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Synergistic selenopeptide formulations for the protection of dermal papilla cells

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ABSTRACT

The present invention discloses selenium peptide based synergistic compositions for the protection (morphology and viable numbers) of dermal papilla cells. The synergistic compositions disclosed in the present invention comprise (a) 1-O-galloyl-β-D-glucose (β-glucogallin) or 1-O-galloyl-β-D-glucose (β-glucogallin) and gallates (b) concentrate of liquid endosperm of *Cocos nucifera* and (c) selenopeptides.

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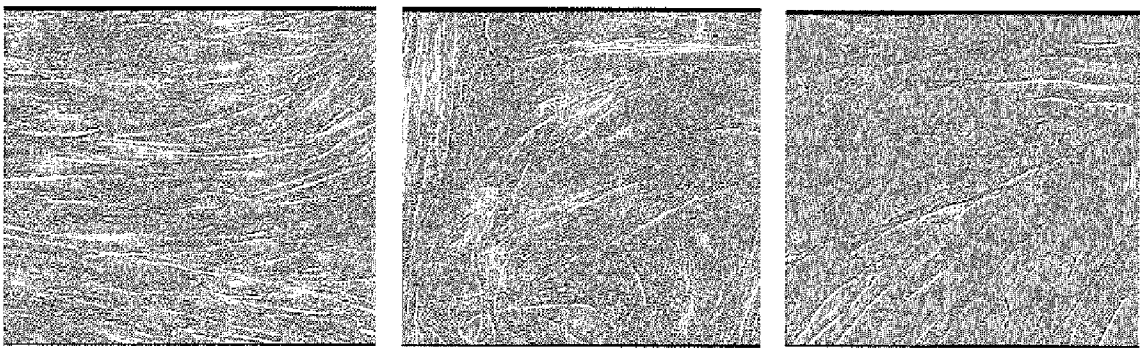
FIG.1

DERMAL PAPILLA CELLS TREATED WITH ONLY 0.5% CONCENTRATE OF LIQUID ENDOSPERM OF *Cocos nucifera*, CONCENTRATE COMPRISING NOT LESS THAN 40%W/W OF TOTAL DISSOLVED SOLIDS.



No UV exposure

UVB exposure of 0.432 J/cm^2

UVB exposure of 0.648 J/cm^2

Cell death shown at 0.432 J/cm^2 and 0.648 J/cm^2

Regulation 3.2

AUSTRALIA

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COMPLETE SPECIFICATION

FOR A STANDARD PATENT

ORIGINAL

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Invention Title:	SYNERGISTIC SELENOPEPTIDE FORMULATIONS FOR THE PROTECTION OF DERMAL PAPILLA CELLS

The following statement is a full description of this invention, including the best method of performing it known to me.

SYNERGISTIC SELENOPEPTIDE FORMULATIONS FOR THE PROTECTION OF DERMAL PAPILLA CELLS

[PARA 001] FIELD OF THE INVENTION

[PARA 002] The present disclosure relates to protective compositions for dermal papilla cells. More specifically, the present disclosure relates to formulations comprising synergistic compositions that include (a) β-glucogallin or β-glucogallin and gallates, (b) concentrate of liquid endosperm of *Cocos nucifera* and (c) selenopeptides, for the protection of dermal papilla cells.

[PARA 003] DESCRIPTION OF PRIOR ART

[Para 004] Dermal papilla cells are mesenchymal cells of the skin that not only regulate development of a hair peg but also constitute a reservoir of multi-potent stem cell lineages (Driskell et al., 2011). These stem cell lineages function as "tissue engineers" and are valued assets in regenerative medicine. Dermal papilla cells expressing the stem cell marker genes Sox 2 (transcription factor essential for the preservation of the pluripotent phenotype of stem cells) evince ability to self-renew, induce hair peg formation and differentiate into fibroblasts that aid the formation of skin extracellular matrix. In fact, dermal papilla plays a very vital role in replacement of senescent fibroblasts with healthy ones thereby maintaining fibroblast numbers. Recent studies (Arnold I. Caplan, Diego Correa. The MSC: An Injury Drugstore. Cell Stem Cell, Volume 9, Issue 1, 11-15, 8 July 2011 DOI: 10.1016/j.stem.2011.06.008) also indicate the role of mesenchymal stem cells (MSCs) as powerful "innate antidotes" in terms of their ability to (i) moderate unwarranted inflammatory responses that follow tissue damage, thus facilitating a conducive environ for automatic tissue repair; and (b) produce proteins that kill bacteria like *Escherichia coli* and *Staphylococcus aureus* and thus enhancing microbial clearance from the body systems. In view of the aforementioned diverse functions of dermal papilla cells, it is important to maintain the healthy state of these cells in terms of numbers and morphology and also protect their stem cell characteristics.

[PARA 005] The ability of selenopeptides gamma-L-glutamyl-Selenomethyl-L-selenocysteine and γ-L-glutamyl-L-Selenomethionine to enhance vascular endothelial growth factor (VEGF) and its 5-alpha reductase activity was documented in US8003614 (Majeed et al.). Surprisingly,

the present inventors note that selenopeptides though being poor protectants of dermal papilla cells by themselves, synergistically enhance the dermal papilla protective ability of the formulations disclosed by Majeed et al. in US 20110033565, said formulations comprising compositions that include (a) 1-O-galloyl-β-D-glucose (β-glucogallin) or β-glucogallin and gallates, and (b) concentrate of liquid endosperm of *Cocos nucifera*.

[PARA 006] As a result, synergistic selenopeptide formulations described herein find considerable application in maintaining morphologically healthy dermal papilla cells in sufficient numbers and thereby protecting the stem cell characteristics of the same.

[PARA 007] It is the principle objective of the present invention to disclose protective formulations comprising synergistic compositions that include (a) β-glucogallin or β-glucogallin and gallates, (b) concentrate of liquid endosperm of *Cocos nucifera* and (c) selenopeptides that protect dermal papilla cells from stress signals and associated applications thereof.

[PARA 008] The present invention fulfills the stated objective and provides further related advantages, and/or provides the public with a useful choice.

[PARA 009] SUMMARY OF THE INVENTION

[PARA 0010] In a first embodiment the present invention provides a synergistic dermal papilla cell protective formulation comprising: (a) at least 10% w/w 1-O-galloyl-β-D-glucose (β-glucogallin), (b) about 0.5% w/w concentrate of liquid endosperm of *Cocos nucifera*, said concentrate comprising at least 40% w/w of total dissolved solidsand (c) about 0.001% w/w of one or more selenopeptides selected from γ-L-glutamyl-Selenomethyl-L-selenocysteine and γ-L-glutamyl-L-Selenomethionine.

[PARA 0010a] In a second embodiment the present invention provides a method of increasing the tolerance of dermal papilla cells to stress signals, said method comprising step of bringing into contact the dermal papilla cells and the protective formulations of the invention.

[PARA 0010b] In a third embodiment the present invention provides a method of maintaining the morphology and numbers of dermal papilla cells during exposure to stress signals, said method comprising step of bringing into contact the dermal papilla cells and the protective formulation of the invention.

[PARA 0010c] Also described are formulations comprising synergistic compositions including (a) β-glucogallin or β-glucogallin and gallates (b) concentrate of liquid endosperm of *Cocos nucifera* and (c) selenopeptides for the protection of dermal papilla cells from stress signals.

[PARA 0011] BRIEF DESCRIPTION OF THE DRAWINGS

[PARA 0012] FIG.1 shows the photomicrographs of dermal papilla cells treated with only 0.5% concentrate of liquid endosperm of *Cocos nucifera*, said concentrate comprising not less than 40%w/w of total dissolved solids. The liquid endosperm was unable to protect dermal papilla cells singly. Fig. 1(a) shows photomicrographs of the cells with no UV exposure. Fig. 1(b) shows photomicrographs of the cells with UVB exposure of 0.432 J/cm^2. Fig. 1(c) shows photomicrographs of the cells with UVB exposure of 0.648 J/cm^2. Figs. 1(b) and 1(c) show cell death.

[PARA 0013] FIG.2 shows the photomicrographs of UVB irradiated dermal papilla cells treated with protective formulation PC-1 (compositions comprising at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin) in comparison with UVB irradiated untreated cells. PC-1 protects dermal papillary cells from UVB exposure of up to 0.43 J/cm^2. PC-1 is unable to protect dermal papilla cells from UVB dosage above 0.43 J/cm^2. Cell damage in PC-1 treated cells occurs at UVB dosage of 0.648 J/cm^2 (shown as part of FIG.4).

[PARA 0014] FIG.3 shows the photomicrographs of UVB irradiated dermal papilla cells treated with protective formulation PC-2 (compositions comprising at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin) and 50% to greater than 50% w/w of gallates) in comparison with UVB irradiated untreated cells. PC-2 protects dermal papillary cells from UVB exposure of up to 0.43 J/cm^2. PC-2 is unable to protect dermal papilla cells from UVB dosage above 0.43 J/cm^2. Cell damage in PC-2 treated cells occurs at UVB dosage of 0.648 J/cm^2 (shown as part of FIG.5).

[PARA 0015] FIG.4 shows the photomicrographs of UVB irradiated dermal papilla cells treated with protective formulation PC-3 (PC-1+0.5% concentrate of liquid endosperm of *Cocos nucifera*, said concentrate comprising not less than 40% w/w of total dissolved solids). Fig. 4(a) shows cell damage upon treatment with PC-1 at 0.648 J/cm^2. Fig. 4(b) shows intact dermal papilla cells upon treatment with PC-3 at 0.648 J/cm^2. PC-3 protects dermal papilla cells from

UVB exposure levels of up to only 0.648 J/cm^2 and not 0.8 J/cm^2. Cell death at 0.8 J/cm^2 is shown in Fig. 4(c).

[PARA 0016] FIG.5 shows the photomicrographs of UVB irradiated dermal papilla cells treated with protective formulation PC-4 (PC-2+0.5% concentrate of liquid endosperm of *Cocos nucifera*, said concentrate comprising not less than 40%w/w of total dissolved solids). Fig. 5(a) shows cell damage upon treatment with PC-2 at 0.648 J/cm^2. Fig. 5(b) shows intact dermal papilla cells upon treatment with PC-4 at 0.648 J/cm^2. PC-4 protects dermal papilla cells at UVB exposure levels of only up to 0.648 J/cm^2 and not 0.8 J/cm^2. Cell death at 0.8 J/cm^2 is shown in Fig. 5(c).

[PARA 0017] FIG.6 shows the photomicrographs of UVB irradiated dermal papilla cells treated with protective formulation PC-5 (PC-3 + 0.001% w/w of γ-L-glutamyl-Selenomethyl-L-selenocysteine). Fig. 6(a) shows cells treated with PC-3 alone at a UVB dose of 0.8 J/cm^2. PC-3 did not show significant protection at this dose. Fig. 6(b) shows cells treated with PC-5 at a UVB dose of 0.8 J/cm^2. PC-5 provides significant (95%) protection to dermal papilla cells exposed to UVB radiation of 0.8 J/cm^2.

[PARA 0018] FIG.7 shows the photomicrographs of UVB irradiated dermal papilla cells treated with protective formulation PC-6 (PC-3 + 0.001% w/w of γ-L-glutamyl-L-Selenomethionine). Fig. 7(a) shows cells treated with PC-3 alone at a UVB dose of 0.8 J/cm^2. Fig. 7(b) shows cells treated with PC-6 at a UVB dose at 1.0 J/cm^2. PC-6 provides significant (95%) protection to dermal papilla cells exposed to UVB radiation of 1.0 J/cm^2.

[PARA 0019] FIG.8 shows the photomicrographs of UVB irradiated dermal papilla cells treated with protective formulation PC-7 (PC-4+0.001%w/w of γ-L-glutamyl-Selenomethyl-L-selenocysteine). Fig. 8(a) shows cell damage upon treatment with PC-4 at 0.8 J/cm^2. Fig 8(b) shows intact dermal papilla cells upon treatment with PC-7 at 0.8 J/cm^2. PC-7 provides significant (95%) protection to dermal papilla cells exposed to UVB radiation of 0.8 J/cm^2.

[PARA 0020] FIG.9 shows the photomicrographs of UVB irradiated dermal papilla cells treated with protective formulation PC-8 (PC-4+0.001% w/w of γ-L-glutamyl-L-Selenomethionine). Fig. 9(a) shows cell damage upon treatment with PC-4 at 0.8 J/cm^2. Fig. 9(b) shows intact

dermal papilla cells upon treatment with PC-8 at 1.0 J/cm^2. PC-8 provides significant (95%) protection to dermal papilla cells exposed to very high doses of UVB radiation of 1.0 J/cm^2.

[PARA 0021] FIG.10 shows the photomicrographs of UVB irradiated dermal papilla cells treated with protective formulation PC-9 (0.001% w/w of γ-L-glutamyl-Selenomethyl-L-selenocysteine (FIG.10a) or 0.001% w/w of γ-L-glutamyl-L-Selenomethionine (FIG.10b)). Both cell samples were treated with a UVB dose of 0.432 J/cm^2. PC-9 provides no protection to dermal papilla cells exposed to even low level of UVB radiation (UVB dose of 0.432 J/cm^2).

[PARA 0022] <u>DESCRIPTION OF THE INVENTION</u>

[PARA 0023] Described herein is a dermal papilla cell protective formulation comprising synergistic composition, said composition including 1-O-galloyl-β-D-glucose (β-glucogallin), concentrate of liquid endosperm of *Cocos nucifera* and selenopeptides.

[PARA 0024] In another embodiment described herein, the synergistic composition comprises at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin).

[PARA 0025] In yet another embodiment described herein, the synergistic composition comprises 0.5% w/w of the concentrate from the liquid endosperm of Cocos nucifera, said concentrate comprising not less than 40% w/w of total dissolved solids.

[PARA 0026] In still another embodiment described herein, the synergistic composition comprises 0.001% w/w of selenopeptides.

[PARA 0027] In still another embodiment described herein, the selenopeptide is γ-L-glutamyl-Selenomethyl-L-selenocysteine.

[PARA 0028] In still another embodiment described herein, the selenopeptide is γ-L-glutamyl-L-Selenomethionine.

[PARA 0029] The present disclosure relates to dermal papilla cell protective formulation comprising synergistic composition, said composition including 1-O-galloyl-β-D-glucose (β-glucogallin) and gallates, concentrate of liquid endosperm of *Cocos nucifera* and selenopeptides.

[PARA 0030] In still another embodiment described herein, the synergistic composition comprises at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin) and 50% to greater than 50% total gallates including mucic acid 1, 4-lactone-5-O-gallate, mucic acid 2-O-gallate, mucic acid 6-methyl ester 2-O-gallate, mucic acid 1-methyl ester 2-O-gallate and ellagic acid.

[PARA 0031] In still another embodiment described herein, the synergistic composition comprises 0.5% w/w of the concentrate from the liquid endosperm of *Cocos nucifera*, said concentrate comprising not less than 40% w/w of total dissolved solids.

[PARA 0032] In still another embodiment described herein, the synergistic composition comprises 0.001% w/w of selenopeptides.

[PARA 0033] In still another embodiment described herein, the selenopeptide is γ-L-glutamyl-Selenomethyl-L-selenocysteine.

[PARA 0034] In still another embodiment described herein, the selenopeptide is γ-L-glutamyl-L-Selenomethionine.

[PARA 0035] The present disclosure also relates to a method of increasing the tolerance of dermal papilla cells to stress signals, said method comprising step of bringing into contact the dermal papilla cells and the protective formulations described herein.

[PARA 0036] The present disclosure also relates to a method of maintaining the morphology and numbers of dermal papilla cells during exposure to stress signals, said method comprising step of bringing into contact the dermal papilla cells and the protective formulation described herein.

[PARA 0037] In still another embodiment described herein, the dermal papilla cells include dermal stem/progenitor cells.

[PARA 0038] In the most preferred embodiment, the present disclosure relates to the following synergistic selenopeptide formulations for the protection of dermal papilla cells.

[PARA 0039] (A) PC-5 comprising synergistic compositions, said compositions including (a) 1-O-galloyl-β-D-glucose (β-glucogallin); (b) concentrate of liquid endosperm of *Cocos nucifera,* said concentrate including at least 40% dissolved solids and (c) γ-L-glutamyl-Selenomethyl-L-

selenocysteine. In specific embodiments, said compositions comprise (a) at least 10% w/w 1-O-galloyl-β-D-glucose (β-glucogallin); (b) 0.5% w/w concentrate of liquid endosperm of *Cocos nucifera,* said concentrate including at least 40% dissolved solids; and (c) 0.001% w/w of γ-L-glutamyl-Selenomethyl-L-selenocysteine.

[PARA 0040] (B) PC-6 comprising synergistic compositions, said compositions including (a) 1-O-galloyl-β-D-glucose (β-glucogallin); (b) concentrate of liquid endosperm of *Cocos nucifera,* said concentrate including at least 40% dissolved solids and (c) γ-L-glutamyl-L-Selenomethionine. In specific embodiments, said compositions comprise (a) at least 10% w/w 1-O-galloyl-β-D-glucose (β-glucogallin); (b) 0.5% w/w of concentrate of liquid endosperm of Cocos nucifera, said concentrate including at least 40% dissolved solids; and (c) 0.001% w/w of γ-L-glutamyl-L-Selenomethionine.

[PARA 0041] (C) PC-7 comprising synergistic compositions, said compositions including, (a) 1-O-galloyl-β-D-glucose (β-glucogallin) and gallates; (b) concentrate of liquid endosperm of *Cocos nucifera,* said concentrate including atleast 40% dissolved solids; and (c) γ-L-glutamyl-Selenomethyl-L-selenocysteine. In specific embodiments, said compositions comprise (a) at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin) and 50% to greater than 50% w/w of total gallates including mucic acid 1, 4-lactone-5-O-gallate, mucic acid 2-O-gallate, mucic acid 6-methyl ester 2-O-gallate, mucic acid 1-methyl ester 2-O-gallate and ellagic acid; (b) 0.5% w/w of concentrate of liquid endosperm of *Cocos nucifera,* said concentrate including at least 40% dissolved solids and (c) 0.001% w/w of γ-L-glutamyl-Selenomethyl-L- selenocysteine.

[PARA 0042] (D) PC-8 comprising synergistic compositions, said compositions including, (a) 1-O-galloyl-β-D-glucose (β-glucogallin) and gallates; (b) concentrate of liquid endosperm of *Cocos nucifera,* said concentrate including atleast 40% dissolved solids; and (c) γ-L-glutamyl-L-Selenomethionine. In specific embodiments, said compositions comprise (a) at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin) and 50% to greater than 50% w/w of total gallates including mucic acid 1, 4-lactone-5-O-gallate, mucic acid 2-O-gallate, mucic acid 6-methyl ester 2-O-gallate, mucic acid 1-methyl ester 2-O-gallate and ellagic acid; (b) 0.5% w/w concentrate of liquid endosperm of *Cocos nucifera,* said concentrate including at least 40% dissolved solids and (c) 0.001% w/w of γ-L-glutamyl-L-Selenomethionine.

[PARA 0043] In other preferred embodiments of the disclosure, other dipeptides occurring as combinations with other amino acids may be used in the aforesaid synergistic dermal papilla cell protective formulations.

[PARA 0044] Also described is a method of increasing the tolerance of dermal papilla cells to stress signals, said method comprising step of bringing into contact dermal papilla cells and the protective formulation PC-5 comprising synergistic compositions, said compositions including, (a) 1-O-galloyl-β-D-glucose (β-glucogallin); (b) concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including at least 40% dissolved solids and (c) γ-L-glutamyl-Selenomethyl-L-selenocysteine. In specific embodiments, said compositions comprise (a) at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin); (b) 0.5% w/w concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including at least 40% dissolved solids; and (c) 0.001% w/w of γ-L-glutamyl-Selenomethyl-L-selenocysteine.

[PARA 0045] Also described is a method of increasing the tolerance of dermal papilla cells to stress signals, said method comprising step of bringing into contact dermal papilla cells and the protective formulation PC-6 comprising synergistic compositions, said compositions including, (a) 1-O-galloyl-β-D-glucose (β-glucogallin); (b) concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including at least 40% dissolved solids and (c) γ-L-glutamyl-L-Selenomethionine. In specific embodiments, said compositions comprise (a) at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin); (b) 0.5% w/w concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including at least 40% dissolved solids; and (c) 0.001% w/w of γ-L-glutamyl-L-Selenomethionine.

[PARA 0046] Also described is a method of increasing the tolerance of dermal papilla cells to stress signals, said method comprising step of bringing into contact dermal papilla cells and protective formulation PC-7 comprising synergistic compositions, said compositions including, (a) 1-O-galloyl-β-D-glucose (β-glucogallin)and gallates; (b) concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including atleast 40% dissolved solids; and (c) γ-L-glutamyl-Selenomethyl-L-selenocysteine. In specific embodiments, said compositions comprise (a) at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin) and 50% w/w to greater than 50% w/w of total gallates including mucic acid 1, 4-lactone-5-O-gallate, mucic acid 2-O-gallate, mucic acid 6-methyl ester 2-O-gallate, mucic acid 1-methyl ester 2-O-gallate and ellagic acid; (b) 0.5% w/w

concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including at least 40% dissolved solids and (c) 0.001% w/w of γ-L-glutamyl-Selenomethyl-L-selenocysteine.

[PARA 0047] Also described is a method of increasing the tolerance of dermal papilla cells to stress signals, said method comprising step of bringing into contact dermal papilla cells and protective formulation PC-8 comprising of synergistic compositions, said compositions including, (a) 1-O-galloyl-β-D-glucose (β-glucogallin) and gallates; (b) concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including atleast 40% dissolved solids; and (c) γ-L-glutamyl-L-Selenomethionine. In specific embodiments, said compositions comprise (a) at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin) and 50% w/w to greater than 50% w/w of total gallates including mucic acid 1, 4-lactone-5-O-gallate, mucic acid 2-O-gallate, mucic acid 6-methyl ester 2-O-gallate, mucic acid 1-methyl ester 2-O-gallate and ellagic acid; (b) 0.5% w/w concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including at least 40% dissolved solids and (c) 0.001% w/w of γ-L-glutamyl-L-Selenomethionine.

[PARA 0048] Also described is a method of maintaining the morphology and numbers of dermal papilla cells during exposure to stress signals, said method comprising step of bringing into contact dermal papilla cells and the protective formulation PC-5 comprising synergistic compositions, said compositions including, (a) 1-O-galloyl-β-D-glucose (β-glucogallin); (b) concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including at least 40% dissolved solids and (c) γ-L-glutamyl-Selenomethyl-L-selenocysteine. In specific embodiments, said compositions comprise (a) at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin); (b) 0.5% w/w concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including at least 40% dissolved solids; and (c) 0.001% w/w of γ-L-glutamyl-Selenomethyl-L-selenocysteine.

[PARA 0049] Also described is a method of maintaining the morphology and numbers of dermal papilla cells during exposure to stress signals, said method comprising step of bringing into contact dermal papilla cells and the protective formulation PC-6 comprising synergistic compositions, said compositions including, (a) 1-O-galloyl-β-D-glucose (β-glucogallin); (b) concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including at least 40% dissolved solids and (c) γ-L-glutamyl-L-Selenomethionine. In specific embodiments, said compositions comprise (a) at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin); (b)

0.5% w/w concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including at least 40% dissolved solids; and (c) 0.001% w/w of γ-L-glutamyl-L-Selenomethionine.

[PARA 0050] Also described is a method of maintaining morphology and numbers of dermal papilla cells during exposure to stress signals, said method comprising step of bringing into contact dermal papilla cells and protective formulation PC-7 comprising synergistic compositions, said compositions including, (a) β-glucogallin and gallates; (b) concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including atleast 40% dissolved solids; and (c) γ-L-glutamyl-Selenomethyl-L-selenocysteine. In specific embodiments, said compositions comprise (a) at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin) and 50% w/w to greater than 50% w/w of total gallates including mucic acid 1, 4-lactone-5-O-gallate, mucic acid 2-O-gallate, mucic acid 6-methyl ester 2-O-gallate, mucic acid 1-methyl ester 2-O-gallate and ellagic acid; (b) 0.5% w/w concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including at least 40% dissolved solids and (c) 0.001% w/w of γ-L-glutamyl-Selenomethyl-L-selenocysteine.

[PARA 0051] Also described is a method of maintaining the morphology and viable numbers of dermal papilla cells during exposure to stress signals, said method comprising step of bringing into contact dermal papilla cells and protective formulation PC-8 comprising of synergistic compositions, said compositions including, (a) β-glucogallin and gallates; (b) concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including atleast 40% dissolved solids; and (c) γ-L-glutamyl-L-Selenomethionine. In specific embodiments, said compositions comprise (a) at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin) and 50% w/w to greater than 50% w/w of total gallates including mucic acid 1, 4-lactone-5-O-gallate, mucic acid 2-O-gallate, mucic acid 6-methyl ester 2-O-gallate, mucic acid 1-methyl ester 2-O-gallate and ellagic acid; (b) 0.5% w/w concentrate of liquid endosperm of *Cocos nucifera*, said concentrate including at least 40% dissolved solids and (c) 0.001% w/w of γ-L-glutamyl-L-Selenomethionine.

[PARA 0052] In a more specific embodiment, the dermal papilla cells mentioned herein above comprise dermal stem/progenitor cells.

[PARA 0053] EXAMPLE I

[PARA 0054] GENERAL PROCEDURE

[PARA 0055] Human dermal papilla cells were plated into a 96 well flat bottomed clear micro plate at a seeding density of 5000 cells per well. The 24 hour monolayer of cells was exposed to UVB dosages ranging from 0.0072 J/cm^2 to 1.0 J/cm^2 (stress signal) with or without sample (protective formulations) treatment. After exposure, the cells were incubated in a CO_2 incubator for 48 hours and developed by NRU (Neutral Red Uptake) staining techniques to analyze cell viability. The absorbance due to viable cells is read at 492nm in a micro plate reader.

[PARA 0056] Sample tested- 0.5% concentrate of liquid endosperm of *Cocos nucifera,* said concentrate comprising not less than 40% w/w of total dissolved solids.

[PARA 0057] FIG.1 shows that the 0.5% concentrate of liquid endosperm of *Cocos nucifera*, said concentrate comprising not less than 40%w/w of total dissolved solids is unable to protect dermal papilla cells singly at 0.43 J/cm^2 and 0.648 J/cm^2 UVB exposure levels.

[PARA 0058] Sample tested: PC-1 in comparison with UVB irradiated untreated cells.

[PARA 0059] FIG.2 shows PC-1 by itself is able to protect dermal papilla cells from UVB exposure levels of only up to 0.43 J/cm^2.

[PARA 0060] Sample tested: PC-2 in comparison with UVB irradiated untreated cells.

[PARA 0061] FIG.3 shows PC-2 by itself is able to protect dermal papilla cells from UVB exposure levels of only up to 0.43 J/cm^2.

[PARA 0062] Sample tested: PC-3

[PARA 0063] FIG.4 shows that PC-3 is able to protect dermal papilla cells from UVB exposure levels of up to 0.648 J/cm^2 and death of PC-3 treated dermal papilla cells is seen at UVB exposure level of 0.8 J/cm^2.

[PARA 0064] Sample tested: PC-4.

[PARA 0065] FIG.5 shows that PC-4 is able to protect dermal papilla cells from UVB exposure levels of up to at 0.648 J/cm^2 UVB exposure levels and death of PC-4 treated dermal papilla cells is seen at UVB exposure level of 0.8 J/cm^2.

[PARA 0066] Sample tested-PC-9

[PARA 0067] FIG.10 shows that neither γ-L-glutamyl-Selenomethyl-L-selenocysteine (FIG.10a) nor γ-L-glutamyl-L-Selenomethionine (FIG.10b) by themselves are able to protect dermal papilla cells even at low levels of UVB exposure (0.432 J/cm^2).

[PARA 0068] Sample tested: PC-5

[PARA 0069] FIG.6 shows that PC-5 provides significant (95%) protection to dermal papilla cells exposed to UVB radiation levels of 0.8 J/cm^2.

[PARA 0070] Sample tested: PC-6

[PARA 0071] FIG.7 shows that PC-6 provides significant (95%) protection to dermal papilla cells exposed to UVB radiation levels of 1.0 J/cm^2.

[PARA 0072] Sample tested: PC-7

[PARA 0073] FIG.8 shows that PC-7 provides significant (95%) protection to dermal papilla cells exposed to UVB radiation levels of 0.8 J/cm^2.

[PARA 0074] Sample tested: PC-8

[PARA 0075] FIG.9 shows that PC-8 provides significant (95%) protection to dermal papilla cells exposed to UVB radiation levels of 1.0 J/cm^2.

[PARA 0076] The effect of various samples used in the invention is presented herein below as Table 1.

Table 1

Samples (Protective formulations tested)	UVB irradiation levels on dermal papilla cells		
	0.432 J/cm^2	**0.648 J/cm^2**	**0.8 or 0.1 J/cm^2**
Concentrate of liquid endosperm of *Cocos nucifera*	No protection (cell death observed)	-	-
PC-1 Compositions comprising at least 10% w/w or greater of 1-O-galloyl-β-D-glucose (β-glucogallin)	Provides protection	No protection (Causes cell damage)	-
PC-2 Compositions comprising at least 10% w/w or greater of β-glucogallin and 50% w/w or greater than 50% w/w of gallates.	Provides protection	No protection (Causes cell damage)	-
PC-3 (PC-1 + concentrate of liquid endosperm of *Cocos nucifera)*	Provides protection	Provides protection	No protection
PC-4 (PC-2 + concentrate of liquid endosperm of *Cocos nucifera)*	Provides protection	Provides protection	No protection
γ-L-glutamyl-Selenomethyl-L-selenocysteine (PC-9)	No protection	-	-
γ-L-glutamyl-L-Selenomethionine (PC-9)	No protection	-	-
PC-5 (PC-3 + γ-L-glutamyl-Selenomethyl-L-selenocysteine)	Provides protection	Provides protection	Provides protection
PC-6 (PC-3 + γ-L-glutamyl-L-Selenomethionine)	Provides protection	Provides protection	Provides protection
PC-7 (PC-4 + γ-L-glutamyl-Selenomethyl-L-selenocysteine)	Provides protection	Provides protection	Provides protection
PC-8 (PC-4 + γ-L-glutamyl-L-Selenomethionine)	Provides protection	Provides protection	Provides protection

[PARA 0077] From the results, it is evident that

A. The selenopeptides and concentrate of liquid endosperm of *Cocos nucifera* singly do not confer protection to dermal papilla cells at even low levels of UVB exposure (0.432 J/cm^2).

B. β-glucogallin or β-glucogallin and gallates are able to provide protection of dermal papilla cells only up to UVB exposure levels of 0.432 J/cm^2.

C. Although the combination of concentrate of liquid endosperm of *Cocos nucifera* and β-glucogallin or β-glucogallin and gallates provides protection to dermal papilla cells from UVB exposure levels up to 0.648 J/cm^2, said protection does not extend beyond this level. Rather, the tolerance level to the tested stress signal is 0.648 J/cm^2.

D. However, the combination of (a) β-glucogallin or β-glucogallin and gallates, (b) concentrate of liquid endosperm of *Cocos nucifera* and (c) selenopeptides extend protection to dermal papilla cells at UVB exposure levels even beyond 0.648 Jcm^{-2}, specifically between 0.8-1.0 J/cm^2. Selenopeptides though being poor protectants of dermal papilla cells by themselves, synergistically enhance the dermal papilla cell protective ability of formulations comprising (a) 1-O-galloyl-β-D-glucose (β-glucogallin) or 1-O-galloyl-β-D-glucose (β-glucogallin) and gallates and (b) the concentrate of liquid endosperm of *Cocos nucifera.* Thus an unexpected improved tolerance of dermal papilla cells to stress signals conferred by the synergistic combination of (a) 1-O-galloyl-β-D-glucose (β-glucogallin) or 1-O-galloyl-β-D-glucose (β-glucogallin) and gallates, (b) concentrate of liquid endosperm of Cocos nucifera and (c) selenopeptides is clear from the present disclosure. The composition described herein shows superior activity when compared to individual components or other combinations.

It is to be understood that though the present invention has been described with reference to specific preferred examples, it is possible for persons having ordinary skill in the art to make modifications and variations without departing from the spirit of the invention. Accordingly, the foregoing disclosure should be interpreted as illustrative only and not in a limiting sense. The present invention is limited only by the scope of appended claims which also includes the scope of equivalents.

[PARA 0079] The term "comprising" as used in this specification and claims means "consisting at least in part of". When interpreting statements in this specification and claims which include "comprising", other features besides the features prefaced by this term in each statement can also be present. Related terms such as "comprise" and "comprised" are to be interpreted in a similar manner.

[PARA 0080] In this specification, where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be construed as an admission that such documents, or such sources of information, in any jurisdiction, are prior art, or form any part of the common general knowledge in the art.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1) A synergistic dermal papilla cell protective formulation comprising: (a) at least 10% w/w 1-O-galloyl-β-D-glucose (β-glucogallin), (b) about 0.5% w/w concentrate of liquid endosperm of *Cocos nucifera*, said concentrate comprising at least 40% w/w of total dissolved solidsand (c) about 0.001% w/w of one or more selenopeptides selected from γ-L-glutamyl-Selenomethyl-L-selenocysteine and γ-L-glutamyl-L-Selenomethionine.

2) The formulation of claim 1, additionally comprising at least 50% w/w total gallates selected from the group comprising mucic acid, 1,4-lactone-5-O-gallate, mucic acid 2-O-gallate, mucic acid 6-methyl ester 2-O-gallate, mucic acid 1-methyl ester 2-O-gallate and ellagic acid.

3) A method of increasing the tolerance of dermal papilla cells to stress signals, said method comprising step of bringing into contact the dermal papilla cells and the protective formulations as claimed in claims 1 or 2.

4) A method of maintaining the morphology and numbers of dermal papilla cells during exposure to stress signals, said method comprising step of bringing into contact the dermal papilla cells and the protective formulation as claimed in claims 1 or 2.

5) The methods of claims 3 or 4, wherein the dermal papilla cells include dermal stem/progenitor cells.

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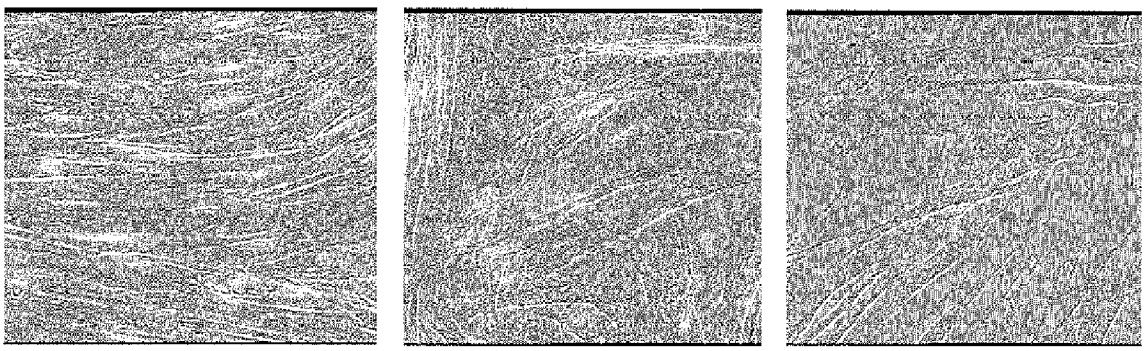
FIG.1



1(a) 1(b) 1(c)

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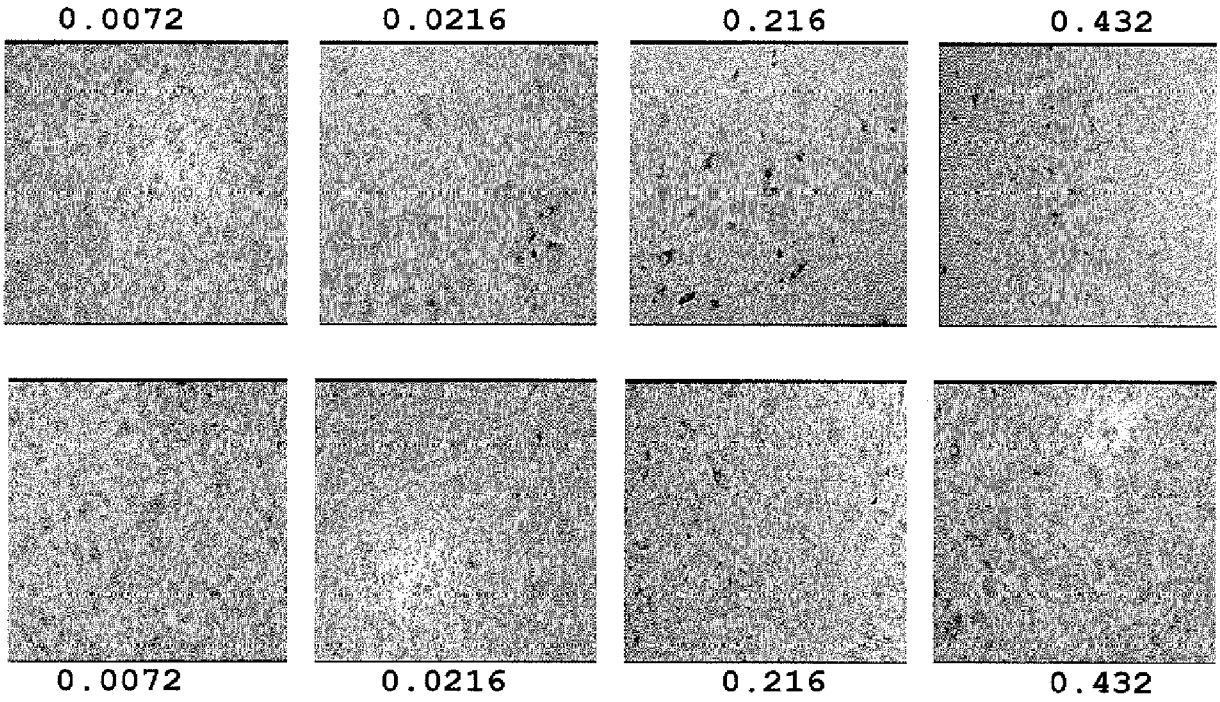
FIG.2

Increasing dosages of UV (J/cm^2)

Row I: Untreated cells



Row II: PC-1 treated cells

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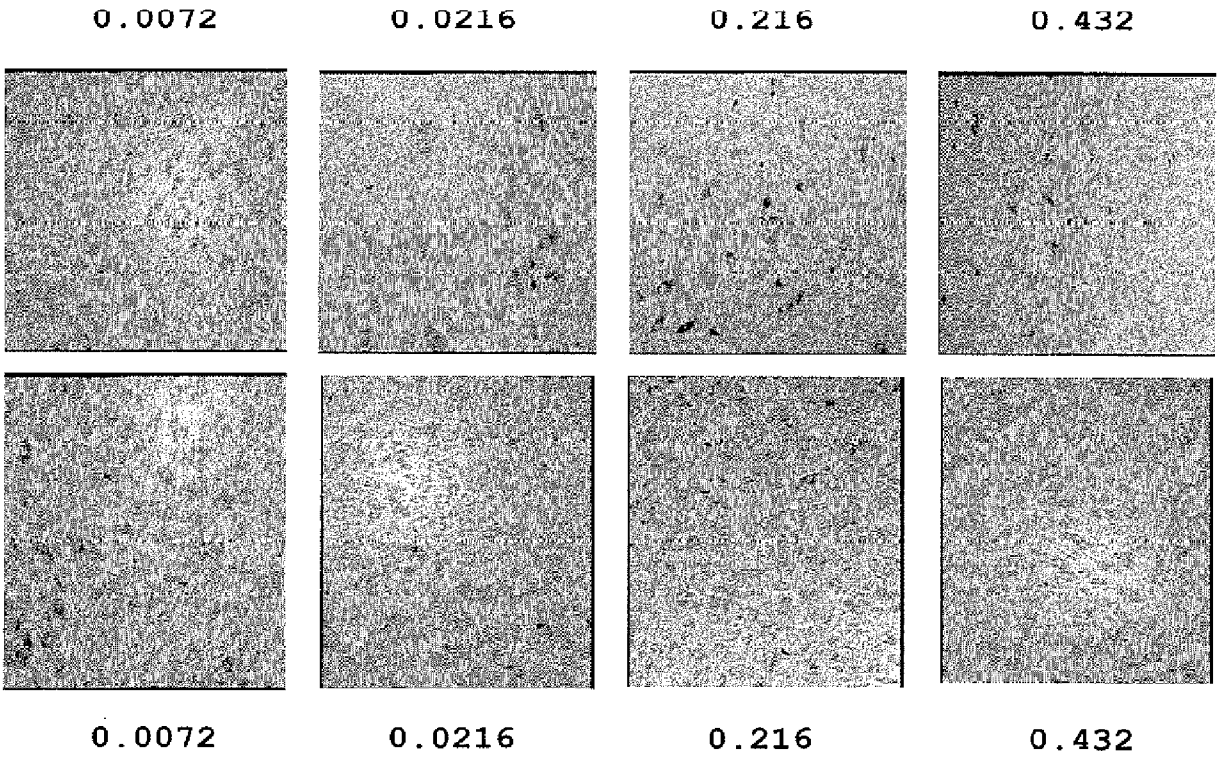
FIG.3

Increasing dosages of UV (J/cm^2)

Row I: Untreated cells



Row II: PC-2 treated cells

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FIG.4

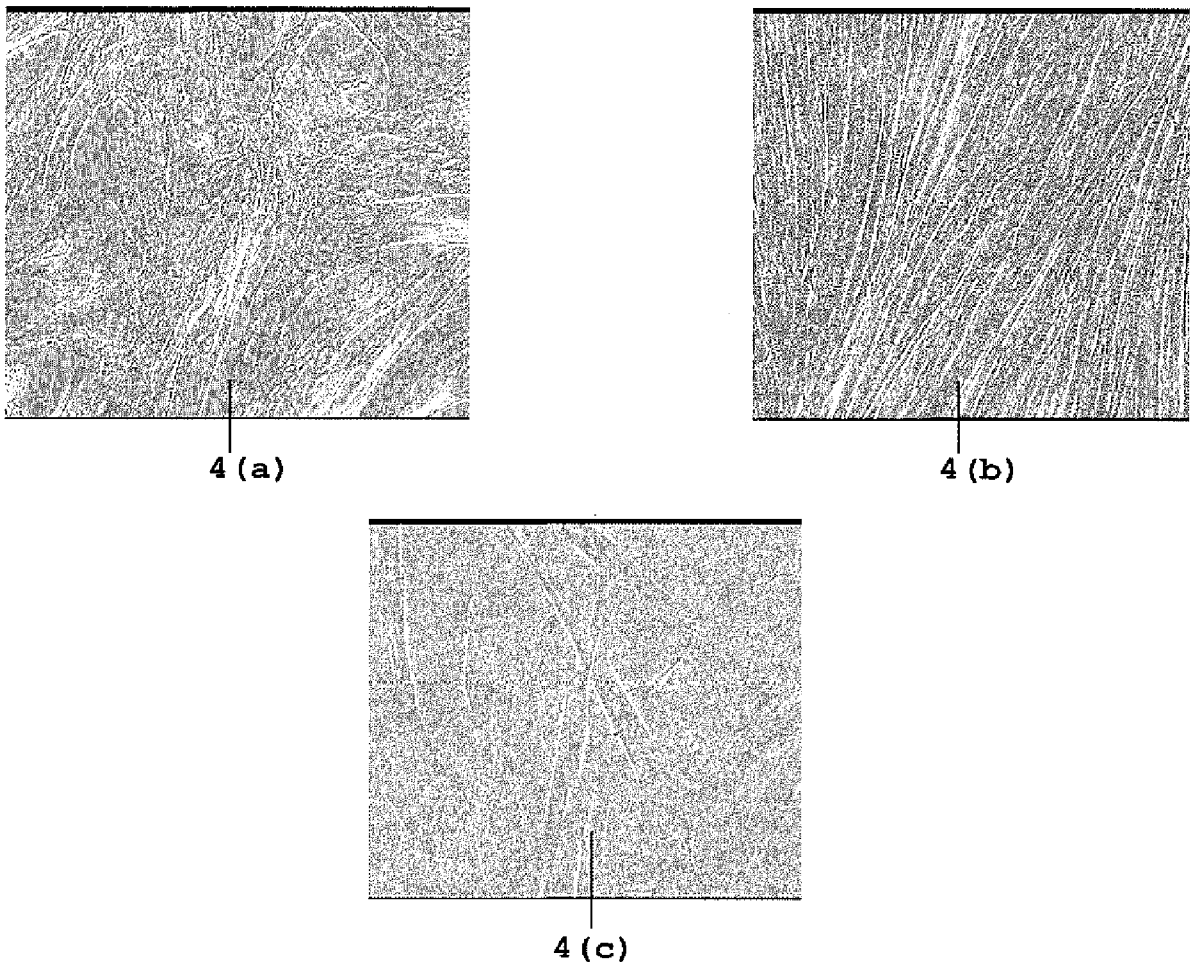

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FIG.5

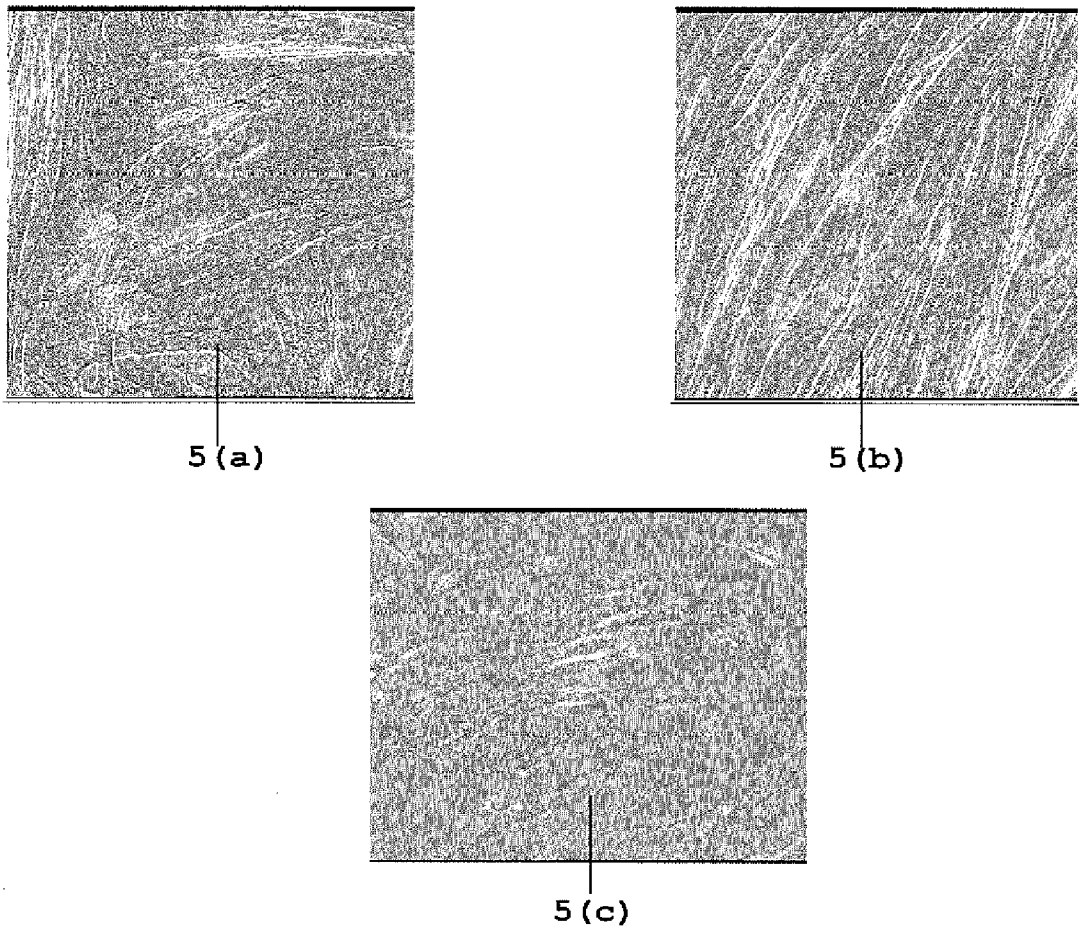

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FIG.6

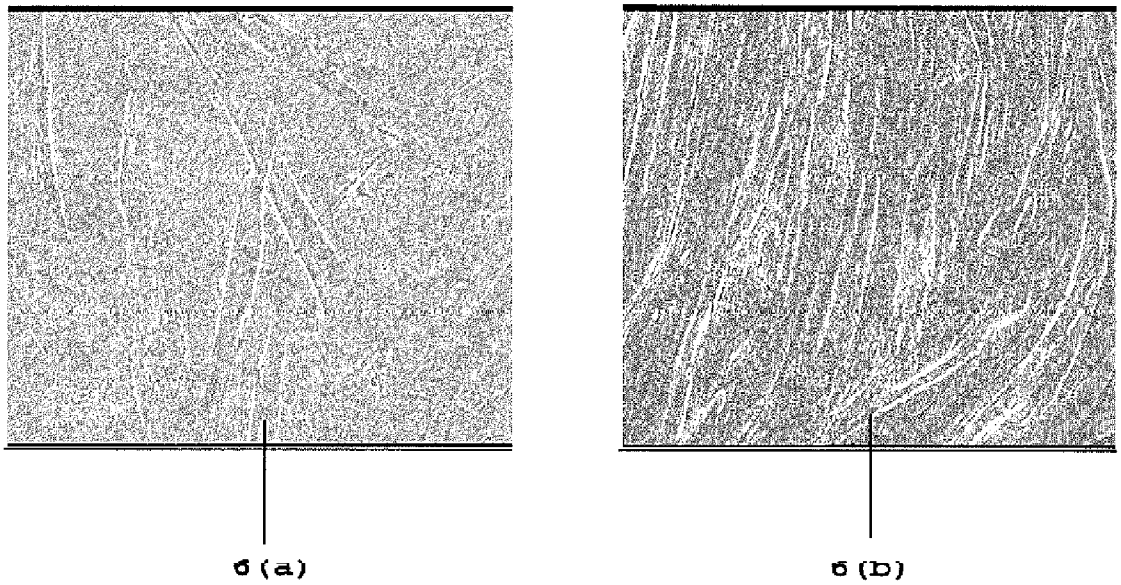

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FIG.7

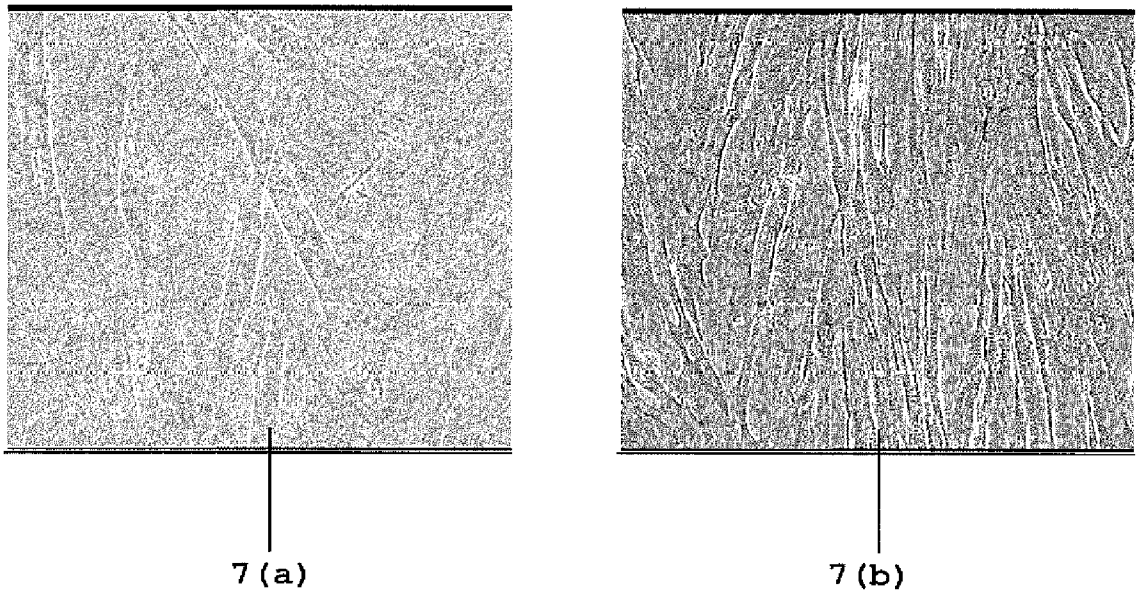

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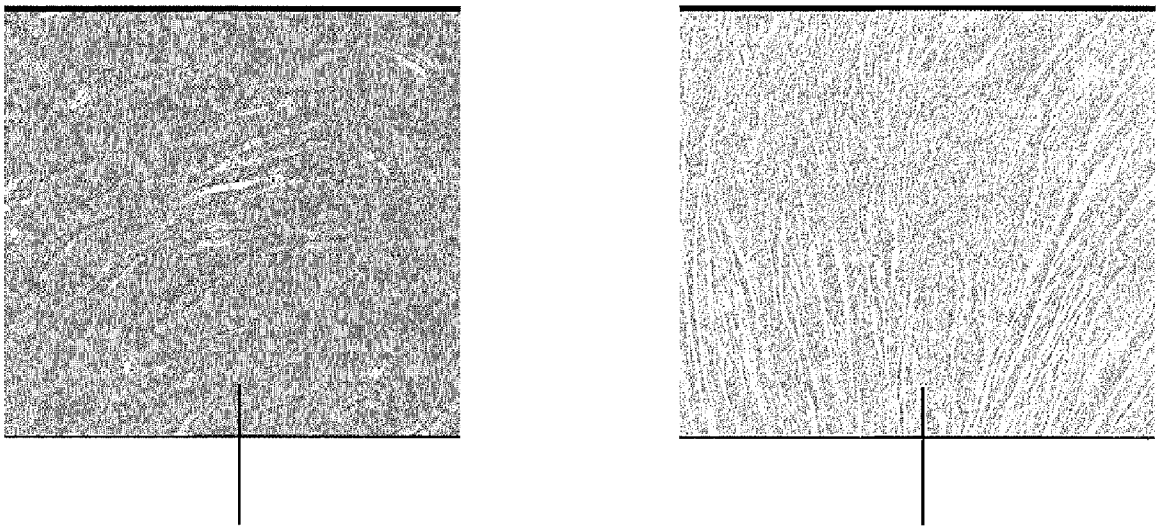
FIG.8



8(a) 8(b)

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FIG.9

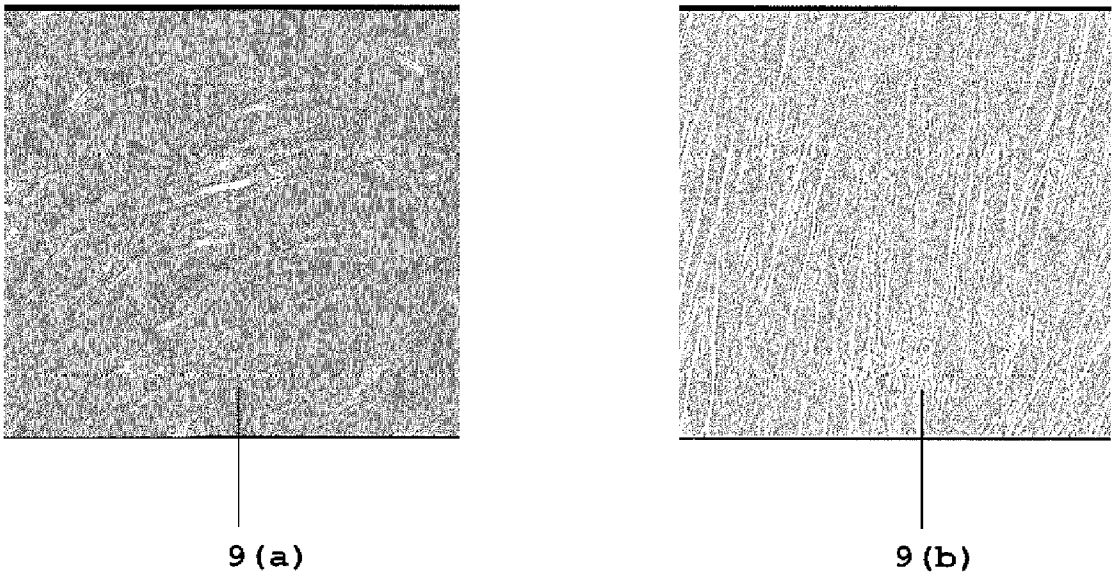

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FIG.10