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(54) Title: NOVEL COMPOSITIONS AND USES THEREOF

(57) Abstract: The present invention provides a composition for stimulating hair growth in a mammal comprising a modified osteo-
pontin polypeptide in which an RGD domain is inactivated; and a pharmaceutically acceptable and/or cosmetically acceptable ex-
cipient, carrier or diluent. The invention further provides methods of stimulating hair growth in a mammal.



5

NOVEL COMPOSITIONS AND USES THEREOF**Field of Invention**

10 The present invention relates to agents for stimulating hair growth in mammals. In particular, there are provided compositions comprising a modified osteopontin polypeptide, and the pharmaceutical and cosmetic use thereof for stimulating hair growth in mammals (including humans).

15

Background

Hair growth is cyclical, occurring in three stages: anagen, the active growth phase; catagen, the degenerative phase; and telogen, the resting phase. After telogen, the old
20 hair fibre is shed and a new hair is generated as part of the repeating cycle.

Alopecia, or hair loss, occurs in both men and women, and is attributed to numerous causes including aging, hormone levels, stress, and chemotherapy. In these circumstances, more and more hair follicles remain in the telogen stage, resulting in a
25 gradual decrease of the hair fibre length and diameter, finally reaching a stage of partial or complete baldness.

Various types of hair loss are known, including alopecia areata, androgenetic alopecia, anagen effluvium, self-induced hair loss, telogen effluvium, and scarring alopecia.
30 Alopecia areata, thought to be an auto-immune disorder, begins with hair loss in a rounded patch on the scalp. Alopecia areata includes mild patchy hair loss on the scalp, as well the loss of all scalp hair, known as alopecia totalis, and the loss of all scalp and body hair, known as alopecia universalis. Androgenetic alopecia, including male and female pattern baldness, is thought to be caused by a combination of genetic
35 predisposition, aging, and androgen hormone levels. Androgenetic alopecia is associated with increased androgen stimulation, which adversely affects the hair follicles.

Increased androgen stimulation can be produced by, among other mechanisms, elevated levels of 5-alpha-reductase, an enzyme that converts testosterone to dihydrotestosterone. Anagen effluvium is hair loss due to chemicals or radiation, such as chemotherapy or radiation treatment for cancer. Self-induced hair loss includes hair loss
5 caused by conscious or unconscious self-inflicted damage to the hair. Two common types of self-induced hair loss are trichotillomania, or hair loss that results when someone continually pulls or plucks out his own hair, and traction alopecia, which is caused by hairstyles such as ponytails or braids that continually pull at the hair. Telogen effluvium is stress-related hair loss caused by events such as, for example, surgery, child
10 birth, or pregnancy termination. Further causes of telogen effluvium include the use of oral contraceptives or other prescription drugs, thyroid abnormality, diabetes, lupus, and emotional stress. Scarring alopecia includes hair loss caused by infection and inflammation of the hair follicles, and hair loss caused by burns or other trauma.

15 Hair loss is a widespread problem that is considered by some to be cosmetically unappealing and often causes emotional distress to the individual concerned. As a consequence, there is great demand for alopecia treatments. Many compositions have been tested for their ability to stimulate hair growth, for example, by promoting or prolonging anagen. Examples of such compositions include potassium channel openers,
20 such as minoxidil (Regaine RTM., Pharmacia Corp.) and diazoxide; 5-alpha-reductase inhibitors, such as finasteride (Propecia RTM., Merck & Co.); and the immunosuppressant cyclosporin A.

However, such known treatments for stimulating hair growth exhibit limited effectiveness
25 and cause unwanted side effects. For example, among other undesirable side effects, potassium channel openers cause cardiovascular effects, finasteride is unsafe for women who are pregnant or may become pregnant, and cyclosporin A suppresses the immune system. Further, even when applied topically to areas in which hair growth is desired, known treatments for alopecia often cause hair growth in undesired areas of the
30 body, such as facial hair on women. Such disadvantages of known compositions for treating alopecia lead many individuals experiencing hair loss to rely on wigs and toupees. Other individuals seek hair transplant surgery, which is expensive, is not fully effective, and sometimes is not possible, for example, for chemotherapy patients.

35 Accordingly, there is a need for new treatments to stimulate hair growth, suitable for use in both medical and cosmetic applications.

Summary of Invention

5 The first aspect of the invention provides a composition for stimulating hair growth in a mammal comprising:

(a) a modified osteopontin polypeptide in which an RGD domain is inactivated; and

10 (b) a pharmaceutically acceptable and/or cosmetically acceptable excipient, carrier or diluent.

Thus, the active component of the compositions of the invention is derived from a naturally-occurring osteopontin protein (*i.e.* the polypeptide comprises an amino acid
15 sequence corresponding to that of a modified, for example mutated, version of a naturally-occurring osteopontin protein).

Osteopontin, also known as bone sialoprotein I (BSP-1 or BNSP), early T-lymphocyte activation (ETA-1), secreted phosphoprotein 1 (SPP1), 2ar and Rickettsia resistance
20 (Ric), is a gene product which is conserved in several mammalian species.

The gene has seven exons, spans 5 kilobases in length and in humans it is located on the long arm of chromosome 4 region 13 (4q13). The protein is composed of ~300 amino acids residues and is rich in acidic residues: 30-36% are either aspartic or glutamic acid.
25 Osteopontin has ~30 attached carbohydrate residues, including 10 sialic acid residues, which are attached to the protein during post-translational modification in the Golgi apparatus.

Osteopontin was first discovered as a novel sialoprotein in bone anchoring osteoclasts
30 onto the mineralized bone matrix (Franzen & Heinegard, 1985, *Biochem. J.* **232**(3)715-24). The name *osteopontin* comes from the presence of the protein in bone (*osteo-*) and its ability to form a bridge (*-pons*) between bone cells and the mineral phase. Sequence analysis and subsequent structural studies showed osteopontin to be a 32 kDa glycoprotein composed of a highly acidic region of some ten aspartic acid residues
35 thought to mediate the mineral binding properties of osteopontin. Furthermore, in the mid portion of the osteopontin molecule there is also a cell attachment domain mediated

through an R-G-D sequence (Oldberg et al., 1986, *Proc. Natl. Acad. Sci. USA* **83**(23):8819-23).

Osteopontin is constitutively expressed in osteoblasts and in several epithelial cell types,
5 resulting in osteopontin being secreted into many body fluids. Bone is the only tissue
type where osteopontin is deposited and from where it can be recovered in large
amounts. The expression of osteopontin can also be induced in vascular smooth muscle
cells, in different cancer cell types and among inflammatory cells (specifically
macrophages and T lymphocytes). Several important cellular functions have been
10 attributed to osteopontin such as adhesion, proliferation, migration, anti-apoptosis and
chemo attraction. Some of these functions are believed to be mediated via the RGD cell-
adhesion domain which interacts with different integrins, mainly with $\alpha v \beta 3$ but also $\alpha v \beta 1$,
and $\alpha v \beta 5$ (for review see Scatena et al., 2007, *Arterio. Thromb. Vasc. Biol.* **27**:2302-
2309).

15 In recent years, osteopontin has emerged as a potent cytokine capable of modulating
several cell types involved in inflammation and immune responses. The broad range of
functions being attributed to osteopontin has been puzzling and cannot all be explained
by the single cell-binding RGD sequence. The explanation came when an eleven amino
20 acid peptide in osteopontin $R^{145}\text{-G-D-S-L-A-Y-G-L-R-S}^{155}$ (**SEQ ID NO:122**)
(corresponding to amino acids 144 to 154 of UniProt code P10923) was identified and
later functionally mapped. In addition to the known $R^{145}\text{-G-D}^{147}$ site mediating binding to
the $\alpha v \beta 3$ integrin, two additional essential regions in the osteopontin molecule were
discovered, namely a highly specific thrombin cleavage site, *i.e.* $R^{154}\text{-S}^{155}$, and a cryptic
25 integrin binding site, *i.e.* $S^{148}\text{-L-A-Y-G-L-R}^{154}$ (**SEQ ID NO:123**), which binds to an $\alpha 9 \beta 1$
integrin (see Scatena *et al.*, *supra*). An additional binding site for $\alpha 4 \beta 1$ has also been
identified (see Scatena *et al.*, *supra*).

30 A characterising feature of the osteopontin-derived polypeptide in the compositions of the
invention is that the RGD domain naturally present in osteopontin is inactivated such that
it is non-functional (at least in part). For example, inactivation of the RGD domain may
prevent the osteopontin-derived polypeptide from binding to one or more of the integrins
which bind the naturally occurring osteopontin protein.

35 Thus, by "modified osteopontin polypeptide" we include polypeptides corresponding to a
modified form of a naturally-occurring osteopontin protein in which the RGD domain is

non-functional (at least in part), as well as fragments and variants thereof which retain a hair-stimulatory property of the 'full length' modified osteopontin. For example, the non-functional RGD domain of the modified osteopontin polypeptide may be unable to bind integrin $\alpha\text{v}\beta 3$ (see Scatena *et al.*, *supra*).

5

Advantageously, the naturally-occurring osteopontin protein is mammalian, e.g. human.

The modified osteopontin polypeptides present in the compositions of the invention are capable of stimulating hair growth in mammals.

10

In one embodiment, the polypeptide is capable of stimulating the growth of human hair.

In a further embodiment, the polypeptide is capable of stimulating the growth of hair *in vivo*.

15

In a further embodiment, the polypeptide is capable of stimulating the growth of hair *in vivo* with greater efficacy than wildtype human osteopontin. By "greater efficacy" in this context, we include a quicker onset of action and/or efficacy at a lower dose and/or greater maximum effect (e.g. greater number of new follicles or density of hair). In one embodiment, the polypeptide is capable of stimulating the growth of hair *in vivo* with a quicker onset of action and a greater maximum hair growth effect than wildtype human osteopontin at the same dose (e.g. see Example B).

20

It will be appreciated by persons skilled in the art that the stimulation of hair growth may be mediated by an effect of existing hair follicles and/or by inducing the formation of new hair follicles.

25

Thus, in one embodiment, the modified osteopontin polypeptide is capable of stimulating existing hair follicles (for example, by prolonging the anagen phase and/or by shortening the telogen phase such that the resting follicles become active).

30

In a further embodiment, the polypeptide is capable of inducing the formation of new hair follicles, or stem cells for producing the same.

Naturally-occurring osteopontin proteins are typically around 300 amino acids in length. However, it will be appreciated by persons skilled in the art that the modified osteopontin polypeptides present in the compositions of the invention may vary from this length.

- 5 Typically, the polypeptide is fewer than 500 amino acids in length, for example fewer than 400, 350, 340, 330, 320, 310, 300, 290, 280, 270, 260, 250, 200, 150, 100, 50, 40, 30, 20, 15, 10 or fewer amino acids in length.

- 10 In one embodiment, the polypeptide is between 250 and 350 amino acids in length, for example between 280 and 300 amino acids in length (e.g. 293 amino acids in length).

- In an alternative embodiment, the polypeptide is between 10 and 20 amino acids in length, for example between 12 and 18 amino acids in length (e.g. 15 amino acids in length).

- 15 As discussed above, the modified osteopontin polypeptides lack the active tri-peptide sequence "arginine-glycine-aspartic acid" normally found in naturally-occurring osteopontin proteins. It will be appreciated that this RGD domain may be inactivated by a number of different strategies.

- 20 In one embodiment, the RGD domain is mutated at one or more amino acids in the modified osteopontin polypeptide, such that it contains one or more deletions, substitutions and/or additions, or combinations thereof, relative to a naturally-occurring osteopontin protein.

- 25 For example, the RGD domain may be deleted, at least in part, such that the arginine and/or glycine and/or aspartic acid residue is absent.

- 30 Alternatively, or in addition, the RGD domain may be substituted at one or more amino acids. For example, the arginine and/or glycine and/or aspartic acid residue may be substituted with another amino acid. Such substitutions may be conservative or non-conservative.

- 35 Likewise, the RGD domain may be inactivated by a combination of substitutions and deletions, including:

- (a) substitution of the arginine residue and deletion of the glycine and aspartic acid residues;
- (b) substitution of the arginine and glycine residues and deletion of the aspartic acid residue (for example, the -RGD- tripeptide can be replaced with the dipeptide sequence -DI-);
- (c) substitution of the arginine and aspartic acid residues and deletion of the glycine residue; or
- (d) deletion of the arginine and aspartic acid residues and substitution of the glycine residue.

In one embodiment, the tripeptide -RGD- sequence is replaced by the dipeptide -DI- sequence.

Without wishing to be bound by theory, it is believed that inactivation of the RGD sequence may result in a conformational change in the osteopontin polypeptide (relative to the corresponding naturally-occurring osteopontin protein), which leads to the creation/exposure of a new site to the surrounding milieu.

In one preferred embodiment, the modified osteopontin polypeptide comprises or consists of the amino acid sequence of SEQ ID NO:1 (in which the region encompassing the inactivated RGD domain is underlined):

MRLAVICFCLFGIASSLPVKVTDSGSSEEKLYSLHPDPIATWLVPDPSQKQNLLAPQNAVSSEEKDDF
 KQETLPNSNESHDMDDDDDDDDGDHAESEDSVDSDESHHSDSEDETVTASTQADTFPTIVP
 TVDVPNGDISLAYGLRSKRSFQVSDEQYPDATDEDLTSHMKSGESKESLDVIPVAQLLSMPDQDNN
 GKGSHESSQLDEPSLETHRLEHSKESQESADQSDVIDSQASSKASLEHQSHKFHSHKDKLVLDPKSKE
 DDRYLKFRISHELESSSSEVN

SEQ ID NO: 1

or a fragment, variant, derivative or fusion thereof (or a fusion of said fragment, variant or derivative) which retains (at least in part) a hair growth stimulatory activity of the amino acid sequence of SEQ ID NO:1.

The term 'amino acid' as used herein includes the standard twenty genetically-encoded amino acids and their corresponding stereoisomers in the 'D' form (as compared to the natural 'L' form), omega-amino acids and other naturally-occurring amino acids,

unconventional amino acids (e.g., α,α -disubstituted amino acids, N-alkyl amino acids, etc.) and chemically derivatised amino acids (see below).

When an amino acid is being specifically enumerated, such as 'alanine' or 'Ala' or 'A', the term refers to both L-alanine and D-alanine unless explicitly stated otherwise. Other unconventional amino acids may also be suitable components for polypeptides of the present invention, as long as the desired functional property is retained by the polypeptide. For the polypeptides shown, each encoded amino acid residue, where appropriate, is represented by a single letter designation, corresponding to the trivial name of the conventional amino acid.

In accordance with convention, the amino acid sequences disclosed herein are provided in the N-terminus to C-terminus direction.

Typically, the modified osteopontin polypeptides used in the compositions of the invention comprise or consist of L-amino acids.

In one embodiment, the polypeptide may comprise or consist of a fragment of the amino acid sequence of SEQ ID NO: 1, or a variant thereof.

By "fragment" we include at least 6 contiguous amino acids of the amino acid sequence of SEQ ID NO: 1, for example at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 100, 150, 200, 210, 220, 230, 240, 250, 255, 260, 265, 270, 275, 280, 285, 286, 287, 288, 289 290, 291 or 292 contiguous amino acids of SEQ ID NO: 1.

Advantageously, the fragment comprises or consists of the inactivated RGD domain sequence of SEQ ID NO:2, namely:

DISLAYGLRS

SEQ ID NO: 2

For example, the fragment may comprise or consist of an amino acid sequence according to any one SEQ ID NOs: 5 to 62.

In one particular embodiment, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 5.

VDVPNGDISLAYGLR

SEQ ID NO: 5

["FOL-004"]

5

In a further embodiment, the polypeptide comprises or consists of a variant of the amino acid sequence of SEQ ID NO: 1, or of a fragment thereof.

By "variant" we mean that the polypeptide does not share 100% amino acid sequence identity with SEQ ID NO: 1, *i.e.* one or more amino acids of SEQ ID NO: 1 must be mutated. For example, the polypeptide may comprise or consist of an amino acid sequence with at least 50% identity to the amino acid sequence of SEQ ID NO: 1, more preferably at least 60%, 70% or 80% or 85% or 90% identity to said sequence, and most preferably at least 95%, 96%, 97%, 98% or 99% identity to said amino acid sequence.

15

Percent identity can be determined by methods well known in the art, for example using the LALIGN program (Huang and Miller, *Adv. Appl. Math.* (1991) 12:337-357) at the Expasy facility site

20

(http://www.ch.embnet.org/software/LALIGN_form.html)

using as parameters the global alignment option, scoring matrix BLOSUM62, opening gap penalty -14, extending gap penalty -4.

25

Alternatively, the percent sequence identity between two polypeptides may be determined using suitable computer programs, for example the GAP program of the University of Wisconsin Genetic Computing Group and it will be appreciated that percent identity is calculated in relation to polypeptides whose sequence has been aligned optimally.

30

By "mutated" we mean that the amino acid at the specified position is altered compared to the amino acid in the polypeptide according to SEQ ID NO: 1. For example, an amino acid at a specified position may be deleted, substituted or may be the site of an insertion/addition of one or more amino acids. It will be appreciated by persons skilled in the art that the substitutions may be conservative or non-conservative.

35

Alternatively, or in addition, the amino acid at a specified position may be chemically modified (derivatised); see below.

5 In one embodiment, the variant polypeptide comprises or consists of an amino acid sequence of SEQ ID NO: 1, or a fragment thereof, in which one or more amino acids is conservatively substituted. By "conservatively substituted" we mean a substitution of one amino acid with another with similar properties (size, hydrophobicity, *etc*), such that the function of the polypeptide is not significantly altered. Thus, by "conservative substitutions" is intended combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln;
10 Ser, Thr; Lys, Arg; and Phe, Tyr.

Advantageously, the variant comprises or consists of the inactivated RGD domain sequence of SEQ ID NO:2, namely:

15 DISLAYGLRS **SEQ ID NO: 2**

The variant polypeptide may also comprise one or more additional amino acids, inserted at the N- and/or C-terminus and/or internally within the amino acid sequence of SEQ ID NO:1. For example, the polypeptide may comprise or consist of at least 2, 3, 4, 5, 6, 7,
20 8, 9, 10, 15 or 20 additional amino acids at the N- and/or C-terminus and/or internally.

Advantageously, the variant polypeptide is non-naturally occurring.

In one preferred embodiment, the modified osteopontin polypeptide is a species
25 homologue of the amino acid sequence of SEQ ID NO:1.

Thus, the polypeptide may comprise or consist of the amino acid sequence of a modified human osteopontin protein, or fragment thereof, in which an RGD domain is inactivated. Suitable human osteopontin protein sequences are well known in the art, for example
30 see Database Accession Nos. AAA59974.1, AAC28619.1, AAA86886.1.

For example, the modified osteopontin polypeptide may comprise or consist of the amino acid sequence of SEQ ID NO:3 (in which the region encompassing the inactivated RGD domain is underlined)

5 MRIAVICFCLLGITCAIPVKQADSGSSEEKQLYNKYPDAVATWLNPDPSQKQNLAPQTLPSKSNESH
DHMDMDDEDDDDHVDSQDSIDSNDSDDVDDTDDSHQSDESHHSDESDELVTDFPTDLPATEVFTPVV
PTVDTYDGDISVVYGLRSKSKKFRRPDIQYPDATDEEDITSHMESEELNGAYKAIPVAQDLNAPSDWDS
RGKDSYETSQ~~LD~~DQSAETHSHKQSRLYKRKANDESNEHSDVIDSQELSKVSREFHSHEFHSHEDMLVV
DPKSKEEDKHLKFRISHELDSASSEVN

10 SEQ ID NO: 3

or a fragment, variant, derivative or fusion thereof (or a fusion of said fragment, variant or derivative) which retains the hair growth stimulatory activity of the amino acid sequence of SEQ ID NO:3.

15

Advantageously, the fragment or variant comprises or consists of the inactivated RGD domain sequence of SEQ ID NO:4, namely:

DISVYGLRS

SEQ ID NO: 4

20

For example, the fragment or variant may comprise or consist of an amino acid sequence according to any one SEQ ID NOs: 63 to 120 (as detailed below).

In one particular embodiment, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 63.

25

VDTYDGDISVYGLR

SEQ ID NO: 63

["FOL-005"]

In a further embodiment, the modified osteopontin polypeptide comprises or consists of a derivative of the amino acid sequence of SEQ ID NO: 1 or 3, or of a fragment, variant or fusion thereof.

Advantageously, the derivative comprises the inactivated RGD domain sequence of SEQ ID NO:3 or 4.

- 5 For example, the polypeptide may comprise or consist of a derivative of the amino acid sequence of SEQ ID NO: 5 or 63

Chemical derivatives of one or more amino acids may be achieved by reaction with a functional side group. Such derivatised molecules include, for example, those molecules
10 in which free amino groups have been derivatised to form amine hydrochlorides, *p*-toluene sulphonyl groups, carboxybenzoxy groups, *t*-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatised to form salts, methyl and ethyl esters or other types of esters and hydrazides. Free hydroxyl groups may be derivatised to form O-acyl or O-alkyl derivatives. Also included as
15 chemical derivatives are those peptides which contain naturally occurring amino acid derivatives of the twenty standard amino acids. For example: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine and ornithine for lysine. Derivatives also include peptides containing one or more additions or deletions
20 as long as the requisite activity is maintained. Other included modifications are amidation, amino terminal acylation (e.g. acetylation or thioglycolic acid amidation), terminal carboxylamidation (e.g. with ammonia or methylamine), and the like terminal modifications.

- 25 It will be further appreciated by persons skilled in the art that peptidomimetic compounds may also be useful, which mimic the conformation and desirable features of the modified osteopontin polypeptides detailed above. Thus, by 'polypeptide' we include peptidomimetic compounds which have the hair growth stimulatory activity of the polypeptide of SEQ ID NO: 1.

30

- For example, the polypeptides of the invention include not only molecules in which amino acid residues are joined by peptide (-CO-NH-) linkages but also molecules in which the peptide bond is reversed. Such retro-inverso peptidomimetics may be made using methods known in the art, for example such as those described in Meziere *et al.* (1997)
35 *J. Immunol.* **159**, 3230-3237, which is incorporated herein by reference. This approach involves making pseudopeptides containing changes involving the backbone, and not the

orientation of side chains. Retro-inverse peptides, which contain NH-CO bonds instead of CO-NH peptide bonds, are much more resistant to proteolysis. Alternatively, the polypeptide of the invention may be a peptidomimetic compound wherein one or more of the amino acid residues are linked by a $-y(\text{CH}_2\text{NH})-$ bond in place of the conventional amide linkage.

In a further alternative, the peptide bond may be dispensed with altogether provided that an appropriate linker moiety which retains the spacing between the carbon atoms of the amino acid residues is used; it may be advantageous for the linker moiety to have substantially the same charge distribution and substantially the same planarity as a peptide bond.

It will be appreciated that the polypeptide may conveniently be blocked at its N- or C-terminus so as to help reduce susceptibility to exoproteolytic digestion.

A variety of uncoded or modified amino acids such as D-amino acids and N-methyl amino acids have also been used to modify mammalian polypeptides. In addition, a presumed bioactive conformation may be stabilised by a covalent modification, such as cyclisation or by incorporation of lactam or other types of bridges, for example see Veber *et al.*, 1978, *Proc. Natl. Acad. Sci. USA* **75**:2636 and Thursell *et al.*, 1983, *Biochem. Biophys. Res. Comm.* **111**:166, which are incorporated herein by reference.

A common theme among many of the synthetic strategies has been the introduction of some cyclic moiety into a peptide-based framework. The cyclic moiety restricts the conformational space of the peptide structure and this frequently results in an increased specificity of the peptide for a particular biological receptor. An added advantage of this strategy is that the introduction of a cyclic moiety into a peptide may also result in the peptide having a diminished sensitivity to cellular peptidases.

Thus, exemplary polypeptides of the invention comprise terminal cysteine amino acids. Such a polypeptide may exist in a heterodetic cyclic form by disulphide bond formation of the mercaptide groups in the terminal cysteine amino acids or in a homodetic form by amide peptide bond formation between the terminal amino acids. As indicated above, cyclising small peptides through disulphide or amide bonds between the N- and C-terminal region cysteines may circumvent problems of specificity and half-life sometime observed with linear peptides, by decreasing proteolysis and also increasing the rigidity

of the structure, which may yield higher specificity compounds. Polypeptides cyclised by disulphide bonds have free amino and carboxy-termini which still may be susceptible to proteolytic degradation, while peptides cyclised by formation of an amide bond between the N-terminal amine and C-terminal carboxyl and hence no longer contain free amino or carboxy termini. Thus, the peptides of the present invention can be linked either by a C-N linkage or a disulphide linkage.

The present invention is not limited in any way by the method of cyclisation of peptides, but encompasses peptides whose cyclic structure may be achieved by any suitable method of synthesis. Thus, heterodetic linkages may include, but are not limited to formation via disulphide, alkylene or sulphide bridges. Methods of synthesis of cyclic homodetic peptides and cyclic heterodetic peptides, including disulphide, sulphide and alkylene bridges, are disclosed in US 5,643,872, which is incorporated herein by reference. Other examples of cyclisation methods includes cyclization through click chemistry, epoxides, aldehyde-amine reactions, as well as and the methods disclosed in US 6,008,058, which is incorporated herein by reference.

Such terminal modifications are useful, as is well known, to reduce susceptibility by proteinase digestion and therefore to prolong the half-life of the peptides in solutions, particularly in biological fluids where proteases may be present. Polypeptide cyclisation is also a useful modification because of the stable structures formed by cyclisation and in view of the biological activities observed for cyclic peptides.

Thus, in one embodiment the polypeptide of the first aspect of the invention is cyclic.

However, in an alternative embodiment, the polypeptide is linear.

In one preferred embodiment, however, the modified osteopontin polypeptide comprises one or more amino acids modified or derivatised by PEGylation, amidation, esterification, acylation, acetylation and/or alkylation.

Persons skilled in the art will appreciate that the modified osteopontin polypeptide may be glycosylated at one or more amino acids. For example, the polypeptide may retain one or more of the glycosylation sites of the corresponding ('parent') osteopontin protein,
5 to which may be attached a carbohydrate group.

In a further embodiment, the modified osteopontin polypeptide comprises or consists of a fusion of the amino acid sequence of SEQ ID NO: 1 or 3, or of a fragment or variant thereof.

10

For example, the polypeptide may comprise a fusion of the amino acid sequence of SEQ ID NO: 5 or 63

By 'fusion' of a polypeptide we include an amino acid sequence corresponding to SEQ ID
15 NO: 1 or 3 (or a fragment or variant thereof) fused to any other polypeptide. For example, the said polypeptide may be fused to a polypeptide such as glutathione-S-transferase (GST) or protein A in order to facilitate purification of said polypeptide. Examples of such fusions are well known to those skilled in the art. Similarly, the said polypeptide may be fused to an oligo-histidine tag such as His6 or to an epitope
20 recognised by an antibody such as the well-known Myc tag epitope. Fusions to any variant or derivative of said polypeptide are also included in the scope of the invention.

The fusion may comprise a further portion which confers a desirable feature on the said polypeptide of the invention; for example, the portion may be useful in augmenting or
25 prolonging the hair growth stimulatory effect. For example, in one embodiment the fusion comprises human serum albumin or similar protein (as disclosed in US 2009/0005312, the disclosures of which are incorporated herein by reference).

Alternatively, the fused portion may be a lipophilic molecule or polypeptide domain that is
30 capable of promoting cellular uptake of the polypeptide, as known to those skilled in the art.

In a further embodiment, the modified osteopontin polypeptide comprises or consists of tandem repeats of an amino acid sequence. For example, the polypeptide may comprise
35 or consist of tandem repeats of SEQ ID NO: 2 and/or 4 (or combinations thereof), which

may be directly adjacent each other or separated by one or more intervening amino acids.

Alternatively, the polypeptide may comprise or consist of tandem repeats of the amino acid sequence of SEQ ID NO: 5 or 63.

In one preferred embodiment, however, the modified osteopontin polypeptide simply comprises or consists of the amino acid sequence of SEQ ID NO: 1 or 3. For example, the polypeptide may consist of the polypeptide according to SEQ ID NO: 1 or 3.

In a further preferred embodiment, the modified osteopontin polypeptide simply comprises or consists of the amino acid sequence of SEQ ID NO: 5 or 63. For example, the polypeptide may consist of the polypeptide according to SEQ ID NO: 5 or 63.

Modified osteopontin polypeptides suitable for use in the compositions of the invention may be made by *in vitro* cell-based expression methods well known to persons skilled in the art (for example, see Sambrook & Russell, 2000, *Molecular Cloning, A Laboratory Manual*, Third Edition, Cold Spring Harbor, New York, which is incorporated herein by reference). The choice of expression vector and host cell to be used may depend on a number of factors. For example, if the modified osteopontin polypeptide is to be glycosylated, a mammalian expression system will be required.

Suitable expression vectors and host cells are commercially available from many sources.

Alternatively, the modified osteopontin polypeptides may be synthesised by known means, such as liquid phase and solid phase synthesis (for example, t-Boc solid-phase peptide synthesis and BOP-SPPS).

It will be appreciated by persons skilled in the art that the present invention also includes pharmaceutically and/or cosmetically acceptable acid or base addition salts of the above described modified osteopontin polypeptides. The acids which are used to prepare the pharmaceutically and/or cosmetically acceptable acid addition salts of the aforementioned base compounds useful in this invention are those which form non-toxic acid addition salts, *i.e.* salts containing pharmaceutically and/or cosmetically acceptable anions, such as the hydrochloride, hydrobromide, hydroiodide, nitrate, sulphate,

bisulphate, phosphate, acid phosphate, acetate, lactate, citrate, acid citrate, tartrate, bitartrate, succinate, maleate, fumarate, gluconate, saccharate, benzoate, methanesulphonate, ethanesulphonate, benzenesulphonate, p-toluenesulphonate and pamoate [i.e. 1,1'-methylene-bis-(2-hydroxy-3 naphthoate)] salts, among others.

5

Pharmaceutically and/or cosmetically acceptable base addition salts may also be used to produce pharmaceutically and/or cosmetically acceptable salt forms of the modified osteopontin polypeptides. The chemical bases that may be used as reagents to prepare pharmaceutically and/or cosmetically acceptable base salts of the present compounds
10 that are acidic in nature are those that form non-toxic base salts with such compounds. Such non-toxic base salts include, but are not limited to those derived from such pharmaceutically and/or cosmetically acceptable cations such as alkali metal cations (e.g. potassium and sodium) and alkaline earth metal cations (e.g. calcium and magnesium), ammonium or water-soluble amine addition salts such as N-
15 methylglucamine-(meglumine), and the lower alkanolammonium and other base salts of pharmaceutically acceptable organic amines, among others.

It will be further appreciated that the modified osteopontin polypeptides of the invention may be lyophilised for storage and reconstituted in a suitable carrier prior to use. Any
20 suitable lyophilisation method (e.g. spray drying, cake drying) and/or reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilisation and reconstitution can lead to varying degrees of activity loss and that use levels may have to be adjusted upward to compensate. Preferably, the lyophilised (freeze dried) polypeptide loses no more than about 20%, or no more than about 25%, or
25 no more than about 30%, or no more than about 35%, or no more than about 40%, or no more than about 45%, or no more than about 50% of its activity (prior to lyophilisation) when rehydrated.

The modified osteopontin polypeptides are provided in the form of a composition
30 comprising the polypeptide and a pharmaceutically acceptable and/or cosmetically acceptable excipient, carrier or diluent, selected with regard to the intended route of administration and standard pharmaceutical or cosmetic practice (for example, see *Remington: The Science and Practice of Pharmacy*, 19th edition, 1995, Ed. Alfonso Gennaro, Mack Publishing Company, Pennsylvania, USA, which is incorporated herein
35 by reference).

By "pharmaceutically acceptable" is included that the formulation is sterile and pyrogen free. Suitable pharmaceutical carriers are well known in the art of pharmacy. The carrier(s) must be "acceptable" in the sense of being compatible with the compound of the invention and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free; however, other acceptable carriers may be used. Thus, "pharmaceutically acceptable carrier" and "pharmaceutically acceptable excipient" includes any compound(s) used in forming a part of the formulation that is intended to act merely as a carrier, *i.e.*, not intended to have biological activity itself. The pharmaceutically acceptable carrier or excipient is generally safe, non-toxic, and neither biologically nor otherwise undesirable. A pharmaceutically acceptable carrier or excipient as used herein includes both one and more than one such carrier or excipient.

Likewise, the term "cosmetically acceptable" is used to denote formulations suitable for use as cosmetic products. Suitable cosmetic carriers are well known in the art, such as those commonly used in shampoos, lotions, creams and other such products.

The excipient may be one or more of carbohydrates, polymers, lipids and minerals. Examples of carbohydrates include lactose, sucrose, mannitol, and cyclodextrines, which are added to the composition, *e.g.*, for facilitating lyophilisation. Examples of polymers are starch, cellulose ethers, cellulose carboxymethylcellulose, hydroxypropylmethyl cellulose, hydroxyethyl cellulose, ethylhydroxyethyl cellulose, alginates, carageenans, hyaluronic acid and derivatives thereof, polyacrylic acid, polysulphonate, polyethyleneglycol/polyethylene oxide, polyethyleneoxide/polypropylene oxide copolymers, polyvinylalcohol/polyvinylacetate of different degree of hydrolysis, and polyvinylpyrrolidone, all of different molecular weight, which are added to the composition, *e.g.*, for viscosity control, for achieving bioadhesion, or for protecting the lipid from chemical and proteolytic degradation. Examples of lipids are fatty acids, phospholipids, mono-, di-, and triglycerides, ceramides, sphingolipids and glycolipids, all of different acyl chain length and saturation, egg lecithin, soy lecithin, hydrogenated egg and soy lecithin, which are added to the composition for reasons similar to those for polymers. Examples of minerals are talc, magnesium oxide, zinc oxide and titanium oxide, which are added to the composition to obtain benefits such as reduction of liquid accumulation or advantageous pigment properties.

The term "diluent" is intended to mean an aqueous or non-aqueous solution with the purpose of diluting the peptide in the pharmaceutical preparation. The diluent may be

one or more of saline, water, polyethylene glycol, propylene glycol, ethanol or oils (such as safflower oil, corn oil, peanut oil, cottonseed oil or sesame oil).

The diluent may also function as a buffer. The term "buffer" is intended to mean an aqueous solution containing an acid-base mixture with the purpose of stabilising pH. Examples of buffers are Trizma, Bicine, Tricine, MOPS, MOPSO, MOBS, Tris, Hepes, HEPBS, MES, phosphate, carbonate, acetate, citrate, glycolate, lactate, borate, ACES, ADA, tartrate, AMP, AMPD, AMPSO, BES, CABS, cacodylate, CHES, DIPSO, EPPS, ethanolamine, glycine, HEPPSO, imidazole, imidazolelactic acid, PIPES, SSC, SSPE, POPSO, TAPS, TABS, TAPSO and TES.

Optionally, the composition may comprise an adjuvant. The term "adjuvant" is intended to mean any compound added to the formulation to increase the biological effect of the peptide. The adjuvant may be one or more of zinc, copper or silver salts with different anions, for example, but not limited to fluoride, chloride, bromide, iodide, thiocyanate, sulfite, hydroxide, phosphate, carbonate, lactate, glycolate, citrate, borate, tartrate, and acetates of different acyl composition.

The pharmaceutical compositions of the invention may also be in the form of biodegradable microspheres. Aliphatic polyesters, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), copolymers of PLA and PGA (PLGA) or poly(ϵ -caprolactone) (PCL), and polyanhydrides have been widely used as biodegradable polymers in the production of microspheres. Preparations of such microspheres can be found in US 5,851,451 and in EP0213303.

The pharmaceutical compositions of the invention may also be in the form of polymer gels, where polymers such as starch, cellulose ethers, cellulose carboxymethylcellulose, hydroxypropylmethyl cellulose, hydroxyethyl cellulose, ethylhydroxyethyl cellulose, alginates, chondroitinans, hyaluronic acid and derivatives thereof, polyacrylic acid, polysulphonate,

The modified osteopontin polypeptides may be formulated at various concentrations, depending on the efficacy/toxicity of the particular polypeptide being used. Preferably, the composition comprises the modified osteopontin polypeptide at a concentration of between 1 nM and 1 M, for example between 0.1 μ M and 1 mM, 1 μ M and 100 μ M, between 5 μ M and 50 μ M, between 10 μ M and 50 μ M, between 20 μ M and 40 μ M and optionally about 30 μ M. For *ex vivo* and *in vitro* applications, compositions may comprise

a lower concentration of a modified osteopontin polypeptide, for example between 0.0025 μM and 1 μM .

5 It will be appreciated by persons skilled in the art that the compositions of the invention may be administered by a variety of routes, for example topical, transdermal, parenteral or oral administration.

Advantageously, the compositions of the invention are suitable for topical administration or intracutaneous administration.

10

Thus, the compositions of the invention may be administered topically to the skin (e.g. scalp). For example, the composition may be provided in the form of an ointment containing the active polypeptide suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. 15 Alternatively, the polypeptide can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

20

Optionally, the composition for topical administration may comprise a penetration enhancer (for example, as described in Osborne & Henke, 1997, *Pharmaceutical Technology*, November: 58-82 and Pathan & Setty, 2009, *Tropical Journal of Pharmaceutical Research* 8 (2): 173-179, the disclosures of which are incorporated 25 herein by reference).

Alternatively, the compositions of the invention may be administered parenterally, for example intracutaneously. Such compositions are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or 30 glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

35 Compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and

solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

It may be beneficial to use a sustained-release system, such as microsphere formulations, for delivering the modified osteopontin polypeptides.

Alternatively, compositions can be administered by a surgically implanted device that releases the active polypeptide directly to the required site (*i.e.* the epidermis).

The compositions of the invention may also be delivered by transdermal methodologies.

For example, electroporation therapy (EPT) and/or iontophoresis systems can be employed for the administration of proteins and polypeptides. In such methods, a device is used to deliver a pulsed electric field to cells, resulting in the increased permeability of the cell membranes to the drug and significant enhancement of intracellular drug delivery.

An alternative transdermal method, electroincorporation, utilises small particles of up to 30 microns in diameter on the surface of the skin experience electrical pulses identical or similar to those used in electroporation. The particles are driven through the stratum corneum and into deeper layers of the skin. The particles can be loaded or coated with drugs or genes or can simply act as "bullets" that generate pores in the skin through which the drugs can enter.

Additional transdermal methodologies have also been developed by PowderJect Pharmaceuticals (now owned by Novartis AG).

Suitable methods for administration of the polypeptides and compositions of the invention are well known in the art, for example, see *Therapeutic Protein and Peptide*

Formulation and Delivery, Zahra Shahrokh *et al.* (Eds), 1997, American Chemical Society, ISBN13: 9780841235281.

A second aspect of the invention provides a polypeptide comprising or consisting of the amino acid sequence of SEQ ID NO: 63 or a fragment, variant, derivative or fusion thereof (or a fusion of said fragment, variant or derivative) which retains a hair growth stimulatory activity of the amino acid sequence of SEQ ID NO: 63.

In one embodiment, the polypeptide is isolated (e.g. outside the mammalian body).

10

The terms "fragment", "variant", "derivative" or "fusion" are as defined above in relation to the first aspect of the invention.

In a related aspect, the polypeptide is a fragment of human osteopontin comprising an RGD domain which is inactivated (by substitution or deletion of one or more of the amino acids of the RGD domain and/or by addition of one or more amino acids within the RGD domain). For example, the three amino acids of the "R-G-D" domain of the wildtype human osteopontin protein may be replaced by two amino acids, "D-I". Preferably, the fragment is between five and thirty amino acids in length, for example between ten and twenty amino acids in length.

For example, the polypeptide may comprise or consist of an amino acid sequence of any one of SEQ ID NOs: 5 to 62, or fragment, variant, derivative or fusion thereof.

25

Preferred 15-amino acid peptides

VDVPNGDISLAYGLR	["FOL-004"]	SEQ ID NO: 5
DVPNGDISLAYGLRS		SEQ ID NO: 6

30

Preferred 14-amino acid peptides:

VDVPNGDISLAYGL	SEQ ID NO: 7
DVPNGDISLAYGLR	SEQ ID NO: 8
VPNGDISLAYGLRS	SEQ ID NO: 9

35

Preferred 13-amino acid peptides:

VDVPNGDISLAYG	SEQ ID NO: 10
DVPNGDISLAYGL	SEQ ID NO: 11
VPNGDISLAYGLR	SEQ ID NO: 12
PNGDISLAYGLRS	SEQ ID NO: 13

40

Preferred 12-amino acid peptides:

5	VDVPNGDISLAY	SEQ ID NO: 14
	DVPNGDISLAYG	SEQ ID NO: 15
	VPNGDISLAYGL	SEQ ID NO: 16
	PNGDISLAYGLR	SEQ ID NO: 17
	NGDISLAYGLRS	SEQ ID NO: 18

10 Preferred 11-amino acid peptides:

	VDVPNGDISLA	SEQ ID NO: 19
	DVPNGDISLAY	SEQ ID NO: 20
	VPNGDISLAYG	SEQ ID NO: 21
15	PNGDISLAYGL	SEQ ID NO: 22
	NGDISLAYGLR	SEQ ID NO: 23
	GDISLAYGLRS	SEQ ID NO: 24

Preferred 10-amino acid peptides:

20	VDVPNGDISL	SEQ ID NO: 25
	DVPNGDISLA	SEQ ID NO: 26
	VPNGDISLAY	SEQ ID NO: 27
	PNGDISLAYG	SEQ ID NO: 28
25	NGDISLAYGL	SEQ ID NO: 29
	GDISLAYGLR	SEQ ID NO: 30
	DISLAYGLRS	SEQ ID NO: 31

Preferred 9-amino acid peptides:

30	VDVPNGDIS	SEQ ID NO: 32
	DVPNGDISL	SEQ ID NO: 33
	VPNGDISLA	SEQ ID NO: 34
	PNGDISLAY	SEQ ID NO: 35
35	NGDISLAYG	SEQ ID NO: 36
	GDISLAYGL	SEQ ID NO: 37
	DISLAYGLR	SEQ ID NO: 38
	ISLAYGLRS	SEQ ID NO: 39

40 Preferred 8-amino acid peptides:

	VDVPNGDI	SEQ ID NO: 40
	DVPNGDIS	SEQ ID NO: 41
	VPNGDISL	SEQ ID NO: 42
45	PNGDISLA	SEQ ID NO: 43
	NGDISLAY	SEQ ID NO: 44
	GDISLAYG	SEQ ID NO: 45
	DISLAYGL	SEQ ID NO: 46
	ISLAYGLR	SEQ ID NO: 47

50

Preferred 7-amino acid peptides:

	VDVPNGD	SEQ ID NO: 48
	DVPNGDI	SEQ ID NO: 49

	VPNGDIS	SEQ ID NO: 50
	PNGDISL	SEQ ID NO: 51
	NGDISLA	SEQ ID NO: 52
	GDISLAY	SEQ ID NO: 53
5	DISLAYG	SEQ ID NO: 54
	ISLAYGL	SEQ ID NO: 55

Preferred 6-amino acid peptides:

10	DVPNGD	SEQ ID NO: 56
	VPNGDI	SEQ ID NO: 57
	PNGDIS	SEQ ID NO: 58
	NGDISL	SEQ ID NO: 59
	GDISLA	SEQ ID NO: 60
15	DISLAY	SEQ ID NO: 61
	ISLAYG	SEQ ID NO: 62

In a preferred embodiment, the polypeptide comprises or consists of an amino acid sequence of SEQ ID NO: 5.

20

In an alternative embodiment, the polypeptide comprises or consists of a variant of an amino acid sequence of SEQ ID NO: 5.

For example, the polypeptide may comprise or consist of an amino acid sequence of any one of SEQ ID NOs: 63 to 120, or a fragment, variant, derivative or fusion thereof.

25

Preferred 15-amino acid peptides:

30	VDTYDGDISVVYGLR	["FOL-005"]	SEQ ID NO: 63
	VDTYDGDISVVYGLS		SEQ ID NO: 64

Preferred 14-amino acid peptides:

35	VDTYDGDISVVYGL	SEQ ID NO: 65
	DTYDGDISVVYGLR	SEQ ID NO: 66
	TYDGDISVVYGLRS	SEQ ID NO: 67

Preferred 13-amino acid peptides:

40	VDTYDGDISVVYG	SEQ ID NO: 68
	DTYDGDISVVYGL	SEQ ID NO: 69
	TYDGDISVVYGLR	SEQ ID NO: 70
	YDGDISVVYGLRS	SEQ ID NO: 71

Preferred 12-amino acid peptides:

45	VDTYDGDISVVY	SEQ ID NO: 72
	DTYDGDISVVYG	SEQ ID NO: 73
	TYDGDISVVYGL	SEQ ID NO: 74

YDGDISVVYGLR
DGDISVVYGLRS

SEQ ID NO: 75
SEQ ID NO: 76

Preferred 11-amino acid peptides:

5 VDTYDGDISV
DTYDGDISVVY
TYDGDISVVY
YDGDISVVYGL
10 DGDISVVYGLR
GDISVVYGLRS

SEQ ID NO: 77
SEQ ID NO: 78
SEQ ID NO: 79
SEQ ID NO: 80
SEQ ID NO: 81
SEQ ID NO: 82

Preferred 10-amino acid peptides:

15 VDTYDGDISV
DTYDGDISV
TYDGDISVVY
YDGDISVVY
20 DGDISVVYGL
GDISVVYGLR
DISVVYGLRS

SEQ ID NO: 83
SEQ ID NO: 84
SEQ ID NO: 85
SEQ ID NO: 86
SEQ ID NO: 87
SEQ ID NO: 88
SEQ ID NO: 89

25 Preferred 9-amino acid peptides:

VDTYDGDIS
DTYDGDISV
TYDGDISV
30 YDGDISVVY
DGDISVVY
GDISVVYGL
DISVVYGLR
ISVVYGLRS

SEQ ID NO: 90
SEQ ID NO: 91
SEQ ID NO: 92
SEQ ID NO: 93
SEQ ID NO: 94
SEQ ID NO: 95
SEQ ID NO: 96
SEQ ID NO: 97

Preferred 8-amino acid peptides:

VDTYDGDI
40 DTYDGDIS
TYDGDISV
YDGDISV
DGDISVVY
GDISVVY
45 DISVVYGL
ISVVYGLR

SEQ ID NO: 98
SEQ ID NO: 99
SEQ ID NO: 100
SEQ ID NO: 101
SEQ ID NO: 102
SEQ ID NO: 103
SEQ ID NO: 104
SEQ ID NO: 105

Preferred 7-amino acid peptides:

50 VDTYDGD
DTYDGDI
TYDGDIS
YDGDISV
DGDISV

SEQ ID NO: 106
SEQ ID NO: 107
SEQ ID NO: 108
SEQ ID NO: 109
SEQ ID NO: 110

GDISVVY
DISVYVG
ISVVYGL

SEQ ID NO: 111
 SEQ ID NO: 112
 SEQ ID NO: 113

5 Preferred 6-amino acid peptides:

	DTYDGD	SEQ ID NO: 114
	TYDGDI	SEQ ID NO: 115
	YDGDIS	SEQ ID NO: 116
10	DGDISV	SEQ ID NO: 117
	GDISVV	SEQ ID NO: 118
	DISVVY	SEQ ID NO: 119
	ISVVYG	SEQ ID NO: 120

15 In a preferred embodiment, the polypeptide comprises or consists of an amino acid sequence of SEQ ID NO: 63.

The polypeptides of the invention may be for medical use in the treatment or prevention of a disease or condition associated with hair loss (as described in detail below).

20

The invention further provides the use of a polypeptide of the invention for stimulating hair growth in a mammal, wherein the use is cosmetic or commercial (as described in detail below).

25 A third aspect of the invention provides compositions according to the first aspect of the invention for use in stimulating hair growth in a mammal.

Thus, the compositions may be for use in stimulating existing hair follicles and/or inducing the growth of new hair follicles (or stem cells for producing the same).

30

In one embodiment, the composition is for use in the treatment or prevention of a disease or condition associated with hair loss, such as alopecia.

Alopecia is typically associated with the loss of anagen hairs. However, it will be appreciated that the compositions of the invention may also be used for treatment of conditions associated with the loss of telogen hairs.

35

In one embodiment, the alopecia is selected from the group consisting of:

- (a) androgenic alopecia (also known as androgenetic alopecia, *alopecia androgenetica*, male pattern baldness or female pattern baldness);
- 5 (b) traction alopecia;
- (c) anagen effluvium;
- (d) telogen effluvium;
- (e) alopecia areata;
- (f) alopecia totalis;
- 10 (g) alopecia universalis;
- (h) alopecia barbae;
- (i) alopecia mucinosa;
- (j) alopecia neoplastica;
- (k) cicatricial alopecia; and
- 15 (l) scarring alopecia.

For example, the alopecia may be androgenic alopecia.

Alternatively, the alopecia may be anagen effluvium. This condition, resulting from the
20 early entry of hairs into the telogen phase, may be due to a variety of causes, including eating disorders, fever, childbirth, chronic illness, major surgery, anemia, severe emotional disorders, crash diets, hypothyroidism, and drugs.

Thus, in one embodiment, the hair loss is induced by radiotherapy and/or chemotherapy
25 agents. For example, hair loss is a common and distressing side effect of treatment with chemotherapeutic drugs such as cisplatin, etoposide and paclitaxel.

Conveniently, the mammal is a human.

30 A related, fourth aspect of the invention provides the use of a modified osteopontin polypeptide as defined above in relation to the first aspect of the invention in the preparation of a medicament for stimulating hair growth in a mammal.

Thus, the medicament may be for stimulating existing hair follicles and/or inducing the
35 growth of new hair follicles (or stem cells for producing the same).

In one embodiment, the medicament is for the treatment or prevention of a disease or condition associated with hair loss, such as alopecia (e.g. associated with the loss of anagen hairs).

5 In one embodiment, the alopecia is selected from the group consisting of:

- (a) androgenic alopecia (also known as androgenetic alopecia, *alopecia androgenetica*, male pattern baldness or female pattern baldness);
- (b) traction alopecia;
- 10 (c) anagen effluvium;
- (d) telogen effluvium;
- (e) alopecia areata;
- (f) alopecia totalis;
- (g) alopecia universalis;
- 15 (h) alopecia barbae;
- (i) alopecia mucinosa;
- (j) alopecia neoplastica;
- (k) cicatricial alopecia; and
- (l) scarring alopecia.

20

For example, the alopecia may be androgenic alopecia.

Alternatively, the alopecia may be anagen effluvium, e.g. induced by radiotherapy and/or chemotherapy (see above).

25

Conveniently, the mammal is a human.

A related, fifth aspect of the invention provides a method for stimulating hair growth in a mammal comprising administering an effective amount of a modified osteopontin polypeptide as defined above in relation to the first aspect of the invention.

30

Thus, the method may be for stimulating existing hair follicles and/or inducing the growth of new hair follicles (or stem cells for producing the same).

35 The polypeptide composition of the invention is administered to the patient in an effective amount. A 'therapeutically effective amount', or 'effective amount', or 'therapeutically

effective', as used herein, refers to that amount which provides a stimulatory effect on hair growth. This is a predetermined quantity of active material calculated to produce the desired therapeutic effect. As is appreciated by those skilled in the art, the amount of a compound may vary depending on its specific activity. Suitable dosage amounts may contain a predetermined quantity of active composition calculated to produce the desired therapeutic effect in association with the required diluent. In the methods and use for manufacture of compositions of the invention, a therapeutically effective amount of the active component is provided. A therapeutically effective amount can be determined by the ordinary skilled medical or veterinary worker based on patient characteristics, such as age, weight, sex, condition, complications, other diseases, *etc.*, as is well known in the art.

In one embodiment, the method is for the treatment or prevention of a disease or condition associated with hair loss, such as alopecia (*e.g.* associated with the loss of anagen hairs).

In one embodiment, the alopecia is selected from the group consisting of:

- (a) androgenic alopecia (also known as androgenetic alopecia, *alopecia androgenetica*, male pattern baldness or female pattern baldness);
- (b) traction alopecia;
- (c) anagen effluvium;
- (d) telogen effluvium;
- (e) alopecia areata;
- (f) alopecia totalis;
- (g) alopecia universalis;
- (h) alopecia barbae;
- (i) alopecia mucinosa;
- (j) alopecia neoplastica;
- (k) cicatricial alopecia; and
- (l) scarring alopecia.

For example, the alopecia may be androgenic alopecia.

Alternatively, the alopecia may be anagen effluvium, *e.g.* induced by radiotherapy and/or chemotherapy (see above).

Conveniently, the mammal is a human.

5 It will be appreciated by persons skilled in the art that the composition of the first aspect of the invention are not limited to medical uses but may also be used as cosmetic agents (in the sense that they do not provide any physical health improvement, as such, but merely provide an aesthetic benefit to the mammal).

10 Thus, a sixth aspect of the invention provides the use of a composition according to the first aspect of the invention for stimulating hair growth in a mammal, wherein the use is cosmetic.

Thus, the cosmetic composition may be for stimulating existing hair follicles and/or inducing the growth of new hair follicles (or stem cells for producing the same).

15

In one embodiment, the cosmetic composition is used for the treatment or prevention of baldness, which may be associated with a receding hairline and/or thinning hair.

20 Such compositions are not limited to use on the scalp, but may also be applied elsewhere on the body (including to the face to encourage the growth of a beard, eyelashes, eyebrows, etc.)

Conveniently, the mammal is a human.

25 It will be appreciated by persons skilled in the art that the compositions of the invention may be used on their own or in combination with other therapeutic or cosmetic agents. For example, the compositions of the invention may be used in a combination therapy with existing treatments to prevent loss of existing hair and/or to stimulate growth of new hair, for example potassium channel openers, such as minoxidil (Regaine RTM., Pharmacia Corp.) and diazoxide; 5-alpha-reductase inhibitors, such as finasteride
30 (Propecia RTM., Merck & Co.); and the immunosuppressant cyclosporin A.

It will be further appreciated by skilled persons that the compositions of the invention may be used *in vivo*, *ex vivo* or *in vitro*.

35

Thus, in addition to being applied or administered directly to a mammal, the compositions may be used to stimulate hair growth *ex vivo*, for example in a skin explant prior to grafting of the skin on to the mammal.

- 5 Alternatively, the compositions may be used to grow hair follicles *in vitro*, e.g. in cell culture, which may then be transplanted to a patient.

Accordingly, a further aspect of the invention provides the use of a polypeptide according to the first aspect of the invention for stimulating hair growth *in vitro* or *ex vivo*.

10

In one embodiment, the polypeptide is used to stimulate the growth of hair follicles (or stem cell precursors of the same).

- A still further aspect of the invention provides a method for making a composition according to the first aspect of the invention, the method comprising admixing a modified osteopontin polypeptide in which an RGD domain is inactivated with a pharmaceutically acceptable and/or cosmetically acceptable excipient, carrier or diluent.

- Preferred, non-limiting examples which embody certain aspects of the invention will now be described, with reference to the following figures:

Figure 1 shows a schematic representation of the sampling area of skin on the mice.

- Figure 2 is a representative photograph of a cross section of skin from a mouse 48 hours after being treated with a modified osteopontin polypeptide. Follicles within the epidermis are indicated by the arrow. The linear density of the follicles was 18.3 follicles per mm (598 follicles in 32.65 mm of epidermis).

- Figure 3 is a representative photograph of a cross section of skin from a mouse 48 hours after being treated with a negative control composition. Follicles within the epidermis are indicated by the arrow. The linear density of the follicles was 11.7 follicles per mm (379 follicles in 32.50 mm of epidermis).

- Figure 4 shows representative photographs of (a) a control-treated animal on day 14 having a hair growth score of 0, (b) an animal treated with exemplary 'full length' polypeptide "Cx" (corresponding to SEQ ID NO: 1 but without the initial sixteen amino

acid signal peptide, 60 nM) on day 14 having a hair growth score of 1.5 (c) an animal treated with exemplary short peptide "FOL-004" (SEQ ID NO: 5, 60 nM) on day 5 having a hair growth score of 2.5 and (d) an animal treated with exemplary short peptide "FOL-005" (SEQ ID NO: 5, 63 nM) on day 5 having a hair growth score of 2.0.

5

Figure 5 shows the effect of exemplary 'full length' polypeptide "Cx" and polypeptides SEQ ID NOs: 5 and 63 of the invention on hair growth.

Figure 6 shows the effect of exemplary polypeptide SEQ ID NO: 5 on hair growth, as compared to the corresponding polypeptide SEQ ID NO: 121 from wildtype mouse osteopontin.

10

EXAMPLE A – *In vivo* study of hair growth effect of mutated mouse osteopontin in mice

Materials and Methods

5

Creation and production of test polypeptide

The modified osteopontin polypeptide sequence SEQ ID NO:1 was cloned into a pCEP4 expression vector by the use of *XhoI* and *BamHI* restriction enzyme sites. The pCEP4 expression vector contains an ampicillin resistance gene for expression in bacteria and a hygromycin resistance gene for expression in eukaryotic EBNA cells. The polypeptide containing vector were transformed into competent XL-10 bacteria cells, multiplied and isolated with Qiagen midi prep kit. Isolated vectors were then transfected into human EBNA cells by the use of Eugene transfection reagent according to manufacture protocol (Invitrogen).

15

Isolation of test polypeptide

Medium from EBNA cell culture containing the produced polypeptide were collected after three to four days of culture. Polypeptides produced by pCEP4 plasmid contain an inserted His-tag, which is used to facilitate the isolation with Ni-sepharose gel chromatography (Invitrogen). Collected medium was diluted with binding buffer (20mM sodium phosphate, 500mM sodium chloride, pH 7.8), Ni-sepharose suspension was added before incubation on shaker overnight at 4°C. The Ni-sepharose gel was pelleted by centrifugation at 1000g for seven minutes and poured into a BioRad disposable mini column. Unbound proteins were removed with binding buffer followed by washing buffer (20mM sodium phosphate, 500mM sodium chloride, pH 6.0). Polypeptides were eluted from the column with 500mM imidazole.

25

General study design

30

The study consisted of four experimental groups each containing five male wtC57BL/6N mice.

On day -1 the necks of all participating mice were shaved carefully in squares of approx. 1.5 x 1.0 cm and small remaining hairpieces were removed. On the following day (day 0),

35

each animal received four intracutaneous injections of 25µl each at separate locations (each approx. 0.5 x 0.5 cm) within the shaved rectangle (see Figure 1).

- Each animal received two injections of 25µl of a composition comprising an exemplary modified osteopontin polypeptide of SEQ ID NO:1 ("Test polypeptide" or "Cx", 60 nmol/l in PBS) and two negative control injections of 25µl PBS (according to the scheme outlined in Table 1).

Table 1: Study Design

Group		Intracutaneous Application		Number of animals
		Volume	Time schedule after application	
1	Test polypeptide + PBS	25µl	Necropsy after 24h (day 1)	5
2	Test polypeptide + PBS	25µl	Necropsy after 48h (day 2)	5
3	Test polypeptide + PBS	25µl	Necropsy after 96h (day 4)	5
4	Test polypeptide + PBS	25µl	Necropsy after 336h (day 14)	5

Animals from Treatment Groups 1 to 4 were sacrificed 24h (Group 1), 48h (Group 2), 96h (Group 3) and 336h (14 days, Group 4) after compound application, respectively.

- In all cases, the marked skin areas in the neck were removed and processed as follows: One of the polypeptide-treated skin samples and one of the PBS-treated control skin samples were fixed in 4% paraformaldehyde and subsequently embedded in paraffin. The two other skin samples (polypeptide-treated and PBS-treated) were snap-frozen in liquid nitrogen and stored appropriately at -80°C.

A Hematoxylin-staining of the paraffin-embedded sections was performed.

Animals

	Rationale	Accepted test system for the purpose of the study
5	Strain	male wtC57BL/6N
	Source	Charles River GmbH Sandhofer Weg 7 D 97633 Sulzfeld
	Total number of animals	20
10	Age at delivery	5-6 weeks
	Body weight and range (at acclimatisation)	approx. 30g
	Identification	Labeling by ear mark
	Acclimatisation	February, 17 th to March, 1 st 2011
15		
	<u>Husbandry</u>	
	Conditions	Optimum hygienic conditions, air-conditioned with 10-15 air changes per hour, and continually monitored environment with target ranges for temperature $22 \pm 3^{\circ}\text{C}$ and for relative humidity 30- 70%, 12 hours artificial fluorescent light / 12 hours dark.
20		
25	Accommodation	max. 3 animals per cage
	Diet	M-Zucht ssniff Spezialdiäten GmbH Ferdinand Gabriel Weg 16 D-59494 Soest
30		
	Water	Community tap water (autoclaved)
35	<u>Test polypeptide</u>	
	Identification	Modified osteopontin polypeptide of SEQ ID NO:1

	Storage conditions	stored in Hepes buffer at 4°C
5	Safety precautions	prevent ingestion, inhaling, wear gloves and skin mask, wash with soap and water after skin contact; no special precautions
10	Sample preparation	delivered stock solution was diluted 15x with PBS to obtain the 60nM working solution before injection

Vehicle

15	Identity	PBS pH 7.4
----	----------	------------

Treatment

	Route of administration:	intracutaneously
20	Frequency of administration	single application on day 0
	Dose volume	25 µl

Observations

25	The following parameters were recorded:
----	---

	Viability / Mortality	daily
	Clinical signs	daily
30		

Protocol for Hematoxylin-staining of paraffin-embedded sections of Group 1

1. Xylen bath 5min
2. Xylen bath 5min
- 35 3. EtOH 100% bath 5min
4. EtOH 95% bath 5min
5. EtOH 70% bath 5min

6. PBS bath 5min
7. Hematoxylin bath about 20sec (Harrys Hematoxylin)^{a),b)}
8. Water bath 3 times 3 min
9. EtOH 70% bath 5min
- 5 10. EtOH 95% bath 5min
11. EtOH 100% bath 5min
12. Xylen bath 5min
13. Xylen bath 5min
14. Mount slide using Permunt and coveslip.

10

a) the time depends on the type of hematoxylin used, 20sec to 10min (which can be determined by the use of test slides)

b) filtrated before use

15 **Results**

The effect of treatment with a modified osteopontin polypeptide (SEQ ID NO:1) on hair follicle density is summarised in Table 1(a) and (b) below.

Table 1(a) – Polypeptide-treated animals

Polypeptide treated Slide 1	Number of hair follicles	Length of epidermis, mm
area a1	18	0.90
area a2	21	0.90
area a3	25	0.95
area b1	13	0.90
area b2	21	0.90
area b3	26	0.95
Sum	124	5.50

Polypeptide treated Slide 2	Number of hair follicles	Length of epidermis, mm
area a1	16	0.90
area a2	18	0.90
area a3	25	0.95
area b1	14	0.90
area b2	18	0.90
area b3	19	0.95
Sum	110	5.50

Polypeptide treated Slide 3	Number of hair follicles	Length of epidermis, mm
area a1	18	0.90
area a2	13	0.90
area a3	19	0.95
area b1	20	0.90
area b2	17	0.90
area b3	14	0.90
Sum	101	5.45

5

Polypeptide treated Slide 4	Number of hair follicles	Length of epidermis, mm
area a1	18	0.90
area a2	17	0.90
area a3	15	0.90
area b1	18	0.90
area b2	17	0.90
area b3	13	0.90
Sum	98	5.40

Table 1(a) – continued

Polypeptide treated Slide5	Number of hair follicles	Length of epidermis, mm
area a1	13	0.90
area a2	10	0.90
area a3	21	0.90
area b1	12	0.90
area b2	10	0.90
area b3	16	0.90
Sum	82	5.40

Polypeptide treated Slide 6	Number of hair follicles	Length of epidermis, mm
area a1	18	0.90
area a2	10	0.90
area a3	10	0.90
area b1	18	0.90
area b2	17	0.90
area b3	10	0.90
Sum	83	5.40

Polypeptide treated Slide7	Number of hair follicles	Length of epidermis, mm
Slide 34	124	5.50
Slide 38	110	5.50
Slide 42	101	5.45
Slide 46	98	5.40
Slide 50	82	5.40
Slide 54	83	5.40
Total	598	32.65

Table 1(b) – Control-treated animals

Control treated Slide 1	Number of hair follicles	Length of epidermis, mm
area a1	9	0.90
area a2	17	0.90
area a3	7	0.90
area b1	11	0.90
area b2	14	0.95
area b3	10	0.90
Sum	68	5.45

Control treated Slide 2	Number of hair follicles	Length of epidermis, mm
area a1	12	0.90
area a2	17	0.90
area a3	7	0.90
area b1	12	0.90
area b2	13	0.90
area b3	10	0.90
Sum	71	5.40

Control treated Slide 3	Number of hair follicles	Length of epidermis, mm
area a1	8	0.90
area a2	7	0.90
area a3	11	0.90
area b1	19	0.95
area b2	8	0.90
area b3	14	0.90
Sum	67	5.45

5

Control treated Slide 4	Number of hair follicles	Length of epidermis, mm
area a1	21	0.90
area a2	5	0.90
area a3	2	0.90
area b1	16	0.90
area b2	11	0.90
area b3	3	0.90
Sum	58	5.40

Table 1(b) – Continued

Control treated Slide 5	Number of hair follicles	Length of epidermis, mm
area a1	13	0.90
area a2	2	0.90
area a3	9	0.90
area b1	12	0.90
area b2	7	0.90
area b3	15	0.90
Sum	58	5.40

Control treated Slide 6	Number of hair follicles	Length of epidermis, mm
area a1	7	0.90
area a2	11	0.90
area a3	10	0.90
area b1	6	0.90
area b2	8	0.90
area b3	15	0.90
Sum	57	5.40

Control treated Slide 7	Number of hair follicles	Length of epidermis, mm
Slide 18	68	5.45
Slide 22	71	5.40
Slide 26	67	5.45
Slide 34	58	5.40
Slide 38	58	5.40
Slide 42	57	5.40
Total	379	32.50

A summary of the results is shown below in Table 2

Table 2

	No. of follicles analysed	No. of follicles per mm
Treatment with SEQ ID NO: 1	356	18.3
Control group	356	11.7

5

Representative tissue sections showing follicles in treated and control animals are shown in Figures 2 and 3.

By comparison, wildtype mouse osteopontin was observed to have no detectable effect
10 on hair growth (data not shown).

Conclusions

The data show that treatment with the exemplary modified osteopontin polypeptide of
15 SEQ ID NO: 1 increases the number of hair follicles/mm epidermis by about 60%.

These findings confirm the usefulness of the osteopontin-like polypeptides of the invention in stimulating hair growth.

EXAMPLE B – *In vivo* study of hair growth effect of SEQ ID NOS: 5 and 63 in mice***Materials and Methods*****5 Animals**

Mice (C57BL/6) were used at an age of 6 to 8 weeks.

Overview of study design

10

- Selection of animals in telogen phase of hair growth.
- Clipping of dorsal back of animals
- Subcutaneous injection of test peptide/vehicle
- Visual analysis: Percentage anagen induction; Mean hair growth score; Visual melanogenesis:
- Histological analysis: Follicle count in subcutis; Morphometry for skin thickness.

15

Treatment groups

20

- Animals were assigned randomly to treatment groups (see Table 3)

Table 3

<i>Treatment</i>	<i>No. of animals</i>	<i>Dose</i>	<i>Volume</i>
"Cx "	5	60 nM	25 µl
"FOL-004" (= SEQ ID NO:5)	5	60 nM	25 µl
"FOL-005" (= SEQ ID NO:63)	5	60 nM	25 µl
Vehicle control	5	-	25 µl

- Animals were administered the specified treatment on Days 1, 5 and 9
- Treatment were administered by subcutaneous injection into dorsal clipped skin

Scoring criteria

5

- The effect of the treatments was observed and scored daily according to the criteria in Table 4

Table 4

<i>Observation of dorsal skin</i>	<i>Hair growth score</i>
No hair growth, pink skin	0
Skin colour changes from pink to gray without visible hair growth	0.5
Skin colour changes from pink to gray or black without visible hair growth, indicating the onset of anagen	1
Sparse hair growth	1.5
Diffuse short hair growth	2
Moderate hair growth	2.5
Dense, normal coat hair	3

10

Results

The effect of the exemplary polypeptides of the invention on hair growth is shown in

15 Table 5.

Table 5

Animal	Day											
	1	2	5	6	7	8	9	12	13	14	15	16
(A) Vehicle (control)												
1	0	0	0	0	0	0	0	0	0	0	NA	NA
2	0	0	0	0	0	0	0	0	0	0	NA	NA
3	0	0	0	0	0	0	0	0	0	0	NA	NA
4	0	0	0	0	0	0	0	0	0	0	NA	NA
5	0	0	0	0	0	0	0	0.5	1	1	NA	NA
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.2	-	-
(B) "Cx" (SEQ ID NO:1 minus the initial 16 amino acid signal peptide)												
1	0	0	0	1	1.5	2	2.5	3	3	3	NA	NA
2	0	0	0	0.5	0.5	0.5	0.5	1	1	1	NA	NA
3	0	0	0	0.5	0.5	0.5	1	1.5	1.5	1.5	NA	NA
4	0	0	0	0.5	0.5	0.5	1	1.5	1.5	1.5	NA	NA
5	0	0	0	0	0	0	0.5	1	1.5	2	NA	NA
Mean	0.0	0.0	0.0	0.5	0.6	0.7	1.1	1.6	1.7	1.8	-	-
(C) FOL-004 (SEQ ID NO: 5)												
1	0	0	0.5	1	1	1	1	1.5	1.5	1.5	2.5	3
2	0	0	2.5	2.5	3	3	3	3	3	3	3	3
3	0	0	2.5	2.5	2.5	3	3	3	3	3	3	3
4	0	0	1.5	2.5	3	3	3	3	3	3	3	3
5	0	0	1.5	2	2.5	2.5	3	3	3	3	3	3
Mean	0.0	0.0	1.7	2.1	2.4	2.5	2.6	2.7	2.7	2.7	2.9	3.0

Animal	Day											
	1	2	5	6	7	8	9	12	13	14	15	16
(D) FOL-005 (SEQ ID NO: 63)												
1	0	0	2	2.5	3	3	3	3	3	3	3	3
2	0	0	2	3	3	3	3	3	3	3	3	3
3	0	0	0.5	0.5	1	1	1.5	2.5	2.5	2.5	3	3
4	0	0	2	2.5	2.5	3	3	3	3	3	3	3
5	0	0	0	0	0	0.5	1	2	2.5	2.5	3	3
Mean	0.0	0.0	1.3	1.7	1.9	2.1	2.3	2.7	2.8	2.8	3.0	3.0

Figure 4 shows representative photographs of (a) a control-treated animal on day 14 having a hair growth score of 0, (b) an animal treated with exemplary 'full length' polypeptide "Cx" (SEQ ID NO: 1, 60 nM) on day 14 having a hair growth score of 1.5 (c) an animal treated with exemplary short peptide "FOL-004" (SEQ ID NO: 5, 60 nM) on day 5 having a hair growth score of 2.5 and (d) an animal treated with exemplary short peptide "FOL-005" (SEQ ID NO: 5, 63 nM) on day 5 having a hair growth score of 2.0.

The results are summarised in Figure 5.

10

Exemplary polypeptides FOL-004 (SEQ ID NO: 5) and FOL-005 (SEQ ID NO: 63) both induced rapid hair growth, which was evident from day 5 and maintained until the end of the assessment period (day 16)..

15 A 'full length' polypeptide of the invention, ("Cx") also induced pronounced hair growth, albeit with a slower onset than the FOL-004 (SEQ ID NO: 5) or FOL-005 (SEQ ID NO: 63).

20 Animals treated with the vehicle control showed little sign of hair growth over the period of the experiment.

None of the polypeptides tested lead to any adverse effects in the animals (no body weight loss or any other discernible adverse symptom).

Conclusions

All mutated osteopontin polypeptides tested showed pronounced hair growth effects in vivo, with the shorter exemplary polypeptides FOL-004 (SEQ ID NO: 5) and FOL-005 (SEQ ID NO: 63) exhibiting a particular fast onset of action.

EXAMPLE C – *In vivo* study of hair growth effect in mice of the wildtype osteopontin fragment equivalent to SEQ ID NO: 5

Materials and Methods

5

Animals, as described above in Example B, were treated as described in Table 6:

Table 6

<i>Treatment</i>	<i>No. of animals</i>	<i>Dose</i>	<i>Volume</i>
"FOL-001"* (= SEQ ID NO:121)	6	60 nM	25 µl

10

* "FOL-001" consists of the following amino acid sequence:

VDVPNGRGDSLAYGLR

[SEQ ID NO: 121]

15

This sequence is a fragment of wildtype mouse osteopontin, and corresponds to the region of the protein from which "FOL-004" is derived (*i.e.* "FOL-004" is a mutated version of wildtype fragment "FOL-001").

20

- Animals were administered the specified treatment on Days 1, 5 and 9
- Treatment were administered by subcutaneous injection into dorsal clipped skin

Scoring criteria

25

- The effect of the treatments was observed and scored daily as described in Example B.

Results

30

The effect of the polypeptide "FOL-001" on hair growth is shown in Table 7 and Figure 6.

Table 7

<i>Animal</i>	<i>Day</i>											
	1	2	5	6	7	8	9	12	13	14	15	16
FOL-001 (SEQ ID NO: 121, 60nM)												
1	0	0	0.5	0.5	0.5	0.5	1	2	2	2.5	2.5	NA
2	0	0	0.5	0.5	0.5	0.5	1	2	2	2.5	2.5	NA
3	0	0	0	0	0	0	0.5	1	1	1	1	NA
4	0	0	0	0	0	0	0	0	0	0	0	NA
5	0	0	0	0	0	0	0.5	1	1	1	1	NA
6	0	0	0	0	0	0	0.5	1	1	1	1	NA
<i>Mean</i>	0	0	0.17	0.17	0.17	0.17	0.58	1.17	1.17	1.33	1.33	-

Peptide FOL-001 (SEQ ID NO: 121) produced a delayed but detectable hair growth
 5 effect in mice, which became evident at about day 9 and reached a plateau at a score of about 1.3.

Conclusions

10 Compared to the corresponding mutated peptide sequence of the invention (FOL-004; SEQ ID NO:5), the equivalent non-mutated wild type sequence exhibited a delayed onset of hair growth stimulation with a maximum effect of less than 50% of that observed with FOL-004.

15 Thus, the exemplary polypeptide shows much greater efficacy than the equivalent non-mutated wild type sequence.

EXAMPLE D – *In vivo* study of anagen induction in peeled skin***Materials and Methods***

- 5 Animals and treatments are as described above in Example B.

All animals were sacrificed on day 16.

Observation of melanogenesis

10

Following completion of the term of hair growth assessment for Example B, the animals were euthanized and dorsal skin peeled and removed for observation of the internal surface.

- 15 **Histological analysis**

Peeled skin sections were prepared for histological analysis as described in Example A. Follicle count in the subcutis and skin thickness were then measured.

- 20 ***Results***

Results are summarised in Tables 8 and 9 below.

Table 8
Observation of melanogenesis

Group	Skin colour (external)	% anagen induction*	Black colour in peeled skin (day 16)	% anagen induction**	No. of animals showing hair growth
Cx (60nM)	Black (5/5 animals)	100	3/5	60	5/5
FOL-004 (60nM)	Black (5/5 animals)	100	1/5	20	5/5
FOL-005 (60nM)	Black (5/5 animals)	100	3/5	60	5/5
Vehicle	Pink (4/5 animals)	20	1/5	20	1/5

5

* with respect to external skin colour change from pink to black

** with respect to black colour in peeled skin

10

Table 9a (all animals)

Group	No. of animals considered	Mean \pm sem	
		Follicle count in subcutis (no.)	Skin thickness
Cx (60nM)	5	22.20 \pm 10.69	308.8 \pm 21.94
FOL-004 (60nM)	5	6.20 \pm 5.80	312.2 \pm 15.60
FOL-005 (60nM)	5	34.80 \pm 14.58	348.80 \pm 40.05
Vehicle	4*	0 \pm 0	246.5 \pm 8.35

* Animal No. 5 was found to be a significant outlier ($p < 0.05$, Grubb's test) and so was ignored in analysis

Table 9b (animals in anagen only)

Group	No. of animals considered	Mean \pm sem	
		Follicle count in subcutis (no.)	Skin thickness
Cx (60nM)	3/5	36.67 \pm 10.69	308.8 \pm 21.94
FOL-004 (60nM)	1/5	29 \pm 5.8	312.2 \pm 15.60
FOL-005 (60nM)	3/5	58 \pm 14.58	348.80 \pm 40.05
Vehicle	0/4*	0 \pm 0	246.5 \pm 8.35

5

The hair growth cycle consists of three phases: a resting telogen phase (C57BL/6 skin is a pale pink colour), an active hair growth anagen phase (where the skin becomes dark gray or black), and finally a catagen phase (where hair growth stops, and the skin transitions back to the telogen phase, returning to a pale pink colour).

10

In the present study, the animals were preselected for pink skin (telogen phase) and the test polypeptide was administered by subcutaneous/topical route. A good hair growth promoter triggers the transition from the resting telogen phase to the active anagen phase, and thus a transition from light skin to dark skin. This dark pigmentation may result from the collection of melanin in the hair follicles, in preparation for new hair growth during the anagen phase. Melanin synthesis of follicular melanocytes is strictly coupled to the anagen phase, ceases during catagen and is absent in telogen phase. Hence a good hair growth promoter causes blackening of dorsal skin. Upon termination, the dorsal skin of the animals was then excised out by peeling and the peeled skin reversed and observed for the induction of melanogenesis indicated by visual blackening. Histological analysis reveals number of follicles and skin thickness. A good hair growth promoter increases no. of follicles in sub cutis and skin thickness.

In the present study, all three test polypeptides led to the appearance of dense hair growth after three subcutaneous injections (see Example B above). It was also observed that in some of the animals, subsequent to full growth of the hair, the skin colour changed from black to gray to pink, a characteristic of catagen phase. The peeled skin collected in the study showed the black patch, indicative of anagen phase (active phase) of hair growth cycle due to melanocyte sequestering. In these animals, the histological

results showed a large number of hair follicles and increase in skin thickness. But in some animals, where the catagen phase had occurred, peeled skin was found to be of pink colour, even though the hair coat was intact. Thus, analysis of such peeled skin sections demonstrated lack of follicles in subcutis but still good increase in skin
5 thickness.

Conclusions

The exemplary mutated osteopontin polypeptides of the invention showed pronounced
10 induction of anagen, as evidenced by an increase in the number of hair follicles in the subcutis and/or enhanced skin thickness.

CLAIMS

1. A composition for stimulating hair growth in a mammal comprising:
- 5
- (a) a modified osteopontin polypeptide in which an RGD domain is inactivated; and
- (b) a pharmaceutically acceptable and/or cosmetically acceptable excipient,
- 10 carrier or diluent.
2. A composition according to Claim 1 wherein the polypeptide is capable of stimulating the growth of human hair.
- 15 3. A composition according to Claim 1 or 2 wherein the polypeptide is capable of stimulating the growth of hair *in vivo*.
4. A composition according to any one of the preceding claims wherein the polypeptide is capable of stimulating existing hair follicles.
- 20 5. A composition according to any one of the preceding claims wherein the polypeptide is capable of inducing the formation of new hair follicles, or stem cells for producing the same.
- 25 6. A composition according to any one of the preceding claims wherein the polypeptide is fewer than 500 amino acids in length, for example fewer than 400, 350, 340, 330, 320, 310, 300, 290, 280, 270, 260, 250, 200, 150, 100, 50, 40, 30, 20, 15, 10 or fewer amino acids in length.
- 30 7. A composition according to Claim 6 wherein the polypeptide is between 250 and 350 amino acids in length, for example between 280 and 300 amino acids in length.
- 35 8. A composition according to Claim 7 wherein the polypeptide is 293 amino acids in length.

9. A composition according to any one of the preceding claims wherein the RGD domain is mutated at one or more amino acids in the modified osteopontin polypeptide.
- 5 10. A composition according to any one of the preceding claims wherein the RGD domain is deleted, at least in part, in the modified osteopontin polypeptide.
11. A composition according to any one of the preceding claims wherein the RGD domain is substituted at one or more amino acids in the modified osteopontin polypeptide.
- 10 12. A composition according to any one of the preceding claims wherein the modified osteopontin polypeptide comprises or consists of the amino acid sequence of SEQ ID NO:1:

15

MRLAVICFCLFGIASSLPVKVTDSGSSEEKLYSLHPDPIATWLVPDPSQKQNLAPQNAVSSSEKD
 DFKQETLPNSNESHDMDDDDDDDDDDGDHAESEDSVDSDESDESHHSEDETVTASTQADTFT
 PIVPTVDVPNGDISLAYGLRSKRSRFQVSDEQYPDATDEDLTSHMKSGESKESLDVIPVAQLLSMP
 SDQDNNGKGSHESSQLDEPSLETHRLEHSKESQESADQSDVIDSQASSKASLEHQSHKFHSHKDKL
 20 VLDPKSKEDDRYLKFRISHELESSSSEVN

SEQ ID NO: 1

or a fragment, variant, derivative or fusion thereof (or a fusion of said fragment, variant or derivative) which retains the hair growth stimulatory activity of the amino acid sequence of SEQ ID NO:1.

25

13. A composition according to any one of the preceding claims wherein the polypeptide comprises or consists of a fragment of the amino acid sequence of SEQ ID NO: 1, or a variant thereof.
- 30 14. A composition according to Claim 13 wherein the polypeptide comprises at least 6 contiguous amino acids of the amino acid sequence of SEQ ID NO: 1, for example at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 100, 150, 200, 210, 220, 230, 240, 250, 255, 260, 265, 270, 275, 280, 285, 286, 287, 288, 289 290, 291 or 292 contiguous amino acids of SEQ ID NO: 1.
- 35

15. A composition according to Claim 13 or 14 wherein the fragment comprises or consists of the inactivated RGD domain sequence of SEQ ID NO:2.
- 5 16. A composition according to Claim 13 or 14 wherein the fragment comprises or consists of an amino acid sequence according to any one SEQ ID NOs: 5 to 62.
17. A composition according to Claim 16 wherein the fragment comprises or consists of an amino acid sequence of SEQ ID NO: 5.
- 10 18. A composition according to any one of the preceding claims wherein the polypeptide comprises or consists of a variant of the amino acid sequence of SEQ ID NO: 1, or of a fragment thereof.
- 15 19. A composition according to Claim 18 wherein the variant comprises or consists of an amino acid sequence with at least 50% identity to the amino acid sequence of SEQ ID NO: 1, more preferably at least 60%, 70% or 80% or 85% or 90% identity to said sequence, and most preferably at least 95%, 96%, 97%, 98% or 99% identity to said amino acid sequence.
- 20 20. A composition according to Claim 18 or 19 wherein the variant comprises or consists of an amino acid sequence of SEQ ID NO: 1, or a fragment thereof, in which one or more amino acids is conservatively substituted.
- 25 21. A composition according to Claim 20 wherein the variant comprises or consists of the inactivated RGD domain sequence of SEQ ID NO:2.
- 30 22. A composition according to any one of Claims 18 to 20 wherein the polypeptide comprises or consists of one or more additional amino acids, inserted at the N- and/or C-terminus and/or internally within the amino acid sequence of SEQ ID NO:1.
23. A composition according to Claim 22 wherein the polypeptide comprises or consists of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 additional amino acids.
- 35 24. A composition according to any one of Claims 18 to 23 wherein the polypeptide is non-naturally occurring.

25. A composition according to any one of Claims 18 to 23 wherein the polypeptide is a species homologue of the amino acid sequence of SEQ ID NO:1, or a fragment thereof.
- 5 26. A composition according to Claim 24 or 25 wherein the polypeptide comprises or consists of the amino acid sequence of a modified human osteopontin protein, or fragment thereof, in which an RGD domain is inactivated.
- 10 27. A composition according to Claim 24 or 25 wherein the polypeptide comprises or consists the amino acid sequence of SEQ ID NO:3:
- MRIAVICFCLLGITCAIPVKQADSGSSEEKQLYNKYPDAVATWLNPDPSQKQNLLAPQTLPSKSNE
SHDHMDMDDEDDDDHVDSQDSIDSNDSDDVDDTDDSHQSDSHHSDESDELVTDFPTDLPATEVF
15 TPVVPTVDTYDGDISVVYGLRSKSKKFRPDIQYPDATDEDITSHMESEELNGAYKAIPVAQDLNA
PSDWDSRGKDSYETSQQLDDQSAETHSHKQSRLYKRKANDESNEHSDVIDSQELSKVSREFHSHEFH
SHEDMLVVDPKSKEEDKHLKFRISHELDSASSEVN
- SEQ ID NO: 3**
- 20 or a fragment, variant, derivative or fusion thereof (or a fusion of said fragment, variant or derivative) which retains the hair growth stimulatory activity of the amino acid sequence of SEQ ID NO:3.
- 25 28. A composition according to Claim 27 wherein the fragment or variant comprises or consists of the inactivated RGD domain sequence of SEQ ID NO:4.
29. A composition according to Claim 26 or 27 wherein the fragment comprises or consists of an amino acid sequence according to any one SEQ ID NOs: 63 to 120.
- 30 30. A composition according to Claim 29 wherein the fragment comprises or consists of an amino acid sequence of SEQ ID NO: 63.

31. A composition according to any one of the preceding claims wherein the polypeptide comprises or consists of a derivative of the amino acid sequence of SEQ ID NO: 1 or 3, or of a fragment, variant or fusion thereof.
- 5
32. A composition according to Claim 31 wherein the polypeptide comprises or consists of a derivative of the amino acid sequence of SEQ ID NO: 5 or 63.
33. A composition according to any one of the preceding claims wherein the polypeptide comprises or consists of a fusion of the amino acid sequence of SEQ ID NO: 1 or 3, or of a fragment or variant thereof.
- 10
34. A composition according to Claim 33 wherein the polypeptide comprises or consists of a fusion of the amino acid sequence of SEQ ID NO: 5 or 63.
- 15
35. A composition according to any one of the preceding claims wherein the polypeptide comprises or consists of tandem repeats.
36. A composition according to Claim 35 wherein the tandem repeats comprise or consist of the amino acid sequence of SEQ ID NO: 2 and/or 4.
- 20
37. A composition according to Claim 35 wherein the tandem repeats comprise or consist of the amino acid sequence of SEQ ID NO: 5 and/or 63.
- 25
38. A composition according to any one of the preceding claims wherein the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 1 or 3.
39. A composition according to any one of the preceding claims wherein the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 5 or 63.
- 30
40. A composition according to any one of the preceding claims wherein the polypeptide consists of the polypeptide according to SEQ ID NO: 1 or 3.
- 35

41. A composition according to any one of the preceding claims wherein the polypeptide consists of the amino acid sequence of SEQ ID NO: 5 or 63.
- 5 42. A composition according to any one of the preceding claims wherein the polypeptide is glycosylated.
43. A composition according to any one of the preceding claims for topical administration.
- 10 44. A composition according to any one of the preceding claims for transdermal administration.
45. A composition according to any one of the preceding claims for intracutaneous administration.
- 15 46. A composition according to any one of the preceding claims wherein the mammal is a human.
- 20 47. A polypeptide comprising or consisting of the amino acid sequence of SEQ ID NO: 63 or a fragment, variant, derivative or fusion thereof (or a fusion of said fragment, variant or derivative) which retains a hair growth stimulatory activity of the amino acid sequence of SEQ ID NO: 63.
- 25 48. A polypeptide according to Claim 47 wherein the polypeptide comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 5 to 62, or fragment thereof.
49. A polypeptide according to Claim 48 wherein the polypeptide comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 5 to 62.
- 30 50. A polypeptide according to Claim 49 wherein the polypeptide consists of an amino acid sequence of any one of SEQ ID NOs: 5 to 62.
- 35 51. A polypeptide according to Claim 50 wherein the polypeptide comprises or consists of an amino acid sequence of SEQ ID NO: 5.

52. A polypeptide according to Claim 51 wherein the polypeptide consists of an amino acid sequence of SEQ ID NO: 5.
53. A polypeptide according to Claim 47 wherein the polypeptide comprises or consists of a variant of an amino acid sequence of SEQ ID NO: 5.
54. A polypeptide according to Claim 53 wherein the polypeptide comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 63 to 120, or fragment thereof.
55. A polypeptide according to Claim 54 wherein the polypeptide comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 63 to 120.
56. A polypeptide according to Claim 55 wherein the polypeptide consists of an amino acid sequence of any one of SEQ ID NOs: 63 to 120.
57. A polypeptide according to Claim 56 wherein the polypeptide comprises or consists of an amino acid sequence of SEQ ID NO: 63.
58. A polypeptide according to Claim 57 wherein the polypeptide consists of an amino acid sequence of SEQ ID NO: 63.
59. A polypeptide according to any one of Claims 46 to 58 for medical use in the treatment or prevention of a disease or condition associated with hair loss.
60. Use of a polypeptide according to any one of Claims 46 to 58 for stimulating hair growth in a mammal, wherein the use is cosmetic
61. A composition according to any one of Claims 1 to 37 for use in stimulating hair growth in a mammal.
62. A composition according to Claim 61 for stimulating existing hair follicles.
63. A composition according to Claim 61 for inducing the growth of new hair follicles, or stem cells for producing the same.

64. A composition according to any one of Claims 61 to 63 for use in the treatment or prevention of a disease or condition associated with hair loss.
- 5 65. A composition according to any one of Claims 61 to 64 for use in the treatment or prevention of alopecia.
66. A composition according to Claim 65 wherein the alopecia is associated with the loss of anagen hairs.
- 10 67. A composition according to Claim 65 wherein the alopecia is associated with the loss of telogen hairs
68. A composition according to any one of Claims 65 to 67, wherein the alopecia is selected from the group consisting of:
- 15 (a) androgenic alopecia (also known as androgenetic alopecia, *alopecia androgenetica*, male pattern baldness or female pattern baldness);
- (b) traction alopecia;
- (c) anagen effluvium;
- 20 (d) telogen effluvium;
- (e) alopecia areata;
- (f) alopecia totalis;
- (g) alopecia universalis;
- (h) alopecia barbae;
- 25 (i) alopecia mucinosa;
- (j) alopecia neoplastica;
- (k) cicatricial alopecia; and
- (l) scarring alopecia.
- 30 69. A composition according to Claim 68 wherein the alopecia is androgenic alopecia.
70. A composition according to Claim 68 wherein the alopecia is anagen effluvium.
71. A composition according to any one of Claims 64 to 70 wherein the hair loss is induced by radiotherapy and/or chemotherapy agents.
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72. A composition according to any one of Claims 61 to 71 wherein the patient is a human.
- 5 73. Use of a modified osteopontin polypeptide as defined in any one of Claims 1 to 37 in the preparation of a medicament for stimulating hair growth in a mammal.
74. A use according to Claim 73 wherein the medicament is for stimulating existing hair follicles.
- 10 75. A use according to Claim 73 wherein the medicament is for inducing the growth of new hair follicles, or stem cells for producing the same.
76. A use according to any one of Claims 73 to 75 wherein the medicament is for the treatment or prevention of a disease or condition associated with hair loss.
- 15 77. A use according to any one of Claims 73 to 76 wherein the medicament is for the treatment or prevention of alopecia.
78. A use according to Claim 77 wherein the alopecia is associated with the loss of anagen hairs.
- 20 79. A use according to Claim 77 wherein the alopecia is associated with the loss of telogen hairs.
- 25 80. A use according to any one of Claims 77 to 79, wherein the alopecia is selected from the group consisting of:
- (a) androgenic alopecia (also known as androgenetic alopecia, *alopecia androgenetica*, male pattern baldness or female pattern baldness);
 - 30 (b) traction alopecia;
 - (c) anagen effluvium;
 - (d) telogen effluvium;
 - (e) alopecia areata;
 - (f) alopecia totalis;
 - 35 (g) alopecia universalis;
 - (h) alopecia barbae;

- (i) alopecia mucinosa;
- (j) alopecia neoplastica;
- (k) cicatricial alopecia; and
- (l) scarring alopecia.

5

81. A use according to Claim 80 wherein the alopecia is androgenic alopecia.

82. A use according to Claim 80 wherein the alopecia is anagen effluvium.

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83. A use according to any one of Claims 76 to 82 wherein the hair loss is induced by radiotherapy and/or chemotherapy agents.

84. A use according to any one of Claims 73 to 83 wherein the patient is a human.

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85. A method for stimulating hair growth in a mammal comprising administering an effective amount of a composition according to any one of Claims 1 to 37.

86. A method according to Claim 85 for stimulating existing hair follicles.

20

87. A method according to Claim 85 for inducing the growth of new hair follicles, or stem cells for producing the same.

88. A method according to any one of Claims 85 to 87 for treatment or prevention of a disease or condition associated with hair loss.

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89. A method according to any one of Claims 85 to 88 wherein the medicament is for the treatment or prevention of alopecia.

90. A method according to Claim 89 wherein the alopecia is associated with the loss of anagen hairs.

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91. A method according to Claim 89 wherein the alopecia is associated with the loss of telogen hairs.

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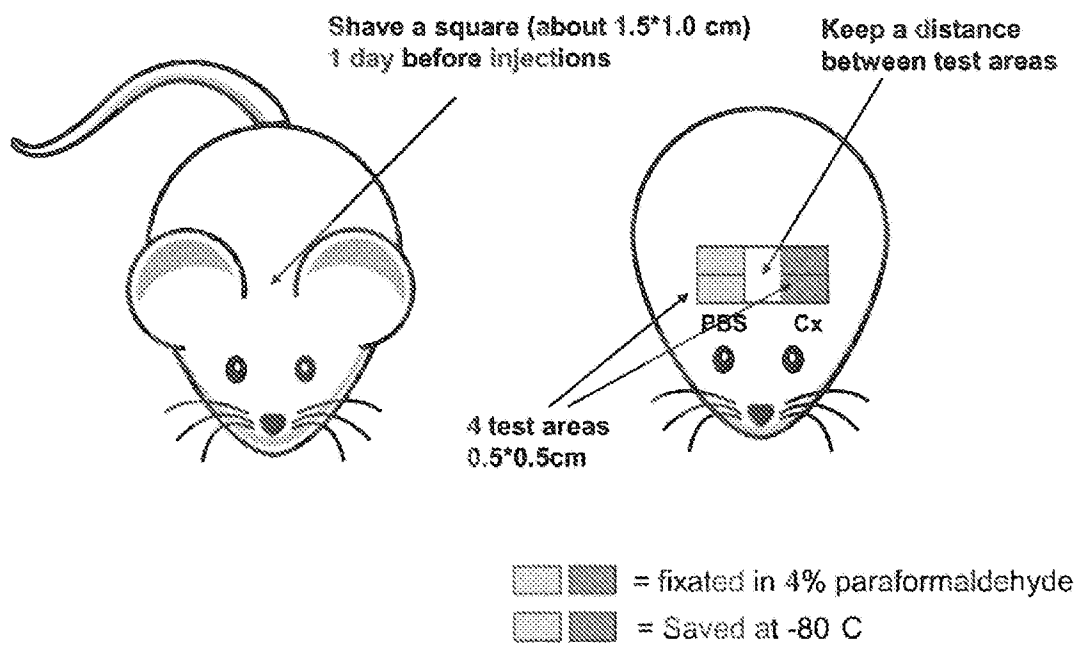
92. A method according to any one of Claims 89 to 91, wherein the alopecia is selected from the group consisting of:

- (a) androgenic alopecia (also known as androgenetic alopecia, *alopecia androgenetica*, male pattern baldness or female pattern baldness);
- (b) traction alopecia;
- 5 (c) anagen effluvium;
- (d) telogen effluvium;
- (e) alopecia areata;
- (f) alopecia totalis;
- (g) alopecia universalis;
- 10 (h) alopecia barbae;
- (i) alopecia mucinosa;
- (j) alopecia neoplastica;
- (k) cicatricial alopecia; and
- (l) scarring alopecia.
- 15
93. A method according to Claim 92 wherein the alopecia is androgenic alopecia.
94. A method according to Claim 92 wherein the alopecia is anagen effluvium.
- 20 95. A method according to any one of Claims 88 to 94 wherein the hair loss is induced by radiotherapy and/or chemotherapy agents.
96. A method according to any one of Claims 85 to 95 wherein the patient is a human.
- 25 97. Use of a composition according to any one of Claims 1 to 37 for stimulating hair growth in a mammal, wherein the use is cosmetic.
98. A use according to Claim 97 for stimulating existing hair follicles.
- 30 99. A use according to Claim 97 for inducing the growth of new hair follicles, or stem cells for producing the same.
100. A use according to any one of Claims 97 to 99 for the treatment or prevention of baldness.
- 35

101. A use according to Claim 100 wherein the baldness is associated with a receding hairline.
- 5 102. A use according to Claim 100 or 101 wherein the baldness is associated with thinning hairline.
103. A use according to Claim 97 or 98 for encouraging growth of a beard, eyelash or eyebrow.
- 10 104. A use according to any one of Claims 97 to 103 wherein the mammal is human.
105. Use of a modified osteopontin polypeptide as defined in any one of Claims 1 to 37 *ex vivo* or *in vitro* for stimulating mammalian hair growth.
- 15 106. A use according to Claim 105 for stimulating hair growth on a skin explant prior to grafting of the explant on to a mammal.
107. A use according to Claim 105 for stimulating hair growth follicles (or stem cell precursors of the same) *in vitro*.
- 20 108. A method for making a composition according to any one of Claims 1 to 30, the method comprising admixing a modified osteopontin polypeptide in which an RGD domain is inactivated with a pharmaceutically acceptable and/or cosmetically acceptable excipient, carrier or diluent.
- 25 109. A composition for stimulating hair growth in mammals substantially as defined herein with reference to the description and figures.
110. A use substantially as defined herein with reference to the description.
- 30 111. A method substantially as defined herein with reference to the description.

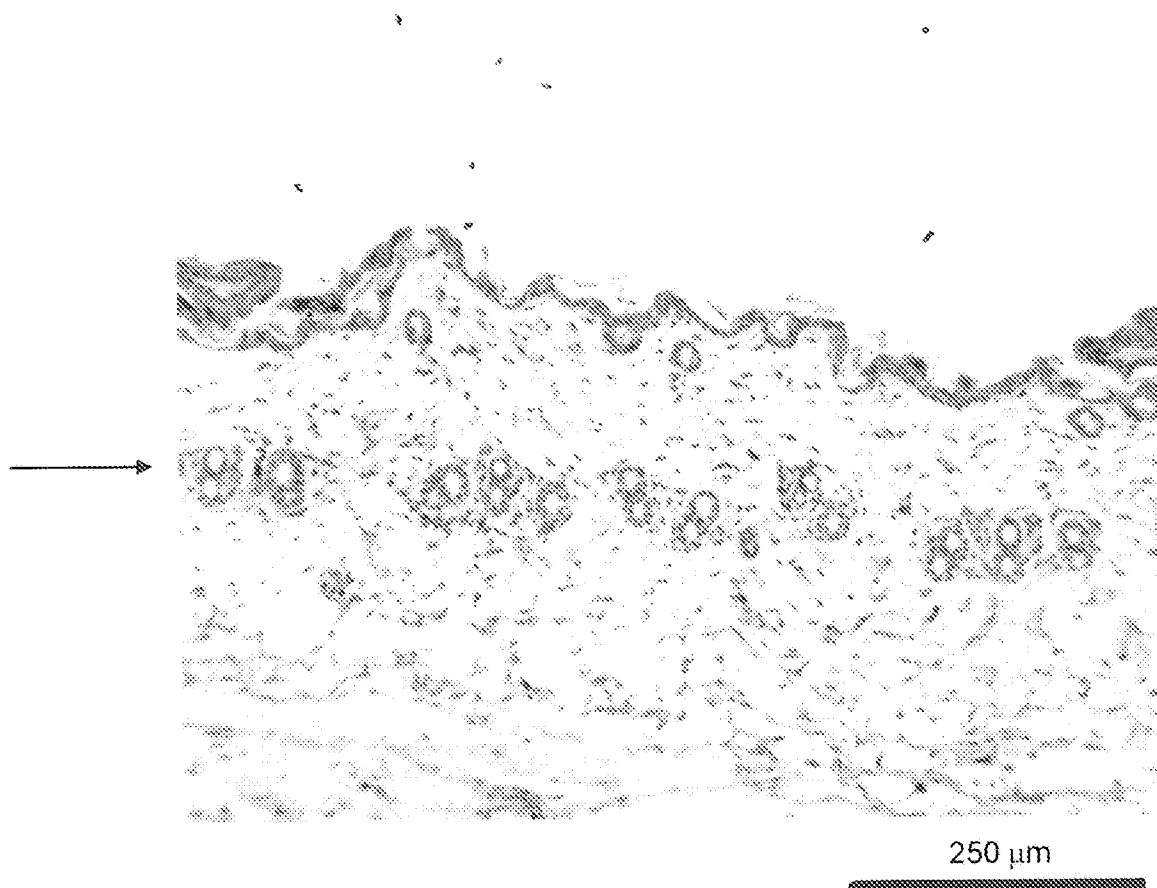
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FIGURE 1



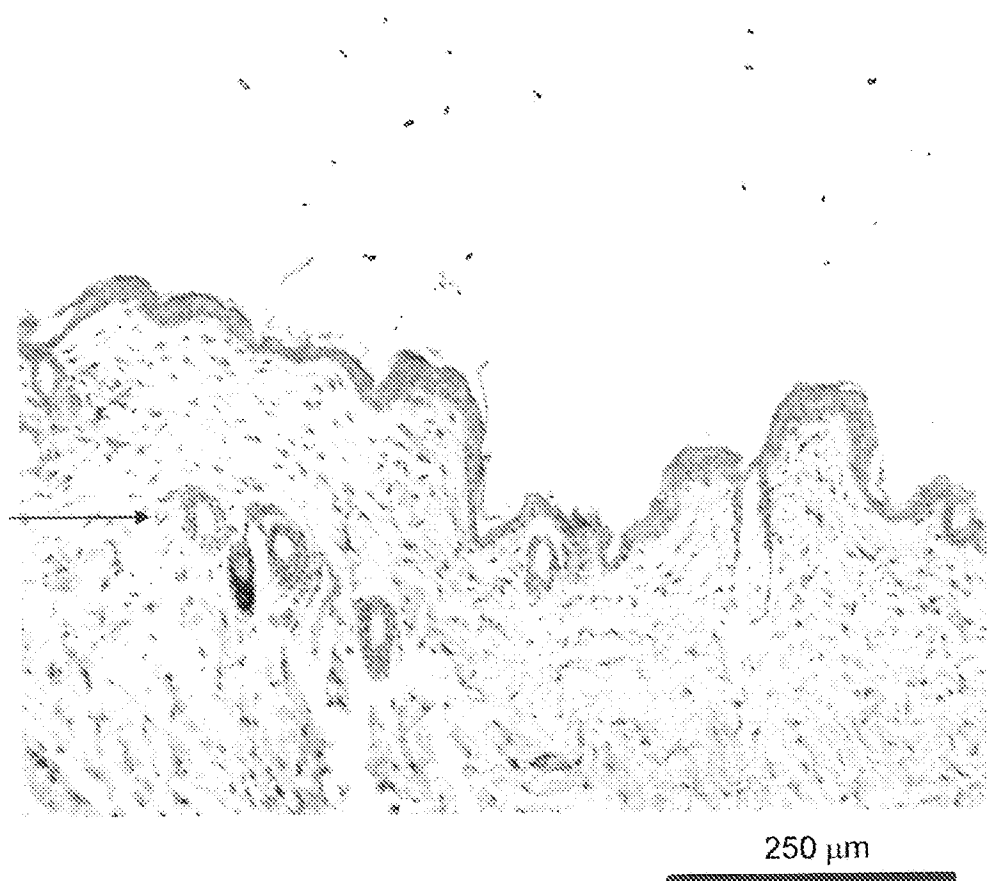
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FIGURE 2



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FIGURE 3



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FIGURE 4(A)



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FIGURE 4(B)



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FIGURE 4(C)



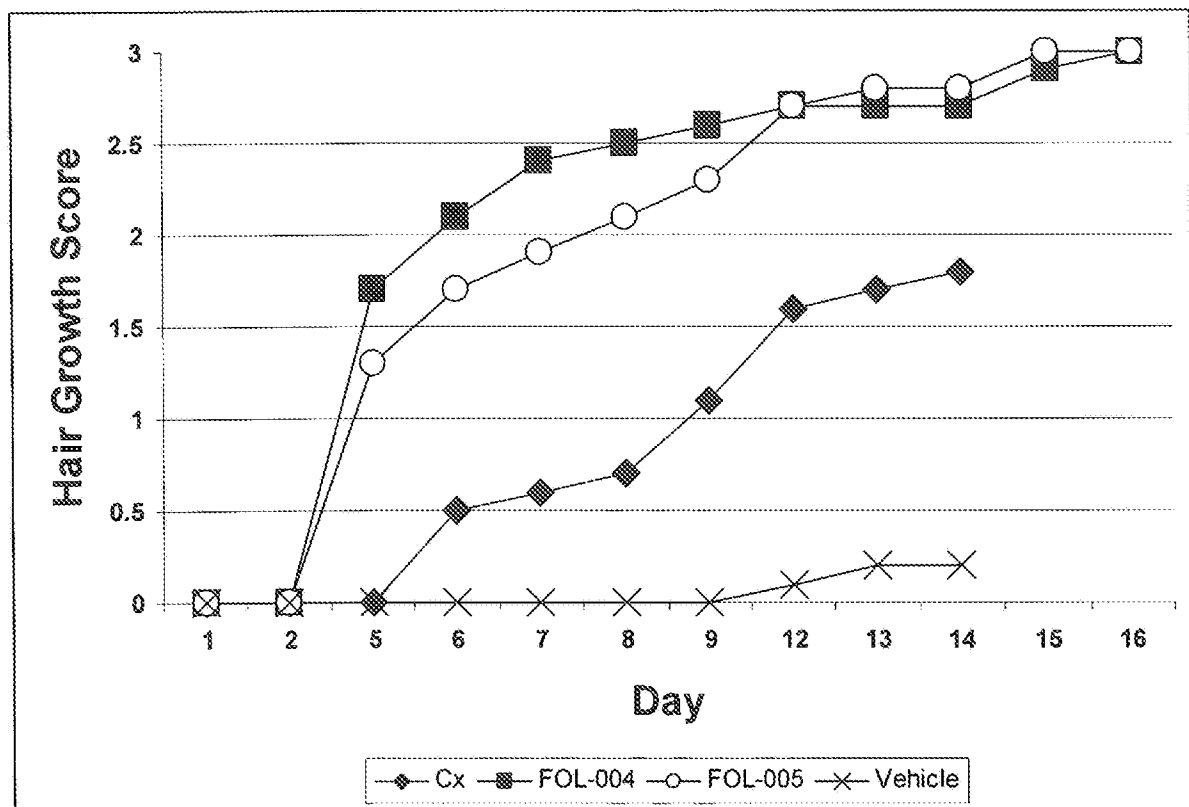
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FIGURE 4(D)



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FIGURE 5



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FIGURE 6

