(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 November 2013 (14.11.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/169273 A1

(51) International Patent Classification:
A61K 38/00 (2006.01) *A61K 8/97* (2006.01)
A61K 8/44 (2006.01) *A61Q 7/00* (2006.01)
A61K 8/64 (2006.01) *A61P 17/00* (2006.01)

(21) International Application Number:
PCT/US2012/038772

(22) International Filing Date:
21 May 2012 (21.05.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
13/465,873 7 May 2012 (07.05.2012) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

WO 2013/169273 A1

(54) **Title**: SYNERGISTIC SELENOPEPTIDE FORMULATIONS FOR THE PROTECTION OF DERMAL PAPILLA CELLS

(57) **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.

SYNERGISTIC SELENOPEPTIDE FORMULATIONS FOR THE PROTECTION OF DERMAL PAPILLA CELLS

[PARA 001] FIELD OF THE INVENTION

[PARA 002] The present invention relates to protective compositions for dermal papilla cells. More specifically, the present invention 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 of the present invention 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.

[PARA 009] SUMMARY OF THE INVENTION

[PARA 0010] The present invention discloses 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 DRAWINGS

[PARA 0012] FIG.1 shows the photomicrographs of 0.5% concentrate of liquid endosperm of *Cocos nucifera*, said concentrate comprising not less than 40%w/w of total dissolved solids unable to protect dermal papilla cells singly.

[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 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 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). 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 the figure.

[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). 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 the figure.

[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). 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). 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). 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). 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)). 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] The present invention relates to 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 of the invention, the synergistic composition comprises at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin).

[PARA 0025] In yet another embodiment of the invention, 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 of the invention, the synergistic composition comprises 0.001% w/w of selenopeptides.

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

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

[PARA 0029] The present invention 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 of the invention, 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 of the invention, 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 of the invention, the synergistic composition comprises 0.001% w/w of selenopeptides.

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

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

[PARA 0035] The present invention 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 as claimed in claims 1 or 7.

[PARA 0036] The present invention 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 as claimed in claims 1 or 7.

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

[PARA 0038] In the most preferred embodiment, the present invention 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 invention, other dipeptides occurring as combinations with other amino acids may be used in the aforesaid synergistic dermal papilla cell protective formulations.

[PARA 0044] In an alternate embodiment, the present invention also relates to 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] In another alternative embodiment, the present invention also relates to 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] In another alternative embodiment, the present invention also relates to 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] In another alternative embodiment, the present invention also relates to 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] In yet another alternative embodiment, the present invention 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 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] In yet another alternative embodiment, the present invention 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 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] In another alternative embodiment, the present invention also relates to 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] In another alternative embodiment, the present invention also relates to 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 instant invention. The composition of the present invention shows superior activity when compared to individual components or other combinations.

[PARA 0078] 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.

Claims:

1) 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.

2) The formulation of claim 1, wherein said synergistic composition comprises at least 10% w/w of 1-O-galloyl-β-D-glucose (β-glucogallin).

3) The formulation of claim 1, wherein said 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.

4) The formulation of claim 1, wherein said synergistic composition comprises 0.001% w/w of selenopeptides.

5) The formulation of claim 1, wherein the selenopeptide is γ-L-glutamyl-Selenomethyl-L-selenocysteine.

6) The formulation of claim 1, wherein the selenopeptide is γ-L-glutamyl-L-Selenomethionine.

7) 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.

8) The formulation of claim 7, wherein said 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.

9) The formulation of claim 7, wherein said 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.

10) The formulation of claim 7, wherein said synergistic composition comprises 0.001% w/w of selenopeptides.

11) The formulation of claim 7, wherein the selenopeptide is γ-L-glutamyl-Selenomethyl-L-selenocysteine.

12) The formulation of claim 7, wherein the selenopeptide is γ-L-glutamyl-L-Selenomethionine.

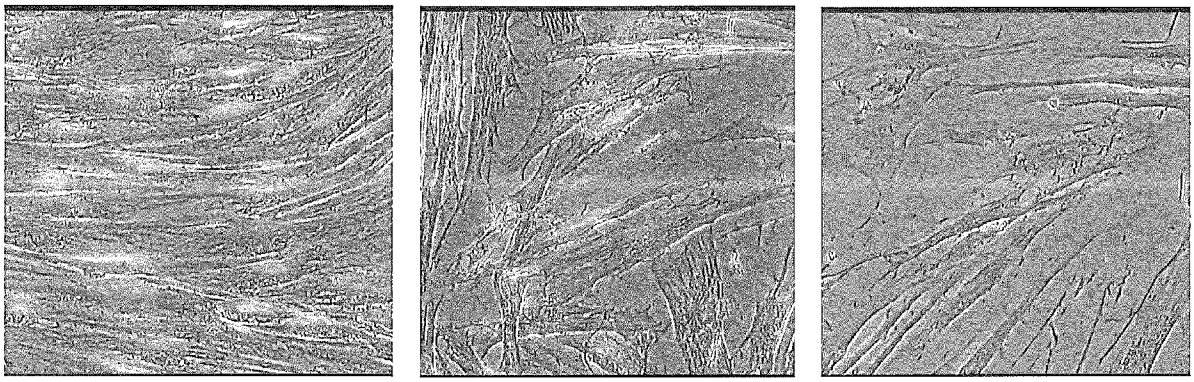
13) A method of increasing the tolerance of dermal papilla cells to stress signals, said method comprising step of bringing into contact the dermal papilla cells and the protective formulations as claimed in claims 1 or 7.

14) 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 7.

15) The methods of claims 13 or 14, wherein the dermal papilla cells include dermal stem/progenitor cells.

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

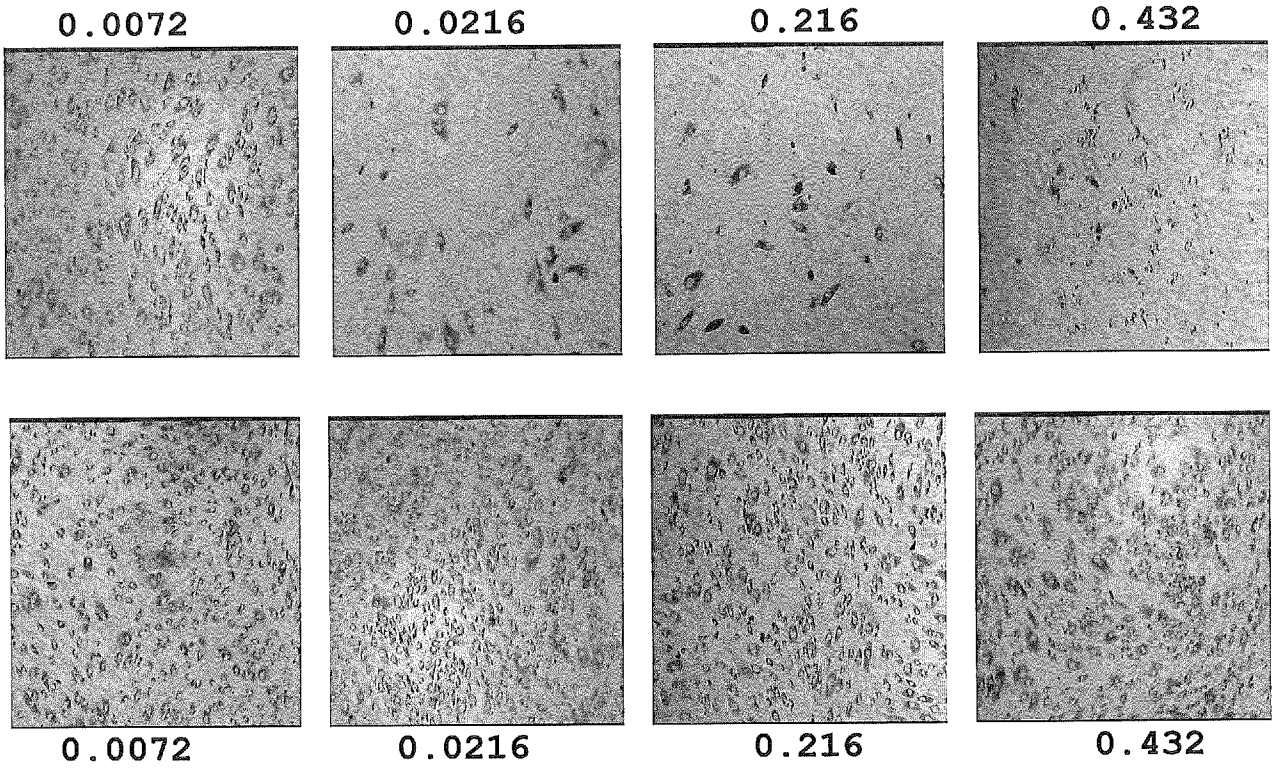
Cell death shown at 0.432 J/cm^2 and 0.648 J/cm^2

FIG.2

UVB IRRADIATED DERMAL PAPILLA CELLS TREATED WITH PROTECTIVE FORMULATION PC-1 (COMPOSITION COMPRISING AT LEAST 10% W/W OR GREATER OF 1-O-galloyl-β-D-glucose (β-glucogallin)) IN COMPARISON WITH UVB IRRADIATED UNTREATED CELLS.

Increasing dosages of UV (J/cm^2)

Row I: Untreated cells



Row II: PC-1 treated cells

PC-1 protects dermal papillary cells from UVB exposure of up to 0.43 J/cm^2.

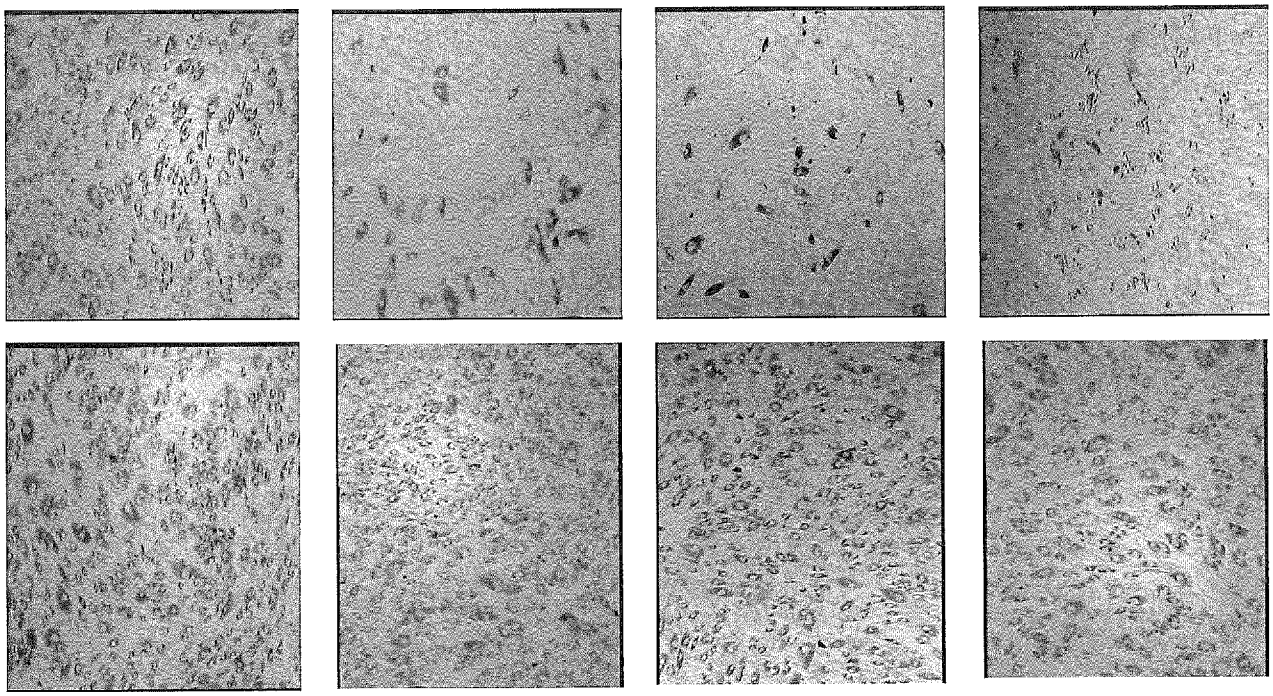
FIG.3

UVB IRRADIATED DERMAL PAPILLA CELLS TREATED WITH PROTECTIVE FORMULATION PC-2 (COMPOSITION COMPRISING AT LEAST 10% W/W OR GREATER OF 1-O-galloyl-β-D-glucose (β-glucogallin) and 50% TO GREATER THAN 50% TOTAL GALLATES IN COMPARISON WITH UVB IRRADIATED UNTREATED CELLS.

Increasing dosages of UV (J/cm^2)

Row I: Untreated cells

0.0072 0.0216 0.216 0.432



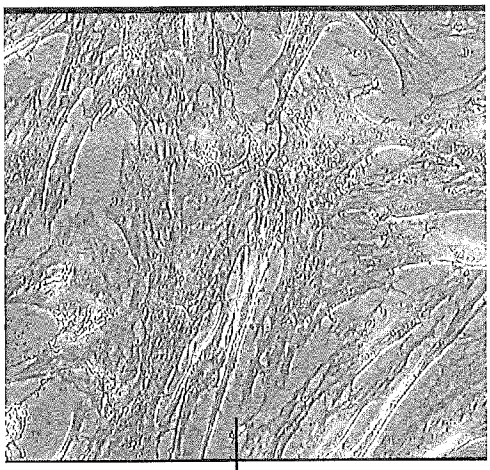
0.0072 0.0216 0.216 0.432

Row II: PC-2 treated cells

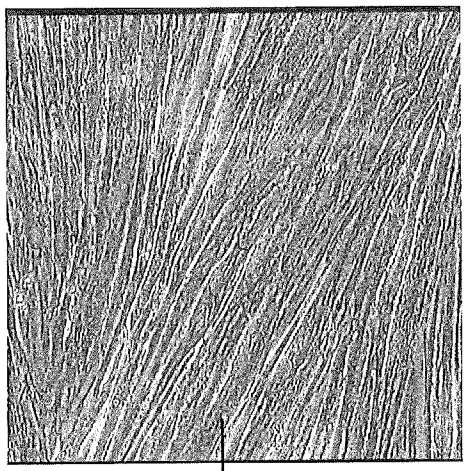
PC-2 protects dermal papillary cells from UVB exposure of up to 0.43 J/cm^2.

FIG.4

UVB IRRADIATED DERMAL PAPILLA CELLS TREATED WITH PROTECTIVE FORMULATION PC-3 (PC-1 + 0.5% CONCENTRATE OF LIQUID ENDOSPERM OF *Cocos nucifera*, CONCENTRATE COMPRISING NOT LESS THAN 40%W/W OF TOTAL DISSOLVED SOLIDS).



Cell damage upon treatment with PC-1 at 0.648 J/cm^2

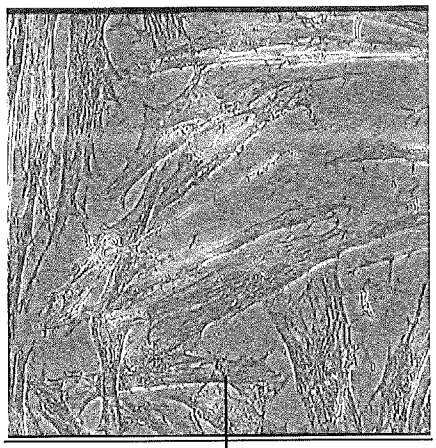


Intact dermal papilla cells upon treatment with PC-3 at 0.648 J/cm^2

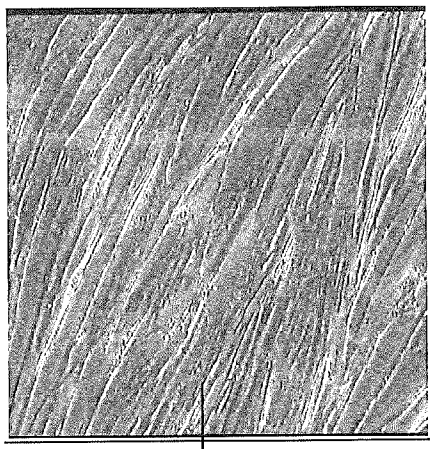
PC-3 protects dermal papilla cells at UVB exposure levels of up to only 0.648 J/cm^2 and not 0.8 J/cm^2 (Cell death at 0.8 J/cm^{2}is shown here).

FIG.5

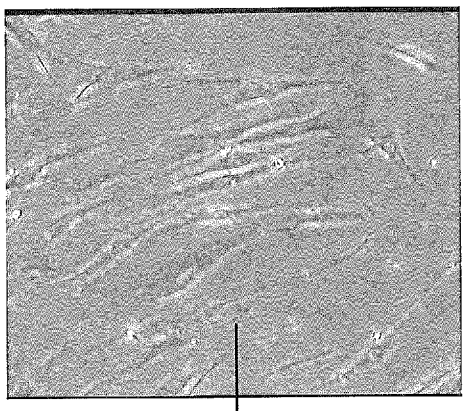
UVB IRRADIATED DERMAL PAPILLA CELLS TREATED WITH PROTECTIVE FORMULATION PC-4 (PC-2+0.5% CONCENTRATE OF LIQUID ENDOSPERM OF *Cocos nucifera*, SAID CONCENTRATE COMPRISING NOT LESS THAN 40%W/W OF TOTAL DISSOLVED SOLIDS)



Cell damage upon treatment with PC-2 at 0.648 J/cm^2



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 here)

FIG.6

UVB IRRADIATED DERMAL PAPILLA CELLS TREATED WITH PROTECTIVE FORMULATION PC-5 (PC-3 + 0.001% OF γ-L-glutamyl-Selenomethyl-L-selenocysteine)

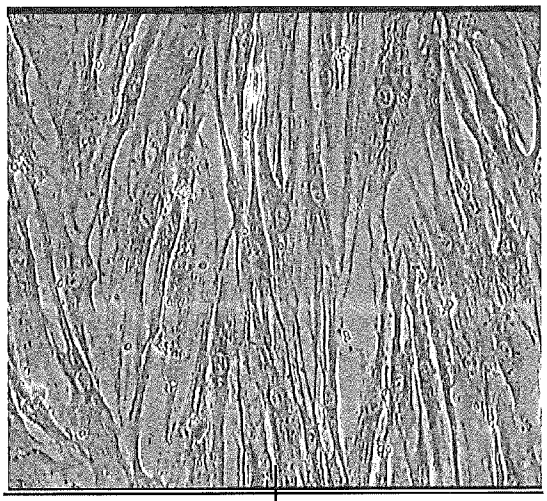
Cells treated with PC-3 alone showing no significant protection from a UV B dose of 0.8 J/cm^2

Cells treated with PC-5 showing significant (95%) protection from a UVB dose of 0.8 J/cm^2

FIG.7

UVB IRRADIATED DERMAL PAPILLA CELLS TREATED WITH PROTECTIVE FORMULATION PC-6 (PC-3 + 0.001% OF γ-L-glutamyl-L-Selenomethionine).

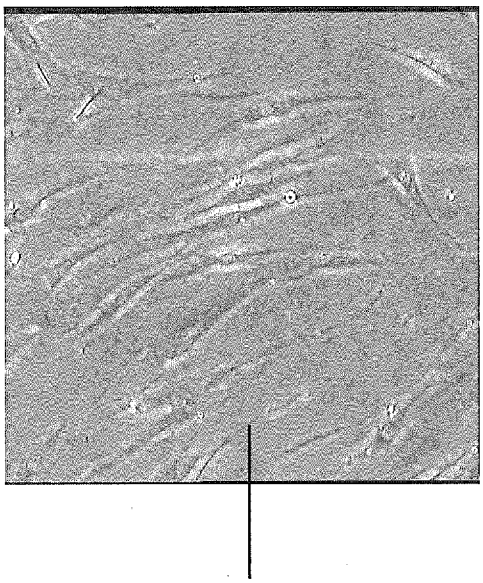
Cells treated with PC-3 alone are not protected from damage by a UV B dose of 0.8 J/cm^2



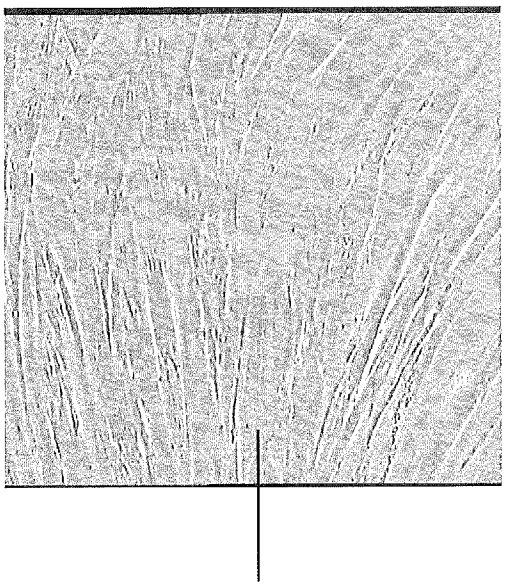
Cells treated with PC-6 are protected from damage by a UVB dose of 1.0 J/cm^2

FIG.8

UVB IRRADIATED DERMAL PAPILLA CELLS TREATED WITH PROTECTIVE FORMULATION PC-7 (PC-4 + 0.001% OF γ-L-glutamyl-Selenomethyl-L-selenocysteine)



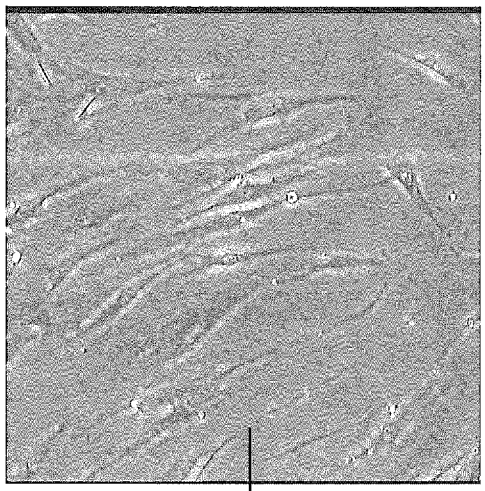
Cell damage upon treatment with PC-4 at 0.8 J/cm^2



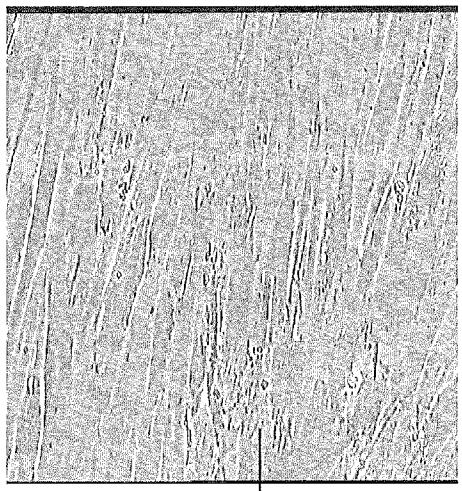
Intact dermal papilla cells upon treatment with PC-7 at 0.8 J/cm^2

FIG.9

UVB IRRADIATED DERMAL PAPILLA CELLS TREATED WITH PROTECTIVE FORMULATION PC-8 (PC-4 + 0.001% OF γ-L-glutamyl-L-Selenomethionine).



Cell damage upon treatment with PC-4 at 0.8 J/cm^2

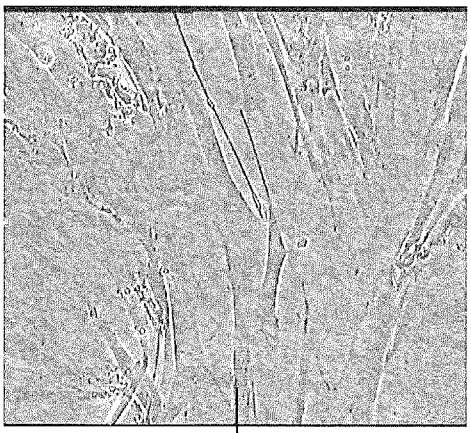


Intact dermal papilla cells upon treatment with PC-8 at 1.0 J/cm^2

FIG.10

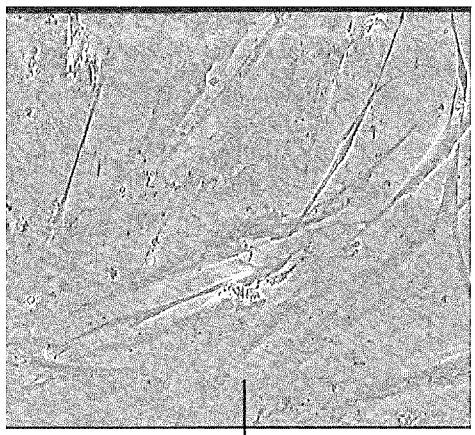
UVB IRRADIATED DERMAL PAPILLA CELLS TREATED WITH PROTECTIVE FORMULATION PC-9 (0.001% OF γ-L-glutamyl-Selenomethyl-L-selenocysteine (FIG.10a) OR γ-L-glutamyl-L-Selenomethionine (FIG.10b)).

FIG.10a



Cells treated with PC-9 showing no significant protection from a lower UVB dose of 0.432 J/cm^2

FIG.10b



Cells treated with PC-9 showing no significant protection from a lower UVB dose of 0.432 J/cm^2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US12/38772

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/00, 8/44, 8/64, 8/97; A61Q 7/00; A61P 17/00 (2012.01)
USPC - 424/401; 514/1.1, 18.6; 520/300, 333
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61Q 7/00 (2012.01)
USPC - 514/1.1, 18.6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-Granted, US-Applications, EP-A, EP-B, WO, JP (bibliographic data only), DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; ScienceDirect; DialogPRO; ACS Publications; Selenopeptide, Selenoprotein, Selenocysteine, selenomethionine, glocogall*, galloyl*, gallin, gallate, gallic, Sabinsa, Sami Labs, papilla, endosperm

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2011/0033565 A1 (MAJEED, M et al.) February 10, 2011, abstract; paragraphs [0002]-[0004], [0017], [0030], [0031]	1-14
Y	US 2008/0026017 A1 (MAJEED, M et al) January 31, 2008, abstract; paragraphs [0021], [0090]-[0092]	1-14
A	US5605929 A (LIAO, S et al.) February 25, 1997, abstract, Claim 1	1-14

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 July 2012 (27.07.2012)	13 AUG 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US12/38772

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 15
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.