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(54) Title: TRANSEPIDERMAL COMPOSITION FOR RESTORATION OF PRE-MATURE AGING SKIN

(57) Abstract: A topical nutritionally balanced and bioactive composition is provided to expeditiously intercede in the biological process of pre-mature solar aging of skin. An ingredient, which re-vitalizes senescent adult human dermal stem cells, has been integrated in the composition. The efficacious bioavailability of this anti-aging composition is assured by means of a bi-functional chemical penetration enhancing compound, which is integrated in this comprehensive formulation. The bi-functional chemical penetration enhancing compound for hosting other guest molecules to facilitate their expeditious bio-availability is presented.

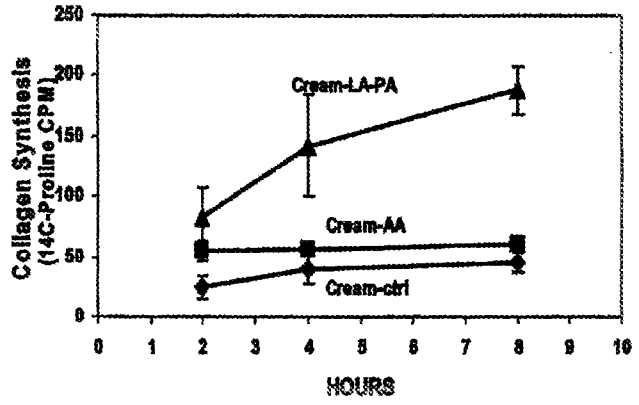

FIGURE 1

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TRANSEPIDERMAL COMPOSITION FOR RESTORATION OF PRE-MATURE AGING SKIN

FIELD OF THE INVENTION

[001] This patent is directed to a bioactive and nutritionally balanced biochemical composition created to expeditiously and effectively intercede in the pre-mature aging process, thereby, restoring the degraded components of the dermal extracellular matrix

BACKGROUND OF THE INVENTION

[002] Premature skin aging, or solar aging, is a manifestation of the sun's UV radiation in up-regulating three collagen and elastin-degrading enzymes, collagenase, 92 kD gelatinase and stromelysin-1. As a direct result, collagen and elastin of the extracellular matrix are diminished in their roles of providing the structural integrity and elasticity of the skin, which, due to the forces of gravity, begins to deform under it's own weight. This results in the fine lines, wrinkles, furrows and skin laxity, which are viewed as common visible signs of aging.

[003] A comprehensive biochemical composition that will effectively and expeditiously intercede in the process of pre-mature aging of skin does not currently exist. The failure to understand the critical nature of skin restoration has been the elusive basis for this issue.

[004] The predominant cell in the dermal layer of skin is the fibroblast. It's primary functionality is that of manufacturing the protein components of the extracellular matrix. The two fibrillar proteins, which play the central role in the skin's structural integrity and elasticity are collagen and elastin, respectively. Just as a factory requires both raw materials and energy to produce a product, the fibroblast does as well. An efficacious composition intended to provide these elements must be both bioactive and nutritionally balanced.
This invention embodies the energy component, as well as the appropriate raw materials in the same ratios as present in normal body fluids.

[005] Viable dermal stem cells will normally differentiate into productive fibroblasts. As skin progresses into pre-mature aging, a substantial quantity of dermal fibroblastic progenitor stem cells will become replicatively senescent due to exceeding their epigenic predetermined life span based largely on the hypothesis that no human cells are immortal. An effective restorative composition must re-vitalize these senescent cells to once again become productive of collagen and elastin. This invention embodies the re-vitalization of the senescent fibroblasts.

[006] There is, however, one as-yet unmet challenge due to the formidable permeability barrier of the stratum corneum. This comprehensive anti-aging composition must be bio-available to the target

site to be efficacious. This invention enables an efficacious and expeditious delivery capability from topical application. All active agents in the formation are delivered simultaneously and require only minimal dosimetry.

SUMMARY OF THE INVENTION

[007] The present invention is directed to a comprehensive anti-aging composition, which addresses the age-related degradation of the critical protein components of the dermal extra-cellular matrix.

[008] The effectiveness of this anti-aging formulation lies in the synergistic effect of it's individual active agents and the expeditious process of transporting these agents to the viable dermal fibroblasts and/or the re-vitalized senescent fibroblasts.

[009] The critical raw materials and the energy sources driving the fibroblastic collagen and elastin bio-synthesis are derived from the following formulation in a synergistic process. Synergism has been defined as the "interaction of two or more agents, which produce a combined effect greater than the sum of their separate effects." The novelty of this invention is largely embodied in this concept.

[010] The building blocks of the proteins are the ten essential amino acids. They are essential because the body doesn't produce them intrinsically. The University of Arizona's Biology Project tells us that "the failure to obtain enough of even one of the essential amino acids has serious implications and can result in the degradation of the body's proteins The body doesn't store excess amino acids for later use."

[011] Two sulfated amino acids (SAAs) are included to maintain the integrity of the dermal reservoir for the hydration and plumpness of the skin. The recommended dietary allowance (RDA) tends to underestimate the body's requirement for sulfated nutrients, especially during times of solar damage. This invention integrates the required concentrations of these SAAs.

[012] The energy to drive the fibroblast functionality is derived from stimulators of cellular biosynthetic activity, as nucleotides (purines and pyrimidines). The nucleotides are the biological molecules, which form the building blocks of the nucleic acids (DNA and RNA) and serve to carry packets of energy within the cell. They play a central role in metabolism, they participate in cell signaling and are incorporated in the co-factors of enzyme reactions.

[013] A lipid-soluble form of vitamin C is included as a co-factor of collagen synthesis and resists oxidation in air and is more readily transported across the epidermal barrier to be bio-available. It also

participates in the hydroxylation of proline to hydroxyproline contributing to the helical configuration of the collagen molecule.

[014] In anti-aging skin restoration, it is not enough to merely initiate collagen and elastin biosynthesis. The spatial arrangement of the three peptide chains in the molecule is unique to collagen, but tropocollagen that has not been polymerized is still quite soluble in cold water. The stabilizing molecular bonds or cross-links are necessary to prevent dissolution by proteolytic enzymes and contribute to the structural integrity of the extracellular matrix. This prevents skin from deforming under it's own weight.

[015] This formulation embodies a bioflavonoid as a robust collagen cross-linker and synthesizer, as well as an active anti-oxidant.

[016] Metallic co-factors are also embodied in this inventive formulation.

[017] Calcium (Ca) is a co-factor of the matrix metalloproteinases (MMPs) and in the conversion of pro-collagen to tropo-collagen, which takes place due to two enzymes, (N- and C-protease), cleaving the N- and the C- terminal of the procollagen. Ca is also involved in the general cell proliferation through it's role in the Ca channels, used in the delivery of stimulant factors into the cells and through it's role in the action of cAMP (cyclic adenosine monophosphate). This is a messenger intracellular signal transducer.

[019] Iron (Fe) is involved in the proliferation of fibroblasts at the chromosomal and DNA replication steps. Fe is also a co-factor in cytochromal enzymes in the mitochondria. It is also utilized in defense against reactive oxygen species (ROS).

[020] Copper (Cu) plays a role related to collagen cross-linking as a co-factor for the enzymes, lysyl oxidase. It is also involved as a catalyst in the free radical-producing reactions (Fenton-type reactions).

[021] A specific growth factor, connective tissue growth factor (CTGF) is a fibrogenic cytokine, which functions as an autocrine growth factor, which means that it acts upon the very same cells that produce it, causing the fibroblasts to proliferate, differentiate and produce collagen.

[022] There is one population of fibroblasts, which has reached the end of it's epigenic predetermined life-span based upon the hypothesis that no human cell is immortal. These cells are rendered replicatively senescent meaning that their progenitor cells, the dermal stem cells, which

populate the dermal papilla at the base of the hair follicle, lose their capability to replicate themselves. They do not undergo apoptotic death but they will cause biological havoc in the aging skin and they no longer differentiate into viable fibroblasts.

[023] This senescence was once thought to be permanent as noted by Hayflick (1965). It has only been a few years since the discovery by Mibelle Biochemistry of Buchs, Switzerland of a metabolic extract derived from a vegetal tissue culture that will revitalize adult human dermal stem cells making them productive once again. This extract is integrated in this composition.

[024] This comprehensive formulation of raw materials and nutritionally balanced agents work together in a synergistic process to enhance the restoration of the age-depleted elements of the dermal extra-cellular matrix. Collagen and elastin are synthesized, thereby, reversing the visible signs of aging, such as fine lines, wrinkles, furrows and skin laxity.

[025] Because of the body's inherent stratum-corneum-induced epidermal barrier to most topically applied agents, a comprehensive composition will not normally effectively breach this barrier to become bio-available to the dermal fibroblasts.

[026] The inventive formulation, however, embodies a novel bi-functional biochemical compound, which is integrated in the total formulation to be applied to the skin surface in a cream, lotion or serum form. This compound is comprised of agents, which hosts various guest molecules and by means of disparate biochemical pathways, will transport the composition effectively and expeditiously to the dermal fibroblasts to drive it's bioactivity.

BRIEF DECRIPTION OF THE DRAWINGS

[027] The following invention will be better understood with references to the specification, appended claims and accompanying drawings, where:

[028] Figure 1 depicts the effect of daily application of a cream containing antioxidants, essential amino acids, and a methionine supplement on collagen synthesis, evaluated by the uptake of C-proline into newly synthesized collagen during a short term organ culture;

[029] Figure 2 depicts the effects of lipoic acid (A) and proanthocyanidin (B) on collagen synthesis by fibroblasts determined by 3H-proline incorporation into collagen. Data were normalized to total DNA content;

[030] Figure 3 the histology of rat dorsal skin at the site of application of the various creams

at the end of the 2- week test period is demonstrated. (A) placebo control, (B) basic formulation containing the amino acid supplements, only, and (C) complete formulation: essential amino acids, methionine supplement, and proanthocyanidin;

[031] Figure 4 is a relationship between cross-linking effectiveness (judged by melting temperature) and PA concentration;

[032] Figure 5 shows cell proliferation rates and collagen synthesis of human fibroblasts cultured on PA-treated or non-treated pericardium tissue;

[033] Figure 6 demonstrates changes in the shrinkage temperature of tissues stored in two different solutions, (a) PBS (solid line); (b) 40% ethanol/PBS (dashed line). Storage temp. 21°C pericardium strips were treated with 0.5% PA for 24 hr before storage;

[034] Figure 7 illustrates the role of dermal stem cells in maintenance and repair of the dermis;

[035] Figure 8 shows the Concentration Mass of iron (Fe) in samples collected at four different time points. Samples were evaluated for elements by PIXI analysis. Donor sample at the concentration used had Fe at a concentration mass of 169.708 (straight line). Experimental samples (with Collagen Biosynthesis compound) started showing an increase in the concentration mass of Fe starting at 30 min and reached a peak value in 120 min. Fe was undetectable in wells incubated with base or PBS;

[036] Figure 9 shows the Concentration Mass of copper (Cu) in samples collected at four different time points. Samples were evaluated for elements by PIXI analysis. Donor sample at the concentration used had Cu at a concentration mass of 3.132 (straight line). Experimental samples (with Collagen Biosynthesis compound) started showing an increase in the concentration mass of Cu starting at 30 min and reached a peak value in 120 min. Cu was undetectable in wells incubated with base or PBS;

[037] Figure 10 illustrates the Amplification plot data using pro-collagen primers and probes. These results show that human dermal fibroblast cells began expressing pro-collagen within 30 min after exposure to Collagen Biosynthesis compound sample. Control samples exposed to base alone did not express pro-collagen at this time point;

[038] Figure 11 is a comparison of lipid profile for Epiderm ™ and normal human skin; and

[039] Figure 12 are theoretical advantages of transdermal delivery which includes less toxicity and improved efficacy. This is due to a reduction in the "peaks" and "valleys", associated with bolus therapy.

DETAILED DESCRIPTION OF THE INVENTION

[040] Certain embodiments of the present invention are directed to topical compositions, which comprise a nutritionally balanced bioactive formulation intended to intercede in the condition of pre-mature or solar skin aging. Skin rejuvenation of this sort requires raw materials crucial to production of the protein components of the dermal extracellular matrix. If one assumes that the predominant

functional dermal cell, the fibroblast, functions as a "factory cell", it's productivity will depend upon not only the raw materials presented to it, but, it's sources of energy to drive this function, as well.

[041] This invention also embodies a biochemical system to provide expeditious and efficacious bio-availability of these agents. This is disclosed as a multi-functional drug delivery system.

[042] In the aging process, fibroblasts will reach and exceed their epigenic determined life-span. This results in replicative senescence. Replicative senescence is defined as an inability to replicate the fibroblastic progenitor adult human dermal stem cells. Normally, the dermal stem cells differentiate into productive fibroblasts, thereby, restoring the age-depleted components of the extracellular matrix. Another preferred embodiment of this invention is a biochemical process for re-vitalizing senescent adult human dermal stem cells, which predispose to restorative collagen and elastin biosynthesis.

Nutritionally Balanced Bio-active Formulation

[043] The two critical fibrillar proteins of the dermal extracellular matrix are collagen and elastin. Collagen, the most abundant protein in the human body, accounts for over 90% of the protein in human dermis. It plays a major role in the strength and structural integrity of the skin. As collagen is age-depleted, skin will deform under it's own weight because gravity will be unopposed.

[044] Collagen biosynthesis and remodeling is complex and involves several post-translational modifications. The dermal fibroblasts synthesize the individual polypeptide chains of Types I and lll collagen as precursor molecules, procollagen. The individual chains, which contain globular amino- and carboxy-terminal domains, assemble into trimeric procollagens. Following complete assembly into a triple helical molecule, procollagens are secreted into the extracellular space (ECM) as soluble proteins. Specific proteases cleave and, thereby remove the carboxy- and amino- terminal domains, which gives rise to pC and pN collagen. These resultant mature collagen fibers consist of a triple helical domain and small, non-helical portions called telopeptides on each end of the molecule. These telopeptide domains are involved in stabilizing the collagen fibers by forming intermolecular covalent cross-links. These cross-links connect C- or N- terminal telopeptide domains to central triple helical domains on adjacent collagen molecules.

[045] Elastin is the other critical fibrillar protein in the dermal extracellular matrix. Elastic fibers are essential extracellular matrix macromolecules comprising an elastic core surrounded by a mantle of fibrillin-rich microfibrils. They endow the skin with the critical properties of elasticity and resilience. The keratinocyte of the epidermis participates with the fibroblast in elastin synthesis. The cross-links between individual elastin molecules is very similar in mechanism to that of collagen. This allows elastin fibers to stretch 100% and still return to their original form.

[046] Matrix metalloproteinase degradation of elastin from solar aging reduces the resiliency of the stretched fibers in the skin while degrading the cross-links.

[047] Amino acids are the building blocks of peptides. In the restoration of age-depleted collagen and elastin, all ten of the essential amino acids must be bio-available to the dermal fibroblast and studies have demonstrated that preferential bioactivity proceeds expeditiously when the amino acids are provided in the exact ratios as in the human body fluids (Figure 1).

[048] Laboratory studies have also revealed that a significant number of individuals, particularly the aged, may be prone to a deficiency of sulfur in their diets. Solar aging will predispose to a degradation in the glycosaminoglycans (GAGs), which function as the dermal reservoir, imbibing great quantities of water per unit weight.

[049] This invention embodies the addition of sulfated amino acids, which function to restore the age-depleted GAGs.

[050] The tissue specific GAGs require a source of inorganic sulfur for their synthesis. One suitable source of sulfur, embodied in this invention, is the sulfur-containing amino acids (SAAs), cysteine and methionine. The importance of the addition of these active ingredients is their previously unrecognized significance in the synthesis of GAGs. The recommended dietary allowance (RDA) for may, in fact, underestimate the bodily needs for these mutually complementary essential nutrients, particularly during periods of increased synthesis of GAGs, such as solar damage of the skin.

[051] The GAGs are key components of the dermal extracellular matrix in facilitating the dermal reservoir, thus providing hydration, which aids in filling and plumping the overlying tissue. This aids in eliminating the appearance of rhytides (wrinkles), as well as imparting a youthful texture to the skin. This proprietary skin restoration composition might well be one of the sole contributors of SAA to the restoration of health to solar-damaged skin.

[052] Collagen and elastin cross-links are also degraded in pre-mature aging. This invention embodies the utilization of remedial cross-linking agents to provide the molecular stability lost during the solar-aging process. The inclusion of proanthocyanidin (PA), a bioflavonoid, has been demonstrated to rapidly enhance collagen cross-linking. Studies have also revealed that PA will increase collagen synthesis and accelerate the conversion of soluble collagen (solar-damaged) to insoluble collagen during restoration.

[053] It is well known that oxidative stress-inducing conditions will cause damage to proteins, lipids and nucleic acids and that surviving an oxidizing environment is actually one of the greatest challenges faced in solar aging.

[054] To diminish oxidative injury, lipoic acid (thiocitic acid) and ascorbic acid (vitamin C), as well as proanthocyanidin, are integrated in this composition and function synergistically as direct radical scavengers (anti-oxidants) (Figure 2).

[055] Lipoic acid functions as a co-factor in the multi-enzyme complexes that catalyzes the oxidative decarboxylation of α-keto acids and in cells, tissues and organs exerts a powerful anti-oxidant effects.

[056] A lipid-soluble form of Vitamin C, such as ascorbyl palmitate will assure adequate permeation to the dermis. Vitamin C is a co-factor for the synthesis of collagen, because it participates in an essential step of the biosynthetic process, that of the hydroxylation of proline to hydroxyproline, a key structural amino acid, which contributes to the helical configuration of the collagen molecule.

Essential Amino Acids

[057] The ten essential amino acids are embodied in this invention in ratios as in human body fluids (Figure 3). The entire amino acid subgroup comprises about 0.50 % (w/w) of the total composition.

1. methionine	3.28%	of the subgroup
2. leucine	10.92%	" "
3. lysine	14.20%	" "
4. phenylalanine	8.20%	" "
5. threonine	12.02%	" "
6. tryptophan	7.65%	" "
7. valine	16.39%	" "
8. histidine	8.20%	" "
9. arginine	10.92%	" "
10. isoleucine	8.20%	" "

Sulfated Amino Acids

[058] Cysteine is included with the methionine as sulfated amino acids (SAAs) at about 0.20% (w/w) of the total composition

Remedial Cross-Linker/Anti-oxidants

[059] Proline-rich proteins, such as collagen have an extremely high affinity for proanthocyanidin (PA) and form especially strong hydrogen bonds with PA. Hydrogen bond formation, by stabilizing the helical structure of collagen fibers will increase the denaturation temperature of collagen. Proanthocyanidin is a bioflavonoid and a robust remedial collagen and elastin cross-linker derived from grape seed extract. It also acts as a natural antioxidant and free radical scavenger (Figs 4,5, 6).

[060] This invention embodies PA in about a 1.0 % to 2.0% (w/w) concentration in the composition.

[061] α-Lipoic acid is integrated in the formulation at about 1.0 % (w/w).

[062] Ascorbyl palmitate is integrated in the formulation at about 0.3% % (w/w).

Nucleotides from DNA & RNA

[063] The stimulators of cellular biosynthetic activity as nucleotides (purine and pyrimidines derived from DNA & RNA) are biological molecules that form the building blocks of nucleic acids (DNA & RNA) and serve to carry packets of energy within the cell (ATP). They play a central role in metabolism, participate in cell signaling and are incorporated into co-factors of enzyme reactions.

[064] The invention embodies the provision in DNA and RNA powder from dry baker's yeast (saccharomyces cerevisiae) at about 0.1 % (w/w).

Metallic Co-Factors

[065] Calcium (Ca) is a co-factor of the MMPs and in the conversion of pro-collagen to tropo-collagen. Ca is also involved in the general cell proliferation through in it's role in the Ca channels, used in the delivery of stimulant factors into the cells and through it's role in the action of cAMP .

[066] Iron (Fe) is involved in the proliferation of fibroblasts at the chromosomal and DNA replication steps. Fe is also a co-factor in cytochromal enzymes in the mitochondria, which mediates the metabolism of the cell. Fe is also active in defense against reactive oxygen species (ROS).

[067] Copper (Cu) plays a role related to collagen cross-linking as a co-factor for the enzyme, lysyl oxidase. Remedial cross-linking aids in the stabilization of the collagen triple helical molecule, thus providing maturation of the collagen. Cu is also involved in the free radical-producing reactions known as Fenton-type reactions.

[068] Ca might be added as Calmodulin at about 1.5% (w/w), Fe as Desfroxamin at about 1.5% (w/w), and Cu as Celeruplasmin at about 1.5 % (w/w).

Connective Tissue Growth Factor

[069] The patent also embodies a specific growth factor, connective tissue growth factor or CTGF. CTGF is a fibrogenic cytokine and, thereby, enhances the synthesis of collagen connective tissue. It is an autocrine growth factor, which indicates that it acts upon the very cells that produce it. CTGF is at about 0.1% (w/w) in the formulation.

The Role of Replicative Senescence

[070] The foregoing embodiments presupposes that the fibroblasts are vital and productive and even though pre-mature aging has resulted in the depletion of the critical dermal proteins, the provision of a comprehensive nutritionally balanced bioactive formulation to the dermal fibroblasts will initiate restorative collagen and elastin biosynthesis.

[071] There is, however, another population of fibroblasts, which have reached the epigenic end of their replicative life-span based upon the hypothesis that no human cells are immortal. The dermal fibroblasts progenitor cells, the adult human dermal stem cells are, thereby, rendered senescent and can no longer replicate themselves to differentiate into viable and productive fibroblasts. It was, thus postulated that this growth arrest was irreversible and senescent cells cannot be stimulated to enter the S-phase of the cell cycle, much less divide, by any combination of physiologic mitogens (Hayflick Limit, 1965). Senescent cells undergo three phenotypic changes: they irreversibly arrest growth, they acquire resistance to apoptotic death and they acquire altered differentiated functions.

[072] At least that is what was understood until 2001, when it was discovered that these adult human dermal stem cells aggregated at the dermal papilla at the base of the hair follicle.

[074] It was further discovered that the replacement of senescent fibroblasts by new fibroblasts cells can only be provided by dermal stem cells (Figure 7). At the end of 2009, Mibelle Biochemistry, Buchs, Switzerland, scientists cultured plant material from an endangered tree, *Argania Spinosa*, growing only in the southwestern regions of Morocco. They produced a metabolic extract of the plant stem cells and, in a cross-species study, discovered that this extract has the capability to revitalize the senescent human dermal stem cells proving Hayflick's postulate invalid. Clinical studies have confirmed this epigenic reversal.

[075] This invention embodies the use of the extract, PhytoCellTec *Argan*™ in a concentration of about 0.8% (w/w) in combination with the Nutritionally Balanced Bio-Active Formulation to

revitalize the senescent population of fibroblasts and to add to the synthesis of collagen and elastin of the extracellular matrix in pre-mature skin aging.

Biochemical Penetration Enhancer

[076] A preferred embodiment of this invention is a multi-functional biochemical penetration enhancing formulation working in synergism with the bioactive composition to synthesize the age-depleted components of the dermal extracellular matrix.

[077] This invention further embodies a method and composition, which significantly enhances the transepidermal delivery of drugs, medicines, agents, formulations and other compositions that are applied topically to the skin surface. The following detailed description is of the best currently contemplated modes of carrying out the invention. The description should not be taken in a limiting sense, but is made merely for the purpose of illustrating the general principles of the invention, since the scope of the invention is best defined by the appended claims.

[078] A multiphasic approach to transepidermal drug delivery is embodied in this invention.

[079] This formulation, which is integrated with the bioactive composition in a topical cream, lotion or serum, is based upon the hypothesis that two or more proven penetrants, which function in a synergistic manner by disparate biochemical pathways to breach the functional stratum corneum-induced epidermal barrier, and that have been demonstrated to be efficacious individually, might provide enhanced capability in the transport of topically applied compositions.

[080] In doing so, this formulation enables the bioactive composition to become bio-available to the dermal target site within minutes of topical administration. Further embodiments permit the use of minimal concentrations, as little as 1/1000th of concentrations of previous alternative processes, while hosting all topical agents simultaneously.

[081] This multi-functional biochemical penetration enhancing formulation is comprised of at least two different biochemical agents including Benzyl Alcohol and Lecithin Organogel.

[082] This invention embodies a novel carrier system for the topical administration pharmaceutically active compounds. One of these agents is benzyl alcohol selected from the group of lower alkyl diols, C10-C20 fatty acids and esters, thereof, and C4-C20 optionally substituted aliphatic alcohols.

[083] More preferably, the transepidermal delivery agent is an optionally substituted aliphatic alcohol. More preferably, the optionally substituted aliphatic alcohol is substituted with an aromatic substituent. Still more preferably, the C4-C20 optionally substituted aliphatic alcohol is benzyl alcohol or phenethyl alcohol. Most preferably, the transepidermal delivery agent is benzyl alcohol. Typically, the benzyl alcohol comprises from about 1.0% (w/w) to about 15.0% (w/w) of the composition. Preferably, the benzyl alcohol comprises from about 1.0% (w/w) to about 2.5% (w/w) of the composition.

[084] Finally, liposomes represent yet another chemical method, frequently employed to enhance drug delivery. However, liposomes appear to enhance transdermal drug delivery solely via the appendageal pathway, but as yet there is no convincing evidence that they penetrate intact stratum corneum.

[085] Liposomes, as carriers, have been found by some investigators to be unable to cross the intact skin. They are unstable and their phospholipids are subject to oxidative degradation. Furthermore, fluorescent micrographs unequivocally have shown dispersion within the stratum corneum without further penetration into the epidermis, dermis or deeper.

[086] Benzyl Alcohol functions by transiently dissolving the lipids in the bilayer membrane of the epidermis. By doing so, the drug or compound dissolved in the Benzyl Alcohol, can have a preferable access to the dermal layer of skin. It also has an advantage over other simple alcohols, such as methanol or ethanol, by virtue of the bipolar nature of the multi-functional biochemical penetration enhancing formulation. Due to the aromatic group (i.e., benzene) present in the Benzyl Alcohol, the molecule has a polar end (the alcohol end) and a non-polar end (the benzene end). This enables Benzyl Alcohol to dissolve a wider variety of drugs and agents, which are non-polar, in general, and carry then into the skin layers by lipid dissolving action of the alcohol end of the molecule.

[087] The embodied Benzyl Alcohol is provided to the composition in about a 2.0% (w/w) concentration.

[088] The second penetrant agent is Lecithin Organogel. It is also a bipolar molecule, however, it's action differs from Benzyl Alcohol. The intended drug molecule is present in the micelle of the Lecithin Organogel such that the non-polar end is towards the center and the polar end is towards the outside. The interaction between the lipid layer of the skin and the polar end of the Lecithin Organogel (the phospholipid group) makes it possible for the Lecithin Organogel to enter the skin layers. The advantage of the Lecithin Organogel over other organic solvents, with regard to the transepidermal transport, is that a wider range of drug molecules can be dissolved by Lecithin

Organogel and can be delivered to the intended site under the skin at much higher concentration. This is due to the fact that there is very little to no diffusion of the drug molecule as it penetrates through the skin.

[089] Lecithin Organogel is dissolved in isopropyl palmitate organic solvent. It is a microemulsion consisting of reversed polymer-like micelles and are readily obtained by adding a minimal amount of water to a solution of lecithin in organic solvents. These gels have the ability to host various guest molecules. Three types of molecules (lipophilic, hydrophilic and amphoteric), including enzymes, can be dissolved in the gels. They are biocompatible and have no restrictions on the chemical structure on the drug to be transported.

[090] Alternative compounds of Lecithin Organogel, not to be confused with the embodied biochemical penetration enhancer, have been formulated with poloxamers, which are nonionic triblock copolymers composed of a central hydrophobic chain of polyoxypropylene flanked by two hydrophilic chains of polyoxyethylene. Poloxamers are known by several trade names and a drug delivery agent known as *Pluronic* ® Lecithin Organogel is formulated with a BASF product of which there are some 49 types available. *Pluronic* ®, originally thought to be a inert carrier molecule, has a very real effect on biological systems independently of the drug they are transporting. They have been shown to have the greatest effect when absorbed by the cell as an unimer rather than as a micelle and have been shown to incorporate into cellular membranes affecting the microviscosity of the membranes. *Pluronic* ® Lecithin Organogel also creates a greasy, tacky and thermally unstable composition. Therefore, *Pluronic* ® is not embodied in the formulation of this invention.

[091] The mechanism of action of the embodied Lecithin Oranogel appears to be related to a relative disorganization of the structure of the skin and, thus permits the permeation of various substances. Since the stratum corneum of the epidermis contains regularly arranged layers of lipids, the organogel interacts with the lipids. The Lecithin Organogel of this invention embodies a vehicle comprising from about 0.5% (w/w) to about 15% (w/w) of the composition. More preferably, the Lecithin Organogel comprises about 0.6% (w/w) of the total composition

[092] More preferably, this carrier system combines two or more penetrants. More preferably, the transepidermal delivery system utilizes a combination or a formulation of Benzyl Alcohol 2% (w/w) with Lecithin Organogel 0.6% (w/w) as penetrant mixture functioning by disparate biochemical pathways but performing in a synergistic manner to enhance the transepidermal transport of topically administered compounds.

[093] Use of the combination of Lecithin Organogel and Benzyl Alcohol, for transepidermal delivery of drug compounds not only uses the advantage of Benzyl Alcohol (i.e., dissolving of the lipid layers and increasing the access to the lower layer much faster), but also can result in the delivery of much higher concentrations of drug by the action of Lecithin Organogel. In this combination, the drug molecule should be dissolved in Lecithin Organogel.

Experimental Example

[094] A study was performed to confirm that the inventive biochemical penetration enhancer was efficacious and expeditious in rendering the topical nutritionally balanced bioactive formulation with the stem cell revitalization agents bio-available to the target site of the dermal extracellular matrix.

Project: Collagen Biosynthesis Product Analyses

Analyses

[095] Two sets of analyses were performed:

Analysis A: To determine whether the formulation does penetrate through human skin equivalent, if so within how many minutes?

Analysis B: If the preparation did penetrate the skin can it induce procollagen synthesis in human fibroblasts?

[096] Materials provided:

1. Two jars of completed formulation
2. Three jars of control base

ANALYSIS A: Percutaneous Absorption Study

[097] Two major evaluations were performed

1. Does the formulation penetrate through human skin equivalent?
2. If it does penetrate how long does it take to reach the dermal layer?

[098] Materials:

1. Collagen Biosynthesis compound
2. Control Base
3. EpiDerm™ Skin Model (EPI-200X) Kit 24 x 2 tissues purchased from MatTek Corp.
4. MTT assay (ET-50) purchased from MatTek Corp.
5. MatTek Permeation device (EPI-100-FIX)
6. PBS for culturing (EPI-100-PBS)
7. Six well plates and other plastic ware and tubes from Costar

Assay Method.

[099] MatTek's patented EpiDermFT Series 200 System consists of normal, human-derived epidermal keratinocytes and normal, human-derived dermal fibroblasts which have been cultured to form a multilayered, highly differentiated model of human dermis and epidermis. The tissues are cultured on specially prepared cell culture inserts using serum free medium, attain levels of differentiation on the cutting edge of *in-vitro* skin technology. Ultrastructurally, the EpiDermFT Skin Model closely parallels human skin, thus providing a useful *in-vitro* means to assess percutaneous absorption or permeability.

[100] MatTek Permeation Device (MPD) was used to measure percutaneous penetration of the Collagen Biosynthesis compound preparations.

[101] The cell culture insert, which contains the EpiDerm™ tissue, is inserted between the two pieces of the MPD and four screws are tightened to create a seal between the bottom rim of the device's inner annulus and stratum corneum. Donor solution with no samples added served as negative controls.

[102] Donor solution (PBS) containing four different concentrations (0.25g/ml, 0.5g/ml, 1g/ml and 2g/ml) of the Collagen Biosynthesis Compound or control base was prepared. Neutral Red (0.001%) was added to give a red tinge to the donor solution.

[103] The donor solution was then added to the center core of the MPD device containing the Skin tissue and the whole assembly was then placed into the wells of a 6 well plate containing 3 ml of PBS.

[104] At definite intervals (15 min, 30 min, 45 min, 60 min, 90 min, 120 min, 150 min, 180 min, 210 min, 240 min, 270 min, 300 min, 330 min, 360 min, 12 hrs, 24 hrs) the assembly was moved to a fresh well containing 3 ml of PBS.

[105] After incubation, PBS from the 6 wells were collected in separate tubes, labeled and stored in -70^0C for further processing.

Elemental analysis

[106] To determine if the "Collagen Biosynthesis compound" has penetrated through the epidermal layer, we subjected the PBS samples collected after incubation of the MPD to elemental analysis. This was done as a subcontract with Elemental Analysis Incorporated, 2101 Capstone Drive, Suite 110, Lexington, KY 40511. Given the cost involved with the analysis of all samples ($150/sample plus

shipping), we decided to analyze selected time points and concentrations. The following 14 samples were chosen for analysis.

Collagen Biosynthesis Compound	– 15 min, 30 min, 60 min and 120 min
Control Base	-- 15 min, 30 min, 60 min and 120 min
PBS control	15 min, 60 min, 120 min
Donor solution sample	15 min
Donor solution base	-- 15 min
PBS	-- 15 min

[107] Samples were analyzed by PIXE analyzer. The analyses measured 74 elements in one run. We were interested mainly in two elements, copper (Cu) and iron (Fe). The results are presented as a graph below. Figure 8 shows values for iron (Fe) and Figure 9 shows values for copper (Cu).

[108] MTT assay on the skin samples after 120 hrs of incubation confirmed that all skin tissue in this study were viable at the end of the study period.

Conclusion

[109] Results of the PIXIE analysis show that (1) the Collagen Biosynthesis compound does penetrate the epidermis (2) within 30 minutes of application. Thus, the compound is available to the deeper layers, especially dermal fibroblasts within 30 minutes of its application to the epidermal surface.

[110] The next question, we asked was whether the compound has any effect on the dermal fibroblasts, especially whether it induces procollagen synthesis in these cells. For this, we performed the second set of analysis.

ANALYSIS B: Pro-collagen synthesis in Dermal Fibroblasts

[111] Pro-collagen synthesis was measured by a real time PCR machine in human dermal fibroblasts following exposure to the compound

[112] Materials:

1. Collagen Biosynthesis compound
2. Control Base
3. Human Dermal fibroblast cell line purchased from Cambrex Bio Sciences Walkersville, Inc. 8830 Biggs Ford Road, Walkersville, MD, 21793
4. Fibroblast growth media

5. FBS
6. Culture flasks and 6 well culture plates
7. Applied Biosystem Real time RT-PCR machine

Assay Method

[113] A real time PCR method was used to determine collagen message levels in the human dermal fibroblast cells lines exposed to "Collagen Biosynthesis compound" (at concentrations of 0.25 mg/ml) and base control (at 0.25 mg/ml concentration). Cells incubated in media alone served as negative controls.

Real time RT-PCR analysis

[114] Absolute quantities of collagen were determined in the fibroblasts using a real time RT-PCR analyses. Briefly, cDNA was prepared from the fibroblasts using a retroscript RT-PCR kit purchased from Ambion Inc. RT reactions without reverse transcriptase served as negative control. Ten nanograms of cDNA were used as template for the RT-PCR reaction. Forward primers, reverse primers and TaqMan probes were purchased from Applied Biosystems (Foster City, CA). Collagen type 1 alpha 1 probe was labeled with the reporter dye FAM (6-carboxyfluorescein) at the 5′ end and a non-fluorescent quencher dye at the 3′ end. Primers remained unlabeled. Master mix for PCR reaction consisted of 10 µl of universal master mix (Applied Biosystems), 900 nM of each primer and 250 nM of probe in a final volume of 20 µl. All PCR reactions were carried out in triplicate wells of a 96-well microamp optical plate (Applied Biosystems). Thermal cycling and data analyses were performed in an ABI Prism 7300 instrument (Applied Biosystems). A standard curve generated using different concentrations (10 ng, 1ng, 0.1ng, 0.01ng and 0.001ng) of collagen plasmid was used for quantitative determination of collagen mRNA in the samples.

[115] These analyses showed that exposure to Collagen Biosynthesis compound induced the expression of collagen in human dermal fibroblasts within 30 minutes (Figure 10). Similar changes were not observed at 30 minutes when the base was applied to fibroblast cultures. These findings thus correlate with the penetration data and clearly suggest that the Collagen Biosynthesis compound cream after penetrating through the epidermal layer of the skin can induce collagen synthesis in human dermal fibroblast cells.

SUMMARY and CONCLUSION

[116] Collagen Biosynthesis compound was tested in an *in-vitro* human skin model system. These studies showed that the compound at a minimum concentration of 0.25gm/ml applied to the

epidermal surface of skin resulted in penetration of compound components through the epidermal layer and reaching the dermal layer within 30 minutes after application (Figure 11).

[117] Functional relevance of this penetration was tested in a human dermal fibroblast cell culture system by measuring the ability of the fibroblasts to produce collagen type 1. These studies clearly indicated that human dermal fibroblasts produce collagen type 1 alpha 1 within 30 minutes after application of a 0.25 mg/ml concentration (this is approximately 1/1000 of the amount we earlier applied to the epidermal surface) of Collagen Biosynthesis Compound.

[118] Certain exemplary embodiments of the present invention have been illustrated and described. However, those of ordinary skill in the art will understand that various modifications and alterations to the described embodiments may be made without departing from the principal , spirit and scope of the invention as defined in the appended claims. For example, it is understood that any methods of topical application, administration or treatment described with respect to one topical composition may generally be used with any other topical composition. In addition, it is understood that any pharmaceutically acceptable carrier and biochemical penetration enhancing compound may be used with any topical composition, and are not limited to use in the topical compositions where they are initially described.

[119] For example, there are other topical compositions, which would benefit from the biochemical penetration enhancing compound herein described, such as retinoids, skin lightening agents, anti-fungal agents, non-steroidal anti-inflammatory drugs, etc.

ADVANTAGES OF THE INVENTION

[120] Compositions, devices, and methods according to the present invention provide an enhanced transepidermal drug delivery method to a patient with increased efficiency and without pain or discomfort normally associated with penetrating injections. Other possible complications of injections include localized swelling or edema, capillary hemorrhage, and inflammation.

[121] Transepidermal Drug Delivery: Theoretical Advantages

1. improved patient compliance
2. improved efficacy, i.e. continuous release
3. reduced toxicity: (a) no peaks and (b) lower total absorbed dose
4. bypass hepatic first-pass metabolism
5. avoid local GI side effects /metabolism
6. decreased dosing frequency
7. avoid painful injections

8. decreased costs to patient due to decreased: (a) total dose, and (b) dosing frequency (increased efficiency)

[122] Transepidermal Drug Delivery: Issues With Current Approaches

1. device or patch-dependent
2. reliance on in-vitro models alone: limited relevance
3. limitations: (a) dose (<10mg/day)
   (b) polarity (primarily lipophilic)
   (c) drug class (peptides excluded)

[123] Therefore, few successful examples, nitroglycerin, scopolamine, clonidine, estrogen, testosterone, nicotinic acid.

[124] The present invention discloses a composition comprising an appropriate dosage of drug and a transepidermal delivery system comprised of one or more penetrants working synergistically. The topical application of the composition by means of ointment, cream, or saturated absorbent cotton pledget permits direct application over target site, thus avoiding inadvertent diffusion into an unwanted site, as well as previously indicated risks and complications (Figure 12).

EQUIVALENTS

[125] The inventions illustratively described herein can suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising," "including," "containing," etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the future shown and described or any portion thereof, and it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions herein disclosed can be resorted by those skilled in the art, and that such modifications and variations are considered to be within the scope of the inventions disclosed herein. The inventions have been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the scope of the generic disclosure also form part of these inventions. This includes the generic description of each invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised materials specifically resided therein.

[126] In addition, where features or aspects of an invention are described in terms of the Markush group, those schooled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group. It is also to be understood that the above description is intended to be illustrative and not restrictive. Many embodiments will be apparent to those of in the art upon reviewing the above description. The scope of the invention should therefore, be determined not with reference to the above description, but should instead be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled. The disclosures of all articles and references, including patent publications, are incorporated herein by reference.

CLAIMS:

1. A topical composition for the treatment of pre-mature solar aging comprised of a nutritionally balanced bioactive formulation to enhance the synthesis of collagen, elastin and other components of the dermal extracellular matrix.

2. The topical composition according to Claim 1, wherein, the bioactive formulation consists of a subgroup consisting of all ten of the essential amino acids, as follows: methionine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, histidine, arginine, isoleucine.

3. The topical composition according to Claim 2, wherein, the entire subgroup of essential amino acids comprise about 0.50% (w/w) of the total composition.

4. The topical composition according to Claim 2, wherein, the bioactive formulation consists of a subgroup of all ten of the essential amino acids, which are presented in a ratio, which is generally the same as their concentration in body fluids, as follows:

methionine	3.28 % (w/w) of the subgroup
leucine	10.92%
lysine	14.20%
phenylalanine	8.20%
threonine	12.02%
tryptophan	7.65%
valine	16.39%
histidine	8.20%
arginine	10.92%
isoleucine	8.20%

5. The topical composition according to Claim 1, wherein, the bioactive formulation consists of an additional sulfated amino acid (SAA) cysteine at about 0.20% (w/w) of the total composition.

6. The topical composition according to Claim 1, wherein, the bioactive formulation consists of a collagen cross-linking agent and anti-oxidant.

7. The topical composition according to Claim 6, wherein, the collagen cross-linking agent and anti-oxidant might be proanthocyanidin (PA).

8. The topical composition according to Claim 7 wherein, the collagen cross-linking agent and anti-oxidant is present in a range of about 1.0% to about 5.0% (w/w).

9. The topical composition according to Claim 7 wherein, the collagen cross-linking agent and anti-oxidant is present in a range of about 1.0% to about 2.0% (w/w).

10. The topical composition according to Claim 1, wherein, the bioactive formulation consists of more than one anti-oxidant.

11. The topical composition according to Claim 10, wherein, the anti-oxidants are selected from a group consisting of α-lipoic acid and ascorbyl palmitate.

12. The topical composition according to Claim 11, wherein, the α-lipoic acid is present in a range of about 1.0 to about 3.0% (w/w).

13. The topical composition according to Claim 11, wherein, the ascorbyl palmitate is present in a range of about 0.1 % to about 0.4% (w/w).

14. The topical composition according to Claim 1, wherein, the bioactive formulation consists of stimulators of cellular biosynthetic activity as nucleotides, such purine and pyrimidine.

15. The topical composition according to Claim 14, wherein, the nucleotides are present in a range of about 0.1% to about 0.2% (w/w).

16. The topical composition according to Claim 1, wherein, the bioactive formulation consists of metallic co-factors, such as calcium (Ca), iron (Fe) and copper (Cu) compounds.

17. The topical composition according to Claim 16, wherein, the Ca co-factor might be present as Calmodulin.

18. The topical composition according to Claim 16, wherein, the Fe co-factor might be present as Desfroxamin.

19. The topical composition according to Claim 16, wherein, the Cu co-factor might be present as Celuruplasmin.

20. The topical composition according to Claim 16, wherein,
each of the metallic co-factors is present in a range from about 1.0% to about 2.0% (w/w).

21.. The topical composition according to Claim 1, wherein,
the bioactive formulation consists of a specific growth factor, connective tissue growth factor (CTGF) present in a range of about 0.1% to about 0.3% (w/w).

22. The topical composition according to Claim 1, wherein,
the bioactive formulation consists of a vegetal metabolic extract, PhytoCellTec *Argan*™ present in a range of about 0.4% to about 1.0% (w/w).

23. The topical composition according to Claim 1, wherein,
the bioactive formulation consists of a bi-functional biochemical penetration enhancer consisting of benzyl alcohol and lecithin organogel.

24. The topical composition according to Claim 23, wherein,
the benzyl alcohol is present in a range of about 1.0% to about 10.0% (w/w).

25. The topical composition according to Claim 23, wherein,
the benzyl alcohol is present in a range of about 1.0% to about 2.0%% (w/w).

26. The topical composition according to Claim 23, wherein,
the lecithin organogel is present in a range of about 0.1% to about 10.0% (w/w).

27. The topical composition according to Claim 23, wherein,
the lecithin organogel is present in a range of about 0.1% to about 1.0 % (w/w).

28. The topical composition according to Claim 23, wherein,
the lecithin organogel is present in a range of about 0.3% to about 0.6 % (w/w).

29. The topical composition according to Claim 23, wherein the bi-functional biochemical penetration enhancer comprising the first and second penetrants, the first and second penetrants working synergistically and following disparate biochemical pathways, wherein the composition has a pH ranging from about 3.0 to about 7.4.

30. The topical composition according to Claim 23, wherein each of the first and second penetrants is independently selected from the group of:

lower alkyl diols

C10-C20 fatty acids and esters, thereof,

C4- C20 substituted aliphatic alcohols,

C4- C20 unsubstituted aliphatic alcohols,

lecithin organogel in isopropyl palmitate organic solvent.

31. The topical composition according to Claim 23, wherein the bi-functional biochemical penetration enhancer might host other guest molecules, thereby facilitating bio-availability.

32. The topical composition according to Claim 31, wherein the bi-functional biochemical penetration enhancer might host such guest molecules selected from the group of:

retinoids,

skin lightening compounds,

non-steroidal anti-inflammatory drugs,

anti-fungal drugs,

optical clearing compounds,

anti-bruising compounds, and

other agents, wherein transepidermal bio-availability would be advantageous.

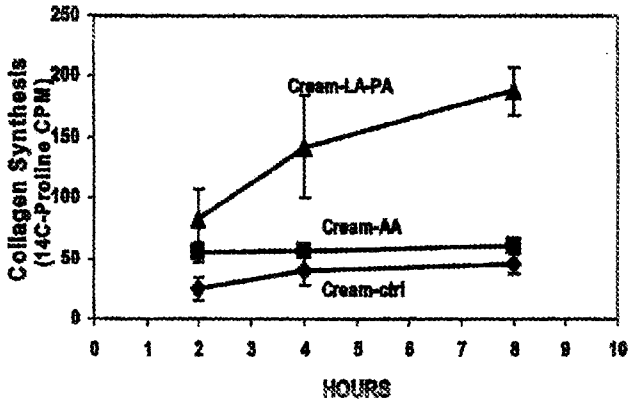

FIGURE 1

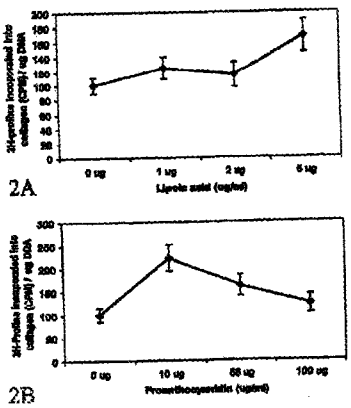

FIGURE 2

FIGURE 3

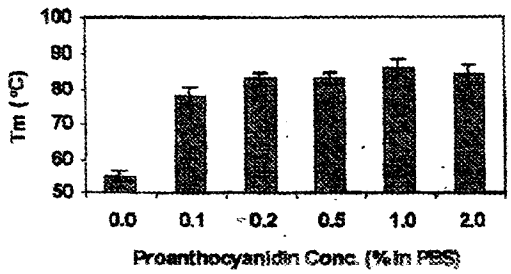

FIGURE 4

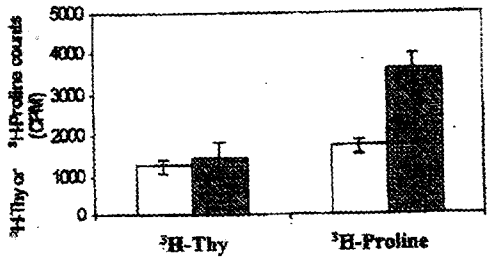

FIGURE 5

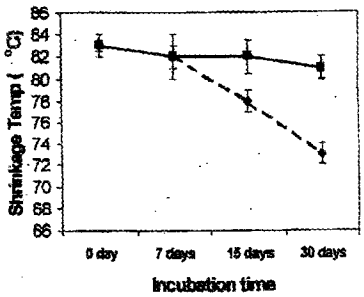

FIGURE 6

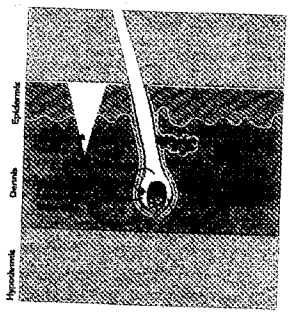

FIGURE 7

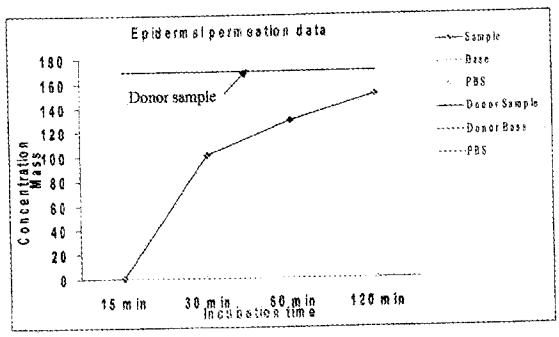

FIGURE 8

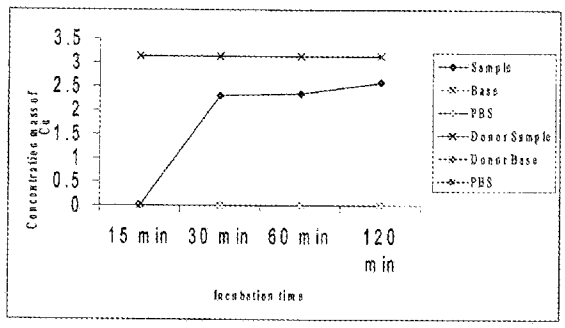

FIGURE 9

Amplification plot to quantitate Pro-collagen

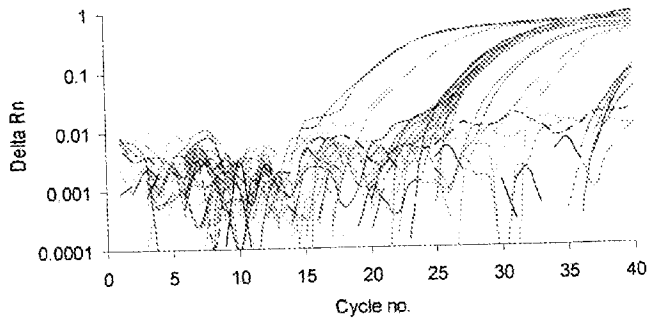

FIGURE 10

Comparison of Lipid Profile for Epiderm and Normal Human Abdominal Skin

	EpiDerm (EPI-100)	EpiDerm (EPI-611)	Human Abdomen
Phospholipids	58.5 ± 3.4	46.8 ± 1.2	41.8
Glucosylceramides (GLS-G)	4.4 ± 0.1	1.8 ± 0.4	4.8
Acylglucosylceramides (GLA)	0.9 ± 0.0	0.7 ± 0.1	1.2
Ceramides (2-6)	5.8 ± 0.5	16.4 ± 1.8	8.7
Acylceramides (CER-1)	0.7 ± 0.1	1.8 ± 0.1	6.6
Cholesterol	12.1 ± 1.4	25.2 ± 2.6	15.7
Fatty Acids	2.9 ± 1.8	0.8 ± 1.2	21.4
Triglycerides	8.1 ± 2.9	8.3 ± 2.9	----
Cholesterol esters	2.9 ± 0.2	2.9 ± 0.2	4.7
Other			1.4

FIGURE 11

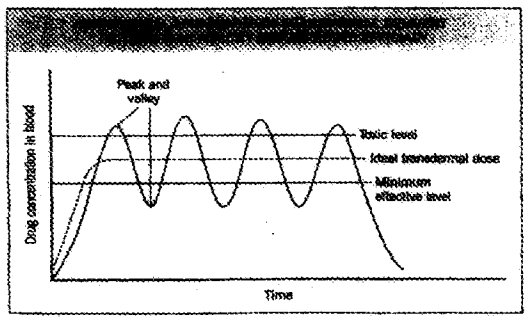

FIGURE 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/43720

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61Q 19/00; A61K 8/67; A61K 47/14; A61K 31/198 (2014.01)
CPC - A61Q 19/00; A61Q 19/004; A61K 8/67; A61K 47/14; A61K 31/198
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)
CPC: A61Q 19/00; A61Q 19/004; A61K 8/67; A61K 47/14; A61K 31/198
IPC: A61Q 19/00; A61K 8/67; A61K 47/14; A61K 31/198 (2014.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 424/59; 514/536; 514/561; 514/626; 514/18.6 (See Search Words Below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PATBASE: Full-text = AU BE BR CA CH CN DE DK EP ES FI FR GB IN JP KR SE TH TW US WO
Google: Scholar/Patents:solar damage topical skin methionine leucine lysine phenylalanine threonine tryptophan valine histidine arginine isoleucine collagen cysteine proanthocyanidin calmodium benzyl alcohol cofactor copper antioxidant PhytoCellTec lecithin organo

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2006/0067959 A1 (NIMNI et al.) 30 March 2006 (30.03.2006) para [0002];[0003];[0006]-[0011]; [0017]; [0021]-[0040]; [0092];[0104];[0105];[0107];[0108];[0110];[0111];[0134];[0135];[0137];[0192];[0256];[0268];[0269]	1-13 --- 14-32
Y	US 5,935,994 A (NIMNI) 10 August 1999 (10.08.1999) Col 2,ln 13-15; Col 2,ln 19-25;Col 4,ln 2	14-15
Y	US 2009/0053290 A1 (SAND et al.) 26 February 2009 (26.02.2009) para [0019];[0045];[0055];[0057];[0059];[0061];[0064];[0068];[0069];[0073];[0088]; [0188]	16-20;23-32
Y	US 2006/0240116 A1 (JOLLEY) 26 October 2006 (25.10.2006) para [0016];[0017];[0025]-[0027]; [0029]; Tables 1 and 2	21
Y	WO 2012/164488 A2 (FOURNIAL et al.) 06 December 2012 (06.12.2012) pg 1,ln 4-7; pg 8,ln 9-19; pg 16,ln 16-17	22

☐ 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
06 September 2014 (06.09.2014)	01 OCT 2014
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: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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