of Binding
1	WWW	-7.4	-7.3	47
2	WWF	-7.2	-7	51
3	WWY	-7.1	-7	48
4	WWR	-7.1	-6.4	36
5	YWW	-7.1	-6.6	36
6	RWY	-7	-6.3	29
7	FWY	-6.9	-6.2	18
8	FWF	-6.8	-6.3	24
9	WWL	-6.7	-6.6	42
10	WWV	-6.7	-6.3	39
	Standard (IPLACCVP)	-5.6	-5.1	68

Conclusion

From these results it can be seen that tripeptides specifically targeting the BMP-2 dimer binding site can be designed, and exemplary tripeptides were identified.

Example 5 – Systemic effects of acylated-tripeptides in abrogating the effect of BMP-2 induced skin inflammaging

The objective of this experiment was to investigate the effect of systemic treatment of exemplary acylated-tripeptides of the present invention, Pal-WWF-OH and Pal-WWW-OH (Pal = palmitoyl), in abrogating the effect of BMP-2 induced skin inflammaging.

Methodology

Tripeptide treatment on primary keratinocytes treated with BMP-2

Tripeptides were treated the same way as the positive controls together with the rhBMP2 at 50, 100, 200 and 500 ng/mL, as described in Example 2.

Systemic treatment in human skin explant induced with BMP-2

Human skin explants (Genoskin) were equilibrated for at least 1 h. In the same day after equilibration, the culture media was then changed and various treatments (Table 6) were treated systemically in the culture media for 24 h. In the next day, the culture media was changed and the same treatment was repeated for another 2 d, total 3 d. On day 4, the skin tissues were fixed in 4% paraformaldehyde and processed for cryosectioning and immunofluorescent staining on collagen 17 (1:200 dilution) according to WI-MD-27 and WI-ECM-04. Changes in collagen 17 protein expression was quantified using NIS software. The experiment was repeated twice on skin tissues from 2 different donors.

Table 6: Final concentrations of various systemic treatments on human skin explants

Treatments	Final concentration (ng/mL)		
	BMP-2	Pal-WWW-OH	Pal-WWF-OH
Untreated Control	100	-	-
10 Pal-WWW-OH	100	10	-
100 Pal-WWW-OH	100	100	-
1000 Pal-WWW-OH	100	1000	-
10 Pal-WWF-OH	100	-	10
100 Pal-WWF-OH	100	-	100
1000 Pal-WWF-OH	100	-	1000

Results and Discussion

As shown in Table 7, systemic treatment with palmitoyl tripeptides of the present invention significantly enhanced the keratinocyte colony forming efficiency at 200 - 500 ng/mL concentration. It also increased the collagen XVII protein expression in the skin explants treated with BMP-2 significantly at 1000 ng/mL (Table 8). This shows that

palmitoyl tripeptides of the present invention are effective in abrogating the effect of BMP-2 induced skin inflammaging.

Table 7: Effect of acylated-tripeptides on keratinocyte colony forming efficiency

Treatment	Keratinocyte colony forming efficiency (% compared to Untreated Control)
Untreated control	100
50 ng/mL Pal-WWF-OH	80.63 ± 26.69
100 ng/mL Pal-WWF-OH	112.68 ± 19.52
200 ng/mL Pal-WWF-OH	134.99 ± 19.44**
500 ng/mL Pal-WWF-OH	121.80 ± 6.44**
50 ng/mL Pal-WWW-OH	108.82 ± 1.71
100 ng/mL Pal-WWW-OH	97.83 ± 6.16
200 ng/mL Pal-WWW-OH	114.67 ± 2.23
500 ng/mL Pal-WWW-OH	93.92 ± 5.12

Data are expressed in mean ± S.E.M of at least two independent experiments (n>2). **p<0.01 significant difference when compared to untreated control using Two-Way ANOVA.

Table 8: Effect of systemic treatment of acylated-tripeptides on collagen XVII protein expression in human skin explant

Treatment	Collagen XVII protein expression (% compared to Untreated Control)
Untreated control	100
10 ng/mL Pal-WWF-OH	106.31 ± 31.95
100 ng/mL Pal-WWF-OH	122.98 ± 15.91
1000 ng/mL Pal-WWF-OH	139.19 ± 32.80****
10 ng/mL Pal-WWW-OH	113.70 ±- 15.66
100 ng/mL Pal-WWW-OH	104.03 ± 19.18
1000 ng/mL Pal-WWW-OH	137.49 ± 24.25***

Data are expressed in mean ± S.E.M of two independent experiments (n=2). ***p<0.001, ****p<0.0001 significant difference when compared to untreated control using Two-Way ANOVA

Conclusion

Palmitoyl tripeptides of the present invention are effective in abrogating the effects of BMP-2 induced skin inflammaging by enhancing the keratinocyte colony forming efficiency and collagen XVII in the skin explants.

Example 6 – Systemic effect of acylated-tripeptides in abrogating the effect of BMP-2 induced skin inflammaging

The objective of this experiment was to validate the effect of an acylated tripeptide of the present invention in abrogating BMP-2 induced skin inflammaging.

Methodology

Systemic treatment in human skin explant induced with BMP2

Human skin explants (Genoskin) were equilibrated for at least 1 h. In the same day after equilibration, the culture media was then changed and various treatments (Table 9) were treated systemically in the culture media for 24 h. In the next day, the culture media was changed and the same treatment was repeated for another 2 d, total 3 d. On day 4, the skin tissues were fixed in 4% paraformaldehyde and processed for cryosectioning and immunofluorescent staining on senescent cells and pro-collagen 1A1. Changes in pro-collagen 1A1 protein expression and senescence cells was quantified using NIS software. The experiment was repeated twice on skin tissues from 2 different donors.

Table 9: Final concentrations of various systemic treatments on human skin explants

Treatments	Final concentration (ng/mL)	
	BMP-2	Pal-WWF-OH
Untreated Control	100	-
10 Pal-WWF-OH	100	10
100 Pal-WWF-OH	100	100
1000 Pal-WWF-OH	100	1000

Results and Discussion

As shown in Table 10, systemic treatment of palmitoyl tripeptides significantly reduced the number of senescent cells induced by BMP-2 in the human skin explants at 100 -1000 ng/mL concentration. Similarly, it also increased the pro-collagen 1A1 protein expression in the skin explants treated with BMP-2. This shows that palmitoyl tripeptides of the present invention are effective in abrogating the effect of BMP-2 induced skin inflammaging.

Table 10: Effect of a palmitoyl tripeptide on the reduction of number of senescent cells and enhancement of pro-collagen 1A1 in human skin explants.

Treatments	No. of Senescent cells (% vs Untreated control)	Pro-collagen 1A1 protein expression (% vs Untreated Control)
Untreated control	100	100
10 ng/mL Pal-WWF-OH	64.58 ± 12.84	169.15 ± 103.48
100 ng/mL Pal-WWF-OH	45.91 ± 8.94*	232.04 ± 195.62****
1000 ng/mL Pal-WWF-OH	40.10 ± 0.74**	228.87 ± 186.42****

Data are expressed in mean ± S.E.M of two independent experiments (n=2). *p<0.05, **p<0.01, ****p<0.0001 when compared to untreated control using Two-Way ANOVA.

Conclusion

Palmitoyl tripeptides of the present invention are effective in abrogating the effects of BMP-2 induced skin inflammaging by reducing the senescent cells and increasing the pro-collagen 1A1 protein expression in the skin explants.

Example 7 – Topical effect of acylated-tripeptides in abrogating the effect of BMP-2 induced skin inflammaging

The objective of this experiment was to validate the effect of topical application of an acylated-tripeptide (palmitoyl tripeptide 101, Pal-WWF-OH) in abrogating the effect of BMP-2 induced skin inflammaging.

Methodology

Composition preparation

A number of exemplary tripeptide topical compositions were prepared from palmitoyl tripeptide Pal-WWF-OH in accordance with Table 11. Premix B was added into water phase (A) and homogenized at 2500 rpm for 5 minutes. Then, premix C was added into the bulk and stirred at 100-200 rpm for 30 minutes.

Table 11: Tripeptide compositions

Component	Ingredient	Formula 1 (wt. %)	Formula 2 (wt. %)	Formula 3 (wt. %)	Formula 4 (wt. %)
A	Deionized water	qs 100	qs 100	qs 100	qs 100
B	Pal-WWF-OH	0	0.001	0.005	0.01
	C12-15 Alkyl Benzoate	5	5	5	5
	PPG-3 Myristyl Ether	5	5	5	5
	Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer (and) Squalane (and) Polysorbate 60	3	3	3	3
C	Ethylhexylglycerin	0.3	0.3	0.3	0.3
	Phenoxyethanol	0.4	0.4	0.4	0.4

Topical treatment in human skin explant induced with BMP2

Human skin explants (Genoskin) were equilibrated for at least 1 h. In the same day after equilibration, the culture media was then refreshed and 100 ng/mL of recombinant BMP-2 protein were added systemically into the culture media. 16 µL of samples (Table 12) were applied topically onto the skin tissues for 24 h. In the next day, the culture media was refreshed and the same treatment was repeated for another 2 d,

total 3 d. On day 4, the skin tissues were fixed in 4% paraformaldehyde and processed for cryosectioning and immunofluorescent staining on senescent cells and pro-collagen 1A1. Changes in pro-collagen 1A1 protein expression and senescence cells were quantified using NIS software. The experiment was repeated twice on skin tissues from 2 different donors.

Results and Discussion

As shown in Table 12, topical treatment of palmitoyl tripeptides at doses ranging from 0.001 – 0.01% significantly increased the collagen IV, collagen VII and pro-collagen 1A1 protein expression in the skin explants treated with BMP-2. This shows that topical application of palmitoyl tripeptides can penetrate into the skin and effectively abrogate the effect of BMP-2 induced skin inflammaging.

Table 12: Topical effect of an acylated tripeptide on the enhancement of collagen IV, collagen VII and pro-collagen 1A1 in human skin explants

Treatments	Palmitoyl tripeptide, Pal-WWF-OH (%)	Collagen IV protein expression (% vs Untreated Control)	Collagen VII protein expression (% vs Untreated Control)	Pro-collagen 1A1 protein expression (% vs Untreated Control)
Untreated control	-	100	100	100
Formula 1	-	98.38 ± 7.02	105.50 ± 4.43	172.45 ± 69.68
Formula 2	0.001	106.89 ± 4.83 **	111.12 ± 6.51 ***	250.26 ± 158.51 ****
Formula 3	0.005	115.03 ± 9.10 ****	121.49 ± 7.58 ****	197.14 ± 55.96 **
Formula 4	0.010	116.64 ± 9.64 ****	121.14 ± 9.89 ****	250.48 ± 107.84 ****

Data are expressed in mean ± S.E.M of two independent experiments (n=2). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 when compared to untreated control using Two-Way ANOVA.

Conclusion

Topical application of an acylated tripeptide, Pal-WWF-OH, can penetrate the skin and effectively abrogate the effect of BMP-2 induced skin inflammaging by enhancing the collagen IV, collagen VII and pro-collagen 1A1 protein expression in human skin explants.

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CLAIMS

1. A peptide comprising an amino acid sequence having the formula (I):

Xaa1-Xaa2-Xaa3 (I)

wherein:

Xaa1 is an amino acid residue selected from the group consisting of Trp, Phe, Tyr, Arg, His, Met, Lys and Asn;

Xaa2 is an amino acid residue selected from the group consisting of Trp, Phe, Tyr, Arg, Asp, Gln, Thr, Pro and Val; and

Xaa3 is an amino acid residue selected from the group consisting of Trp, Phe, Tyr, Arg, Leu, Val, Ile, His, Ala, Gln, Lys, Asp and Thr; and

wherein either:

(i) the peptide is a tripeptide;

Xaa1 is an N-terminal residue which is optionally derivatised; and

Xaa3 is a C-terminal residue which is optionally derivatised;

or (ii) Xaa1 and Xaa3 are cyclised to form a cyclic peptide;

or a salt thereof.

2. A peptide as claimed in claim 1, wherein Xaa1 is a Trp, Tyr, Arg or Phe residue, which residue is optionally derivatised.

3. A peptide as claimed in claim 1 or claim 2, wherein Xaa1 is derivatised by being acylated, alkylated, biotinylated, PEGylated or phosphorylated.

4. A peptide as claimed in claim 3, wherein Xaa1 is acylated at the N-terminus, optionally wherein Xaa1 is acylated at the N-terminus with an acyl group selected from acetyl, palmitoyl and myristoyl.

5. A peptide as claimed in any of claims 1 to 4, wherein Xaa2 is a Trp residue.

6. A peptide as claimed in any of claims 1 to 5, wherein Xaa3 is a Trp, Phe, Tyr, Arg, Leu or Val residue, which residue is optionally derivatised.

7. A peptide as claimed in any of claims 1 to 6, wherein Xaa3 is derivatised by being esterified, amidated, alkylated, biotinylated, PEGylated or phosphorylated.

8. A peptide as claimed in any of claims 1 to 6, wherein Xaa3 is not derivatised.

9. A peptide as claimed in any of claims 1 to 8, wherein the peptide is selected from the group consisting of:

Trp-Trp-Trp;
Trp-Trp-Phe;
Trp-Trp-Tyr;
Trp-Trp-Arg;
Tyr-Trp-Trp;
Arg-Trp-Tyr;
Phe-Trp-Tyr;
Phe-Trp-Phe;
Trp-Trp-Leu; and
Trp-Trp-Val;

wherein the N-terminal residue Xaa1 is optionally acylated;
or a salt thereof.

10. A peptide as claimed in any of claims 1-9, wherein the N-terminal residue is palmitoylated.

11. A peptide as claimed in claim 10, wherein the peptide is

N-palmitoyl-Trp-Trp-Phe; or
N-palmitoyl-Trp-Trp-Trp.

12. A pharmaceutical or cosmetic composition comprising i) a peptide as claimed in any of claims 1 to 11, or a salt thereof; and ii) a pharmaceutically and/or cosmetically acceptable excipient.

13. A pharmaceutical or cosmetic composition as claimed in claim 12, wherein the composition is formulated for topical administration.

14. A pharmaceutical or cosmetic composition as claimed in claim 12 or claim 13, wherein the composition comprises a further compound which inhibits bone morphogenetic protein (BMP) activity, optionally wherein the further compound is selected from the group consisting of a peptide which exhibits noggin-like activity, a peptide which inhibits a BMP, a Wnt promoter, and an anti-inflammatory agent.

15. A pharmaceutical or cosmetic composition as claimed in any of claims 12 to 14, wherein the composition comprises one or more of the following: a further anti-aging agent, a further anti-inflammatory agent, a sunscreen, an antioxidant, and a moisturizing agent.

16. A pharmaceutical or cosmetic composition as claimed in any of claims 12 to 15, wherein the composition comprises one or more of the following: an extracellular matrix enhancer, an autophagy enhancer, a stem cell enhancer, a skin exfoliator, a senolytic, a senomorphic, a DNA repair enhancer, an agent to reduce telomere attrition or promote telomere elongation, and an agent which promotes cellular adaptation to stress.

17. A pharmaceutical or cosmetic composition as claimed in any of claims 12 to 16, wherein the peptide of formula (I) or salt thereof is present in the composition in an amount of from 0.00001 to 1 wt. %, optionally from 0.0001 to 0.1 wt.%.

18. A composition as claimed in any of claims 12 to 17, wherein the composition is in the form of a cream, lotion, paste, wax, liquid, semisolid composition or a sprayable composition.

19. A method of inhibiting bone morphogenetic protein 2 (BMP-2) in a subject, comprising administering an effective amount of a peptide of formula (I) or a salt thereof as claimed in any of claims 1 to 11, or of a pharmaceutical or cosmetic composition as claimed in any of claims 12 to 18, to the subject.

20. Use of a peptide of formula (I) or a salt thereof as claimed in any of claims 1 to 11, or of a pharmaceutical or cosmetic composition as claimed in any of claims 12 to

18, for the manufacture of a medicament, for inhibiting bone morphogenetic protein 2 (BMP-2).

21. A peptide of formula (I) or a salt thereof as claimed in any of claims 1 to 11, or of a pharmaceutical or cosmetic composition as claimed in any of claims 12 to 18, for use in inhibiting bone morphogenetic protein 2 (BMP-2).

22. Use of a peptide of formula (I) or a salt thereof as claimed in any of claims 1 to 11, or of a pharmaceutical or cosmetic composition as claimed in any of claims 12 to 18, for inhibiting bone morphogenetic protein 2 (BMP-2).

23. A method of treating or preventing a disease or disorder associated with bone morphogenetic protein 2 (BMP-2) expression in a subject, comprising administering an effective amount of a peptide of formula (I) or a salt thereof as claimed in any of claims 1 to 11, or of a pharmaceutical or cosmetic composition as claimed in any of claims 12 to 18, to the subject.

24. Use of a peptide of formula (I) or a salt thereof as claimed in any of claims 1 to 11, or of a pharmaceutical or cosmetic composition as claimed in any of claims 12 to 18, for the manufacture of a medicament, for treating or preventing a disease or disorder associated with bone morphogenetic protein 2 (BMP-2) expression.

25. A peptide of formula (I) or a salt thereof as claimed in any of claims 1 to 11, or of a pharmaceutical or cosmetic composition as claimed in any of claims 12 to 18, for use in treating or preventing a disease or disorder associated with bone morphogenetic protein 2 (BMP-2) expression.

26. A cosmetic method of treating and/or preventing one or more of skin aging, wrinkles, fine lines sagged skin, hyperpigmented spots and uneven skin tone in a subject, comprising administering an effective amount of a peptide of formula (I) or a salt thereof as claimed in any of claims 1 to 11, or of a cosmetic composition as claimed in any of claims 12 to 18, to a subject in need thereof, optionally wherein the method is for treating

and/or preventing one or more of cosmetic skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone in a subject.

27. Use of a peptide of formula (I) or a salt thereof as claimed in any of claims 1 to 11, or of a cosmetic composition as claimed in any of claims 12 to 18, for the manufacture of a medicament for non-therapeutic treatment and/or prevention of one or more of skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone.

28. A peptide of formula (I) or a salt thereof as claimed in any of claims 1 to 11, or of a cosmetic composition as claimed in any of claims 12 to 18, for use in the non-therapeutic treatment and/or prevention of one or more of skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone.

29. Use of a peptide of formula (I) or a salt thereof as claimed in any of claims 1 to 11, or of a cosmetic composition as claimed in any of claims 12 to 18, for non-therapeutic treatment and/or prevention of one or more of cosmetic skin aging, wrinkles, fine lines, sagged skin, hyperpigmented spots and uneven skin tone.

30. A method, use, or peptide, salt or composition for use, as claimed in any of claims 19 to 29, wherein the composition is applied topically to the skin of the subject.