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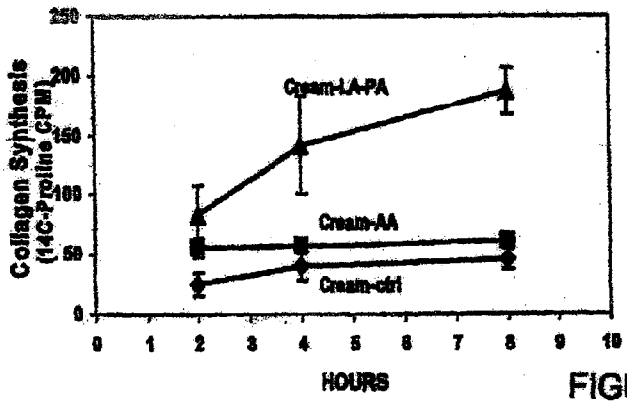


FIGURE 1

(57) Abstract: A topical nutritionally balanced and bioactive composition is provided to expeditiously intercede in the biological processes of pre-mature skin aging. Included in this comprehensive anti-aging treatment are agents directed to the revitalization of senescent adult human dermal stem cells. Methods, apparatus and compositions targeting the loss of tissue volume associated with premature skin aging is also embodied in this patent. The efficacious bioavailability of the embodied anti-aging compositions is facilitated by means of a multi-functional chemical penetration enhancing compound, which is integrated in the embodied comprehensive formulation.

RESTORATION OF PRE-MATURE AGING SKIN

FIELD OF THE INVENTION

[001]This patent is directed to bioactive and nutritionally balanced biochemical compositions created to expeditiously and effectively intercede in the pre-mature aging process, thereby, restoring the degraded components of the dermal extracellular matrix. The effectiveness of this composition is facilitated by methods and apparatus designed to facilitate this process.

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, skin laxity, and loss of volume, all of which are viewed as the common visual signs of aging.

[003]The predominant functional 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 (ECM). The two fibrillar proteins, which play the central role in the skin's structural integrity, tone and elasticity are collagen and elastin, respectively. Using the analogy of a factory requirement of both raw materials and energy to produce an end-product, the fibroblast mirrors these requirements, as well. An efficacious composition intended to restore these elements in premature skin aging must be both bioactive and nutritionally balanced.

[004]Aging skin is, however, also characterized by the presence of an increasing number of senescent fibroblasts, which are no longer productive of the components of the ECM. Normal cells are said to have a finite division potential or replicative life-span. This was first formally described about four decades ago by Hayflick (1965). Campisi, reporting in the J Investig Dermatol Symp Proc: Vol. 3, No.1; 1-5; (1998), first illuminated the role of cellular senescence in skin aging.

[005]Exhaustion of the available pool of fibroblastic precursors, the adult human dermal stem cells, limits the life-span of this tissue. These stem cells are, thereby, limited to the number of replicative cell divisions that these precursor cells can undergo based upon the hypothesis that no human cell is immortal. This epigenic process is referred to as replicative senescence.

[006]This collateral process, also contributing to premature skin aging, was once thought to be irreversible but the recent studies of Biernaskie, Cell Stem Cell.Vol. 5, No.6; 610-623, (2009), of Mimeault M et al, J Cell Mol Med, Vol 14, Nos. 1-2; 116-134, (2010), and those of the investigators of Mibelle Biochemistry; Buchs, Switzerland (2012), have brought forth a cross-species process, which has been demonstrated in clinical studies, to revitalize these senescent human dermal precursor cells.

[007]The background foundation upon which this patent is based would not be complete without illuminating the role of the atrophic diminution in dermal volume and thickness in premature

skin aging. Loss of dermal thickness is about 20% in aging individuals with the loss of volume from an average of about 1.1 mm at the age of 20 to about 0.8 mm by the age of 80. Biomechanical properties also change during aging as the skin becomes increasingly rigid, inelastic and slower to recover from compressive forces. Silver et al, reporting in Skin Res & Tech, Vol 7.18-23 (2001), describe decreased elastic spring constant during skin aging consistent with the disruption of the elastic fibers and loss of α-helical structure.

[008]A comprehensive biochemical composition, process and apparatus that will effectively and expeditiously intercede in the process of pre-mature aging of skin does not currently exist. The failure of the prior art in understanding these critical issues underlying skin restoration is presented and their remedies are embodied in this patent.

[009]This invention embodies the energy component, as well as the appropriate raw materials, in the same ratios as present in normal body fluids, for fibroblastic restoration of age-depleted collagen and elastin.

[010]Also embodied in this patent is the stem cell re-vitalization of the senescent fibroblasts.

[011]Methods, apparatus and compositions for restoration of age-related dermal volume loss completes the comprehensive approach to the treatment and reversal of the visible signs of premature skin aging. Glutaric anhydride (GA) in conjunction with a chemical penetration enhancing compound causes collagen matrices to bind water and, thereby increase tissue volume at selected sites. The penetrant provides expeditious transepidermal delivery of the acylating agent (GA) to the ECM.

[012]U.S. Patent No. 6,161,544 of Devore and Oefinger (Dec. 12, 2000) disclose the use of a chemical softening agent, glutaric anhydride, for destabilizing cross-links between collagen molecules in the treatment of corneal refractive conditions but not in the context of enhancing the collagen thickness in the treatment of age-related tissue volume loss.

[013]U.S. Patent Application Publication No. US 2005/0106270 in May 2005 disclosed a chemical treatment of in vivo tissue to alter charge and net charge density characteristics. This patent application suggested a chemical method for the purpose of increasing dermal thickness and pliability.

[014]There was, however, one as-yet unmet challenge due to the formidable permeability barrier of the stratum corneum. The comprehensive anti-aging compositions, including the collagen volumizing composition, must breach this barrier and be rendered effectively and expeditiously bio-available to the target sites. A preferred embodiment of this invention is the efficacious transepidermal delivery capability from topical application. All active agents in the formulation are delivered simultaneously requiring only minimal dosimetry.

SUMMARY OF THE INVENTION

[015]The present invention is directed to methods, apparatus and comprehensive anti-aging compositions, which address the age-related degradation of the critical protein components and resultant visible signs of skin aging associated with the dermal extra-cellular matrix (ECM).

[016]The effectiveness of this anti-aging composition, methods and apparatus lies in the combined synergistic effect of the individual active agents and components. The novelty is also manifested in the expeditious process of transporting these agents to the target sites within the ECM.

[017]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 critical agents, as raw materials, energy sources and processes, driving the fibroblastic collagen and elastin remodeling are derived from the following formulation in a synergistic process.

[018]Embodied in this patent is a comprehensive biochemical composition, which is directed to two populations of fibroblasts; the currently productive fibroblasts, which require expeditious delivery of all raw materials with energy sources to enhance restoration of the age-depleted components of the ECM and the non-productive replicatively senescent fibroblasts, which initially require revitalization to a productive state as a prerequisite to enhancement by means of the appropriate raw materials and energy sources required for restorative synthesis of age-depleted components of the ECM.

[019]Collagen and elastin biosynthesis will partially restore the loss of structural integrity, tone and flexibility in the reversal of premature skin aging. The current use of injectable dermal fillers and volumizers will temporarily augment the age-related loss of volume while treating wrinkles and skin laxity but all are biodegradable and require multiple tissue interventions.

[020]This patent further embodies a non-interventional comprehensive treatment protocol with protracted aesthetic enhancements described as a topical volumizer. This adjunctive topical process yields prompt tissue augmentation, which can be supplemented with the daily topical application of the ECM restoration composition.

BRIEF DECRIPTION OF THE DRAWINGS

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

[022]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

[023]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.

[024]Figure 3 illustrates 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.

[025]Figure 4 is a relationship between cross-linking effectiveness (judged by melting

temperature) and PA concentration.

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

[027]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.

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

[029]Figure 8 illustrates the compact single barrel-dual chamber syringe containing the micronized GA powder in the proximal chamber and the buffered penetrant solution in the distal chamber.

[030]Figure 9 demonstrates the solubilization of collagen threads by acetic anhydride in alkaline buffer.

[031]Figure 10 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.

[032]Figure 11 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.

[033]Figure 12 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.

DETAILED DESCRIPTION OF THE INVENTION

[034]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", its productivity will depend upon not only the raw materials presented to it, but, it's sources of energy to drive this function, as well.

[035]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.

[036]This patent also embodies a composition, methods and apparatus directed to the immediate restoration of age-depleted tissue volumetric loss and reduction in wrinkles, furrows and skin laxity.

[037]It is intended that the topical ECM restoration formulations and the topical volumizer will address the visible signs of premature skin aging synergistically in the most comprehensive treatment protocol. The application of these separate but synergistic modalities might also be used effectively in stand-alone anti-aging rejuvenation procedures.

[038]This patent is also directed to a biochemical system,which provides expeditious and efficacious bio-availability of these agents. This is disclosed as a multi-functional drug delivery system.

[039]This patent embodies the following active agents in restoration of age-depleted components of the ECM.

Nutritionally Balanced Bio-active Formulation

[040]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, tone and structural integrity of the skin. As collagen is age-depleted, skin will deform under its own weight because gravity will be unopposed.

[041]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.

[042]Elastin, 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.

[043]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. These amino acids are essential because the body doesn't produce them intrinsically. The University of Arizona Biology Project has reported 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 does not store excess amino acids for later use."

[044]Our studies have revealed that the preferential bioactivity proceeds expeditiously when the amino acids are provided in the exact ratios as in the human body fluids (Figure 1).

[045]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. The recommended dietary allowance (RDA) tends to underestimate the body's requirement of sulfated nutrients. Solar aging will predispose to a degradation in the sulfate-dependent glycosaminoglycans (GAGs), which function as the dermal reservoir, imbibing great quantities of water per unit weight.

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

[047]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 has been previously unrecognized in the synthesis of GAGs.

[048]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.

[049]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 bioflavanoid, 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.

[050]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.

[051]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).

[052]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.

[053]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

[054]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

[055]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

[056]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 stabilizes the helical structure of collagen fibers and increases the denaturation temperature of collagen.

[057]Proanthocyanidin is a bioflavanoid 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 (Figures 4,5 & 6).

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

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

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

Nucleotides from DNA & RNA

[061]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.

[062]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

[063]Calcium (Ca) is a co-factor of the matrix metallopproteinases (MMPs) and in the conversion of pro-collagen to tropo-collagen, which takes place due to two enzymes (N- and C-proease), cleaving the N- and the VC- terminal of the procollagen molecule. 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 (cyclic adenosine monophosphate). This is a messenger intracellular signal transducer.

[064]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 mitocondria, which mediates the metabolism of the cell. Fe is also active in defense against reactive oxygen species (ROS).

[065]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.

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

[066]The patent also embodies a specific growth factor, connective tissue growth factor (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 in Premature Skin Aging

[067]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.

[068]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.

[069]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.

[070]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.

[071]This invention embodies the use of the extract, PhytoCellTec Argan™ in a concentration of about 0.4% to 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.

Tissue Volumizer

[072]Collagen and elastin biosynthesis will partially restore the loss of structural integrity, tone and flexibility in the reversal of premature skin aging. The current use of injectable dermal fillers and volumizers will temporarily augment the age-related loss of volume while treating wrinkles and skin laxity but all are biodegradable and require multiple tissue interventions.

[073]This patent further embodies a non-interventional comprehensive treatment protocol with protracted aesthetic enhancements described as a topical volumizer. This adjunctive topical process yields prompt tissue augmentation, which can be supplemented with the daily topical application of the ECM restoration composition.

[074]This patent also embodies a topical formulation, which is composed of an acylating agent defined as an agent that transfers an acyl group to another nucleophile, examples of specific agents include, but are not limited to glutaric anhydride, succinic anhydride or malleic anhydride, since each of these anhydrides hydrolyze into rather innocuous compounds.

[075]The GA (C5H6O3) is obtained from SIGMA-ALDRICH of St. Louis, MO. It is supplied in a white to light yellow powder or crystals or chunks of a molecular weight of 114.1 g/mol. It has been determined that rapid solubility is desirable in the embodied process. The GA is referred to a company, such as Powdersize, Inc. of Quakertown, PA for the purpose of particle size reduction and consistency. They employ processes, such as "high potency milling/micronization, jet milling, mechanical milling and micropulverizing." These technologies improve the solubility of poorly soluble chemical entities with average particle size ranges from 2 to 10 microns.

[076]The present invention embodies a process for reacting specific acylation agents with collagen fibrils in intact tissue to alter the net charge and net charge density of the treated tissue. The exposed tissue surface is pre-treated for 30 seconds to 2 minutes with a chemical penetration enhancer adjusted to alkaline pH to bring the pH of the tissue to between about 8.5 and about 9.5 resulting in deprotonation of ε-amino groups of lysine residues on exposed proteins.

[077]The multi-functional penetration enhancer is mixed with the acylating agent (micronized GA) for about 5 seconds bringing the GA to a concentration of between 10 mg/mL and 100 mg/mL, preferably between 10 mg/mL and 50 mg/mL in the buffered penetration enhancer.

[078]The resultant formulation is then applied to the exposed targeted tissue site such that the chemical composition immediately reacts with the exposed pre-treated tissue surface resulting in co-valent bonding of the pendant chemical moiety to the deprotonated ε-amino groups of lysine residues on exposed proteins.

[079]This is followed by thorough rinsing of the total tissue surface to remove unreacted chemical agent and masking the deprotonated free amino group with the desired pendant group to alter the net charge and the net charge density of the treated tissue. The predominant protein to react with the acylation chemicals is collagen.

[080]Acylation agents are applied to skin in order to obtain an increase in tissue hydration producing a thicker dermal skin layer with increased pliability. The chemical binding of water to the dermal collagen fibers will result in 3-D volumetric enhancement, thereby, correcting the deficits from facial fat loss, fat movement and skeletal remodeling associated with pre-mature skin aging.

Experimental Example No. 1

[081]A preliminary clinical evaluation was conducted on two subjects. An area on the forearm was selected and washed with isopropyl alcohol. The penetration enhancer composed of a combination of two chemical penetration enhancers that was adjusted to a pH of 9.5 was then applied to the forearm of each subject following minimal dermabrasion. One milliliter of the pH adjusted penetration enhancer was added to a vial containing 20 mg of GA micronized powder. The mixture was instantaneously dissolved in the proximal chamber of the embodied application device and was quickly applied to the forearm area pre-treated with the penetration enhancer. After approximately 1 minute, the treated area was rinsed with buffer solution. Immediately after the GA application, the treated area was examined both visually and by tactile inspection. Within 1 to 2 minutes, visual examination and tactile inspection revealed an obvious increase in skin thickness. This increase in skin thickness was maintained beyond two weeks duration.

Application Method and Apparatus:

[082]Another novel embodiment of this patent, which distinguishes this patent from the prior art is the method and apparatus utilized to facilitate application of the biochemical reagents.

[083]This patent also embodies the application of three single-barrel syringes. The first syringe is a single chamber syringe, which contains the multi-functional penetration enhancer buffered with an alkaline solution, such as sodium hydroxide or disodium phosphate to a pH of about 10. The targeted skin site is pre-treated for 1 to 2 minutes with the composition from within this

syringe, with or without slight skin abrasive action to effectively to bring the treated tissue to a pH range from about 7.5 to about 10.0.

[084]A second single-barrel syringe is a highly customized dual-chambered system fabricated by Unilife Corporation This novel device is pre-filled with the dry-micornized powdered acylating agent (GA) in the proximal chamber, which is sealed from inadvertent fluid contamination. The distal chamber is pre-filled with the liquid multi-functional penetration enhancer. This agent is buffered with an alkaline solution, such as sodium hydroxide, to a pH of about 10. (Figure 8).

[085]The liquid-to-dry micronized drug reconstitution takes place as the syringe plunger is pulled forth resulting in a negative pressure, thereby, aspirating the liquid (penetrant) compound into the proximal chamber and instantaneously admixing and blending the two drugs in solution. The pre-buffered liquid maintains the final mixture at an alkaline pH bringing the GA to a concentration of between 10 mg/mL and 100 mg/mL, preferably between 10 mg/mL and 50 mg/mL

[086]As the plunger is compressed, the resultant formulation is then rapidly applied to the exposed tissue site such that the chemical composition promptly reacts with the exposed pre-treated tissue. The penetrant enables expeditious transepidermal delivery and bio-availability of the GA resulting in co-valent bonding of the pendant chemical moiety to the deprotonated ε-amino groups of lysine residues on exposed proteins.

[087]Subsequent water-binding of the fibrillar collagen enhances the dermal collagen matrix volume (Figure 9).

[088]The third syringe in the system provides a buffer solution facilitating rinsing and neutralizing of the total tissue surface, thereby, removing un-reacted chemical agents.

[089]Other devices might be substituted in the application of the GA-penetrant mixture.

Biochemical Penetration Enhancer

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

[091]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, such as the embodied GA/penetrant mixture. 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.

[092]A multiphasic approach to transepidermal drug delivery is embodied in this invention. 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 corneal-induced epidermal barrier, and that have been demonstrated to be efficacious individually, might provide enhanced capability in the transport of topically applied compositions.

[093]It would appear that the common denominator in the equivocal results reported by others using various agents alone or in combination is the poor bioavailability of the drugs administered topically. Although a wide variety of methods have been deployed to enhance transepidermal delivery of the topically applied agents to render them expeditiously bioavailable to the targeted sites of bruising, they have been minimally effective. Studies have relied upon in-vitro models, which limit relevance.

[094]A major problem with most of these approaches is that they have been assessed in-vitro, using devitalized human skin, for the most part. Non-viable samples do not mount a metabolic response against barrier perturbations, and in-vivo repair responses, inevitably, restrict the efficacy of any enhancing method.

[095]Accordingly, an alternative approach is to enhance the efficacy of standard enhancers by inhibiting the repair metabolic) response in-vivo. Such a metabolic response could be used in conjunction with another method to further increase efficacy. Some of these methods can abrogate the barrier of intact skin by "opening the permeation window", thereby, obviating the requirement for pretreatment or co-treatment with a primary enhancer.

[096]The concept of biochemical approach to enhance transepidermal drug delivery came from pharmacological studies aimed at inhibiting key metabolic sequences that restore and maintain the barrier function, e.g., epidermal lipid synthesis. These methods all either alter the critical molar ratio of the three ket stratum corneum (SC) lipids, or induce discontinuities in the lamellar bi-layer system.

[097]Whereas, these studies determined metabolic events required for permeability barrier homeostasis, these inhibitors possess the potential to either cause the "window to open longer", and/or they actually can "open the window" for transepidermal drug delivery. Thus all of the pharmacological "knock-out" studies support the concept that interference with the biosynthesis of any of the key SC lipids can lead to a temporary increase in transepidermal water loss (TEWL), with obvious implications for transepidermal delivery.

[098]This patent embodies a transepidermal drug delivery system, whereby, the concentrations of biochemical penetration enhancers is determined by the molecular mass of the proposed penetrating agents.

[099]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.

[100]This multi-functional biochemical penetration enhancing formulation is comprised of at least two or more different biochemical agents, which function synergistically by disparate

biochemical pathways to effectively and expeditiously deliver the topically administered composition directly to the target site in the dermis.

[101]One active penetrant consists of a micro-emulsion-based organic gel defined as a semi-solid formation having an external solvent phase immobilized within the spaces available of a three-dimensional networked structure.

[102]This micro-emulsion-based organic gel in liquid phase is characterized by 1,2-diacyl-sn-glycero-3-phosphatidyl choline, an organic solvent, selected from a group consisting of:

1. ethyl laureate
2. ethyl myristate
3. isopropyl myristate
4. isopropyl palmitate
5. cyclopentane
6. cyclooctane
7. trans-decalin
8. trans-pinane
9. n-pentane
10. n-hexane
11. n-hexadecane
12. tripropylamine

in concentrations of from about 0.5% to 30% (w/w),
and a polar component, selected fro the following agents:

1. water
2. glycerol
3. ethylene glycol
4. formamide

and one or more anti-oxidants, selected from the following group:

1. vitamin C
2. vitamin E
3. proanthocyanidin
4. α-lipoic acid
5. l-ergothionene

in concentrations of from about 0.1 % to about 2.5% (w/w).

[103]This permeation enhancer is a bipolar molecule with the intended drug molecule present in the micelle of this agent, 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 phosphatidyl choline+ organic solvent formulation makes it possible for this bipolar molecule to enter the skin layers.

[104]The advantage of this penetrant over other organic solvents, with regard to the transepidermal transport, is that a wider range of drug molecules can be dissolved by it and can be delivered to the intended site under the skin at much higher concentrations. This is due to the fact that there is very little to no diffusion of the drug molecule as it penetrates through the skin.

[105]The micro-emulsion-based organic gel is dissolved in isopropyl palmitate or other organic solvents. 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 phosphatidyl choline 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 visco-elastic, bicompatible and isotropic gels consisting of a 3-dimensional network of entangled reverse cylindrical, polymer-like) micelles, which have no restrictions on the chemical structure on the drug to be transported.

[106]This penetration enhancer has the advantages of ease of preparation and scale-up, easier quality monitoring, thermodynamic stability, and enhanced topical perforation qualities.

Salient Features of this Penetrant:

[107] 1. Template vehicle: provides opportunities for incorporation of a wide range of substances with diverse physiochemical properties (e.g., chemical nature, solubility, molecular weight, size)

[108] 2. Process benefits: spontaneity of gel formation, by virtue of self-assembled supra-molecular arrangement of surfactant molecules makes the process simple and easy to handle.

[109] 3. Structural/physical stability: being thermodynamically stable, the structural integrity of the gels is maintained for longer periods of time.

[110] 4. Chemical stability: the gels are moisture insensitive and being organic in character, they also resist microbial contamination.

[111] 5. Safety: use of biocompatible. biodegradable and non-immunogenic materials makes them safe for long-term applications.

[112]Alternative compounds, not to be confused with the embodied biochemical penetration enhancer, have been formulated with poloxamers, which are nonionic triblock coploymers 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.

[113]Another embodied penetrant of the formulation is selected from a group of lower alkyl diols, C10-C20 fatty acids and esters, thereof, and C4-C20 optionally substituted aliphatic alcohols. Preferably, the transepidermal delivery agent is a optionally substituted aliphatic alcohol. More preferably, the optionally substituted aliphatic alcohols is substituted with an aromatic substituent.

[114]This permeation enhancer functions by transiently dissolving the lipids in the bilayer membrane of the epidermis.

[115]In doing so, the drug or compound dissolved in the penetrant 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.

[116]Due to the aromatic group (i.e., benzene) present in the penetrant, the molecule has a polar end (the alcohol end) and a non-polar end (the benzene end). This enables this penetrant 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.

[117]This penetration enhancer is provided to the formulation in a range from about a 0.5 % (w/w) to about 15% (w/w) concentration.

[118]Other penetration enhancers are known in the art but not in the context of two or more agents facilitating expeditious transepidermal delivery by means of synergistic biochemical activity.

[119]Although recent efforts in using chemical enhancers have focused on substances categorized as generally recognized as safe (GRAS), early work included a wide range of materials. In a review of the technical and patent literature, more than 275 different chemical compounds were found to be cited as skin penetration enhancers. This patent also embodies other chemical permeation enhancing agents.

[120]Chemical mixtures offer several opportunities to overcome the limitations of single chemical enhancers through a variety of different ways.

[121]Other permeation-enhancing chemical combinations embodied in this patent include:

1. fatty acids (linoleic acid, oleic acid, valeric acid, and lauric acid),
2. alcohols and organic solvents (acetone, ethanol, pentanol, lauryl alcohol, propylene glycol and glycerol),
3. amines (diethanolamine and triethanolamine),
4. esters (isopropyl palmitate isopropyl myristate and ethyl acetate),
5. amides (1-dodecylazacycloheptane-2-one, urea, dimethylacetamide, dimethylformamide and pyrrolidone derivatives),
6. hydrocarbons (alkanes and squalene),
7. surfactants (sodium laureate, cetylmethylammonium bromide and sodium cholate),
8. terpenes (D-limonene, carvone, and anise oil),and
9. sulfoxides (dimethyl sulfoxide)

Experimental Example No. 2

[122]A study was performed to confirm that the inventive biochemical penetration enhancers are efficacious and expeditious in rendering the topical nutritionally balanced bioactive and bio-available to the target site of the dermal extracellular matrix.

Analyses

[123]Two sets of analyses were performed:

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

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

Materials provided:

[126]1. Two jars of completed formulation

[127]2. Three jars of control base

ANALYSIS A: Percutaneous Absorption Study

[128]Two major evaluations were performed:

[129]A. Does the formulation penetrate through human skin equivalent?

[130]B. If it does penetrate how long does it take to reach the dermal layer?

Materials:

[131]1. Collagen Biosynthesis compound

[132]2. Control Base

[133]3. EpiDermTM Skin Model (EPI-200X) Kit, 24 x 2 tissues purchased from MatTek Corp.

[134]4. MTT assay (ET-50) purchased from MatTek Corp.

[135]5. MatTek Permeation device (EPI-100-FIX)

[136]6. PBS for culturing (EPI-100-PBS)

[137]7. Six well plates and other plastic ware and tubes from Costar.

Assay Method

[138]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.

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

[140]The cell culture insert, which contains the EpiDermTM 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.

[141]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.

[142]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.

[143]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.

[144]After incubation, PBS from the 6 wells were collected in separate tubes, labeled and stored in –700C for further processing.

Elemental Analysis

[145]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

[146]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 10 shows values for iron (Fe) and Figure 11 shows values for copper (Cu).

[147]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

[148]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.

[149]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

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

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

[151]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

[152]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.

[153]These analyses showed that exposure to Collagen Biosynthesis compound induced the expression of collagen in human dermal fibroblasts within 30 minutes 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

[154] 1. Collagen Biosynthesis compound, supplied by Bruce Sand, M.D., 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.

[155] 2. 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 (Figure 12).

[156]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.

[157]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, optical clearing compounds, anti-bruising compounds, hair restoration formulations, topical vaccines, and other agents, wherein effective and expeditious transepidermal bio-availability would be advantageous.

ADVANTAGES OF THE INVENTION

[158]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.The present invention discloses a composition comprising an appropriate dosage of drug and a transepidermal delivery system comprised of two 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.

EQUIVALENTS

[159]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.

[160]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.

WHAT IS CLAIMED IS:

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:
a. methionine
b. leucine
c. lysine
d. phenylalanine
e. threonine
f. tryptophan
g. valine
h. histidine
i. arginine
j. 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:

a. methionine	3.28 % (w/w) of the subgroup
b. leucine	10.92%
c lysine	14.20%
d. phenylalanine	8.20%
e. threonine	12.02%
f. tryptophan	7.65%
g. valine	16.39%
h. histidine	8.20%
i. arginine	10.92%
j. isoleucine	8.20%

5. The topical composition according to Claim 1, wherein, the bioactive formulation consists of an additional sulfated amino acid (SAA) 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 method for treating animal tissue, comprising the steps of:

(a) providing an exposed surface area of animal tissue;

(b) applying an alkaline-penetration enhancing solution to the exposed tissue surface area

to form a tissue protein having a different net charge;

(c) providing a quantity of acylating compound powder, which has been milled to particle size of about 2 microns to about 10 microns, and

(d) applying an acylating /alkaline-penetration enhancing solution to the exposed surface area so that the acylating/alkaline-penetration enhancing solution penetrates the epidermis and reacts with the tissue protein to form a protein complex having a different charge than the net charge of the tissue protein formed in above step (b).

24. The method of Claim 23, further comprising the step of rinsing the exposed surface area with a neutral or alkaline solution after step (c).

25. The method of Claim 23, wherein the tissue protein is collagen.

26. The method of Claim 23, wherein the animal tissue is human tissue.

27. The method of Claim 23, wherein the animal tissue is human tissue and the human tissue is treated in vivo.

28. The method of Claim 27, wherein the tissue is skin tissue.

29. The method of Claim 28, wherein the treatment with the acylating/ alkaline-penetration enhancing solution makes the skin more hydrophilic

30. The method of Claim 23, wherein the alkaline solution comprises a compound selected from the group consisting of disodium phosphate, sodium pyrophosphate, and sodium borate and penetration enhancing solution.

31. The method of Claim 28, wherein the alkaline penetration enhancing solution has a pH in the range of about 7.5 to about 9.5.

32. The method of Claim 23, wherein the acylating agent comprises a compound selected from the group consisting of sulfonic acids, sulfonyl chlorides, and acid chlorides.

33. The method of Claim 23, wherein the net charge of the tissue protein formed in step (b) is positive, and the net charge of the protein complex formed in step (c) is negative.

34. The method of Claim 23, wherein the net charge of the tissue protein formed in step (b) is positive, and the net charge of the protein complex formed in step (c), is neutral.

35. The method of Claim 23, wherein the tissue protein is treated with an acylating/alkaline-penetrating enhancing solution that causes the protein complex formed in step (c), to have increased net positive charge over the tissue protein formed in step (b).

36. The method of Claim 23, wherein the tissue protein is treated with an acylating/penetration enhancing solution that causes the protein complex formed in step (c) to have increased net negative charges over the tissue protein formed in step (b).

37. The method of Claim 23, wherein the combination acylating/penetration enhancing solution is mixed in the special designed single-barrel/dual chamber syringe with micronized acylating powder contained in the proximal chamber and the buffered penetration enhancing solution is contained in the distal chamber prior to mixing.

38. The topical composition according to Claim 1, wherein, the bioactive formulation is further comprised of two or more biochemical penetration enhancers, one of which consists of a micro-emulsion-based organic gel in liquid phase characterized by 1,2-diacyl-sn-glycero-3-phosphatidyl choline and an organic solvent selected from a group consisting of:

a. ethyl laureate
b. ethyl myristate
c. isopropyl myristate
d. isopropyl palmitate
e. cyclopentane
f. cyclooctane
g. trans-decalin
h. trans-pinane
i. n-pentane
j. n-hexane
k. n-hexadecane
l. tripropylamine

in concentrations of from about 0.5% to about 35% (w/w), as determined by the molecular mass of the penetrating active agent or agents, and a polar component, selected fro the following agents:

a. water
b. glycerol
c. ethylene glycol
d. formamide

and one or more anti-oxidants, selected from the following group:

a. vitamin C
b. vitamin E
c. proanthocyanidin
d. α-lipoic acid
e. l-ergothionene

39. The topical composition according to Claim 38, wherein, another biochemical penetration enhancers is selected from a group of lower alkyl diols, C10-C20 fatty acids and esters, thereof, and C4-C20 optionally substituted aliphatic alcohols.

40. The transepidermal delivery agent according to Claim 39, wherein the agent is a optionally substituted aliphatic alcohol.

41. The optionally substituted aliphatic alcohols according to Claim 40, wherein the agent is substituted with an aromatic substituent present in a range of about 1.0% to about 20.0% (w/w).

42. The topical composition according to Claim 41, wherein, the agent is present in a range of

about 1.0% to about 5.0%% (w/w).

43. The topical composition according to Claim 38, wherein the multi-functional biochemical penetration enhancer comprising a first, second or other penetrants functioning synergistically and following disparate biochemical pathways with a pH ranging from about 3.0 to about 7.4.

44.. The topical composition according to Claim 38, wherein the multi-functional biochemical penetration enhancer is independently selected from the group of;

a. lower alkyl diols
b. C10-C20 fatty acids and esters, thereof,
c. C4- C20 substituted aliphatic alcohols,
d. C4- C20 unsubstituted aliphatic alcohols,
e. 1,2-diacyl-sn-glycero-3-phosphatidyl
f. choline and an organic solvent

45. The topical composition according to Claim 38, wherein the multi-functional biochemical penetration enhancer might host other guest molecules, thereby facilitating bio-availability.

46. The topical composition according to Claim 45, wherein the multi-functional biochemical penetration enhancers might be selected from lists of:

a. fatty acids (linoleic acid, oleic acid, valeric acid, and lauric acid),
b. alcohols and organic solvents (acetone, ethanol,pentanol, lauryl alcohol, propylene glycol and glycerol),
c. amines (diethanolamine and triethanolamine),
d. esters (isopropyl pamitate isopropyl myristate and ethyl acetate),
e. amides (dodecyllazacycloheptane-2-one, urea, dimethylacetamide, dimethylformamide and pyrrolidone derivatives),
f. hydrocrbons (alkanes and squalene),
g. surfactants (sodium laureate, cetylmethylammonium bromide and sodium cholate),
h. terpenes (D-limonene, carvone, and anise oil),and
i. sulfoxides (dimethyl sulfoxide)

47. The topical composition according to Claim 45, wherein the multi-functional biochemical penetration enhancers might host such guest molecules selected from the group of:

a.retinoids,
b. skin lightening compounds,
c. non-steroidal anti-inflammatory drugs,
d. anti-fungal drugs,
e. optical clearing compounds,
f. anti-bruising compounds, and
g. other agents, wherein effective and expeditious transepidermal

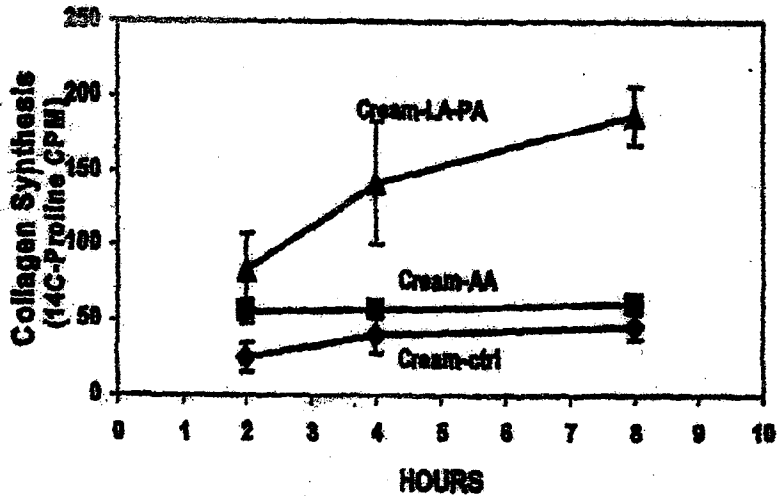
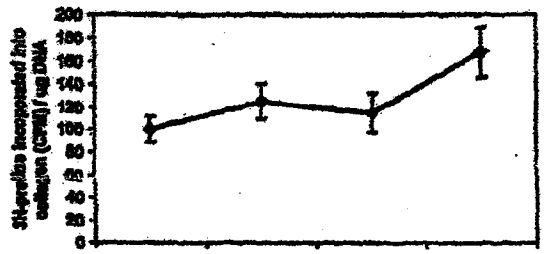
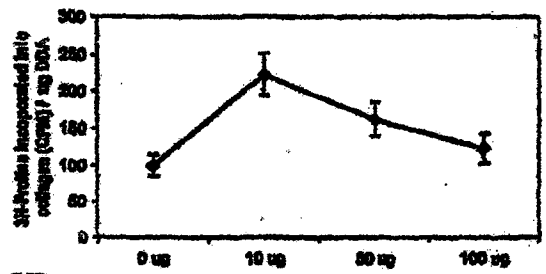


FIGURE 1



2A



2B

FIGURE 2

FIGURE 3

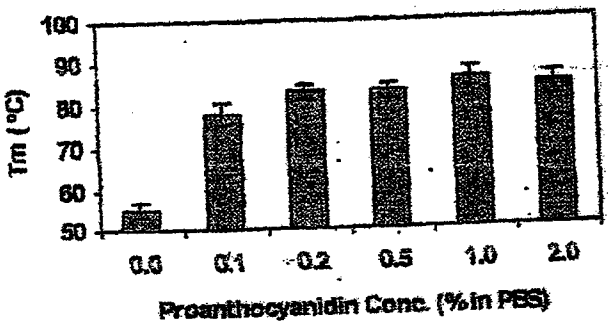


FIGURE 4

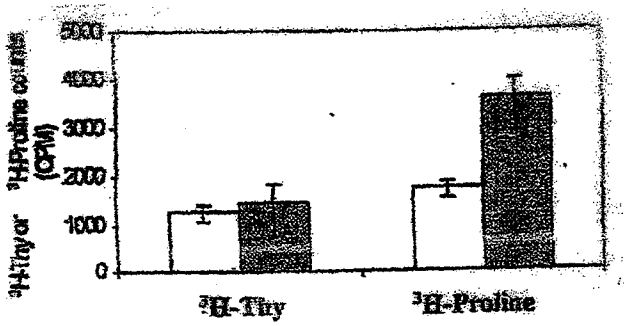


FIGURE 5

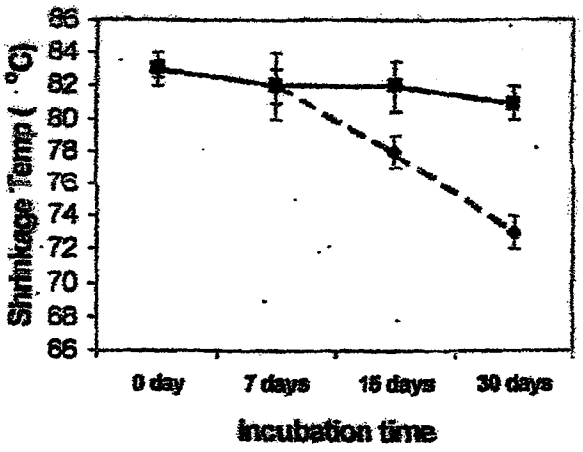


FIGURE 6

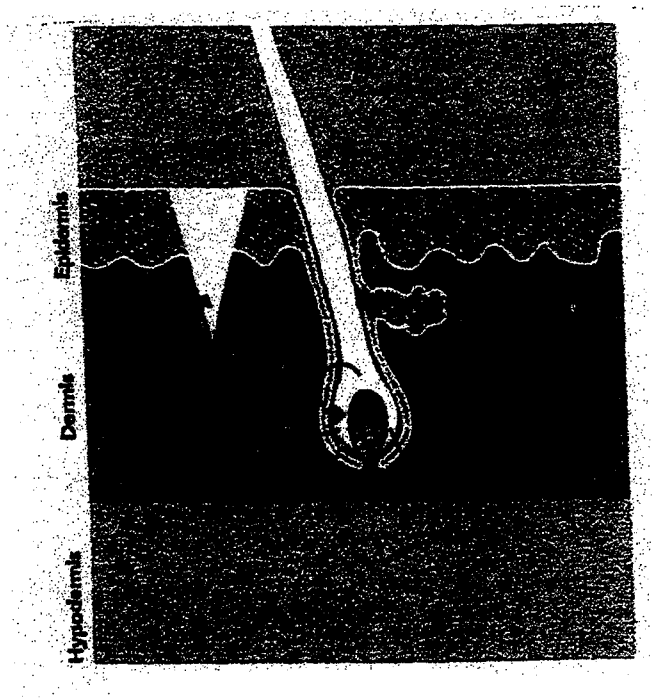


FIGURE 7

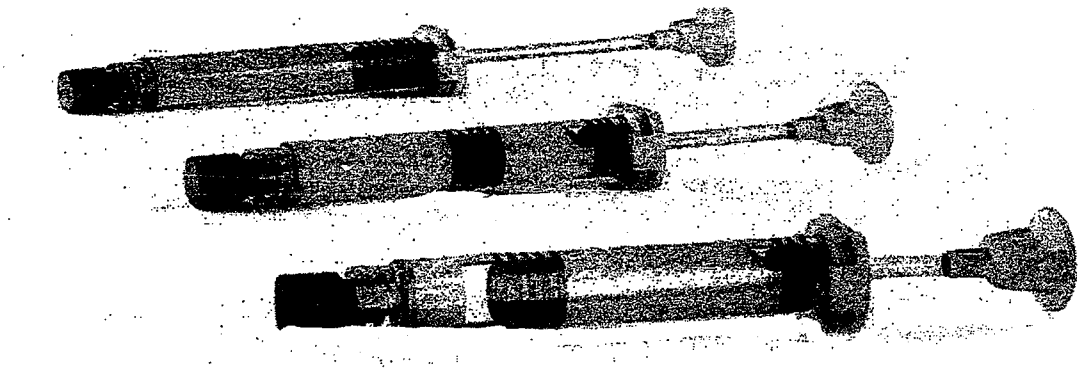


FIGURE 8

Solubilization of collagen threads by Acetic Anhydride in alkaline buffer

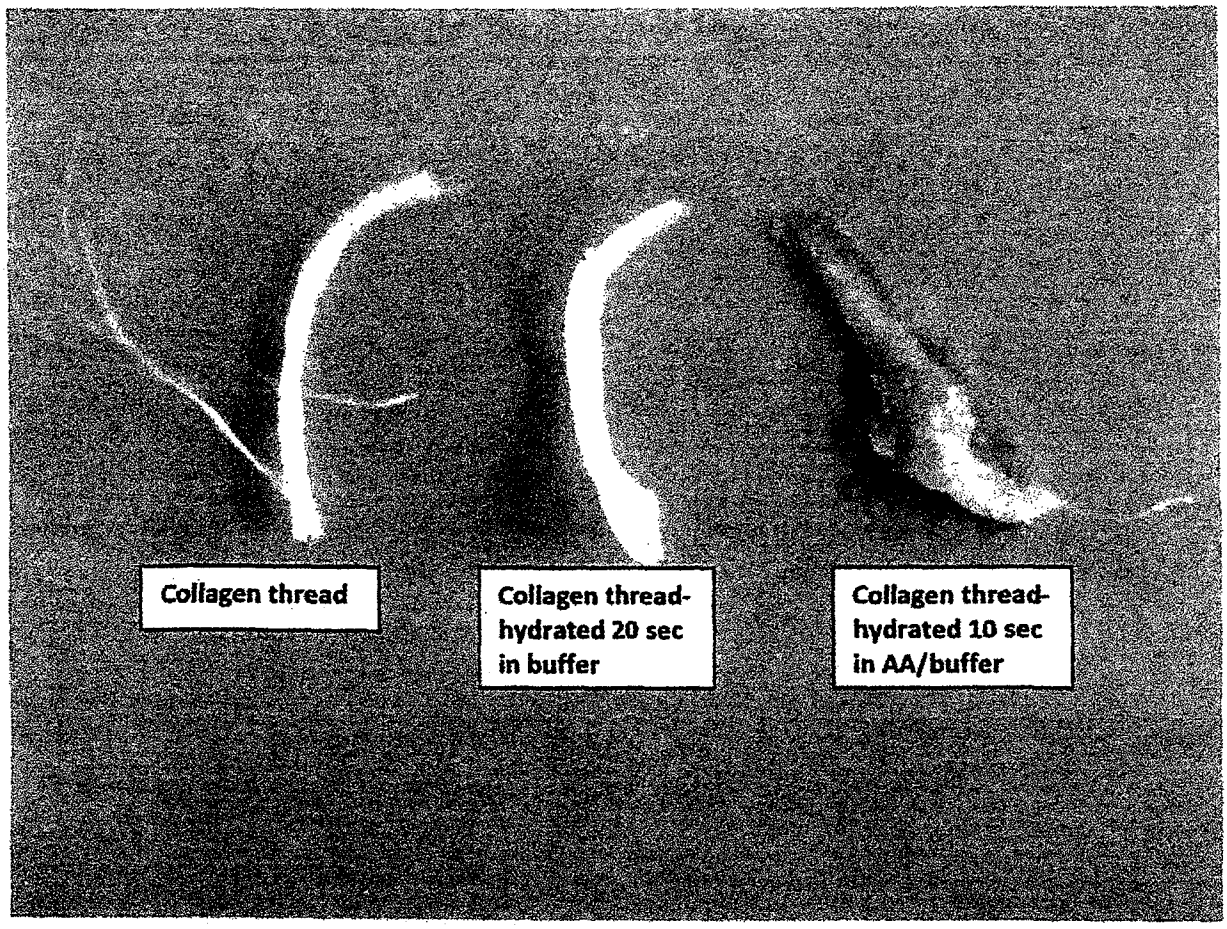


FIGURE 9

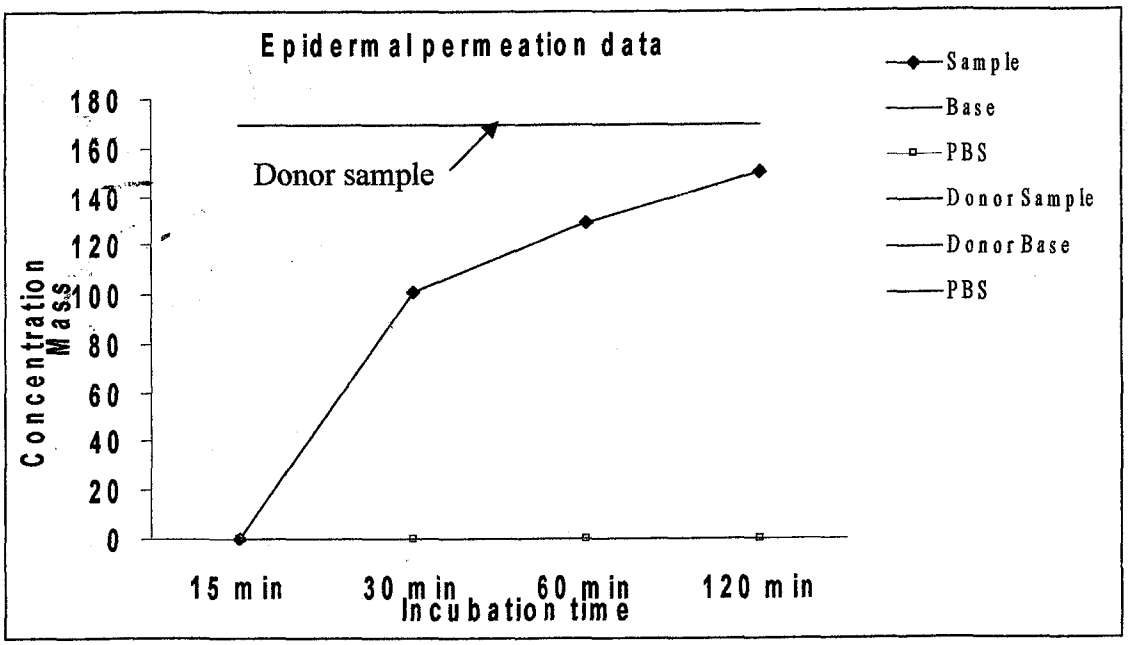


Figure 10. Concentration Mass of iron (Fe) in samples collected at four different time points. Samples were evaluated for elements by PIXI analysis. Donor sample at the concentration used had Fe at a concentration mass of 169.708 (straight line). Experimental samples (with **Collagen Biosynthesis compound**) 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.

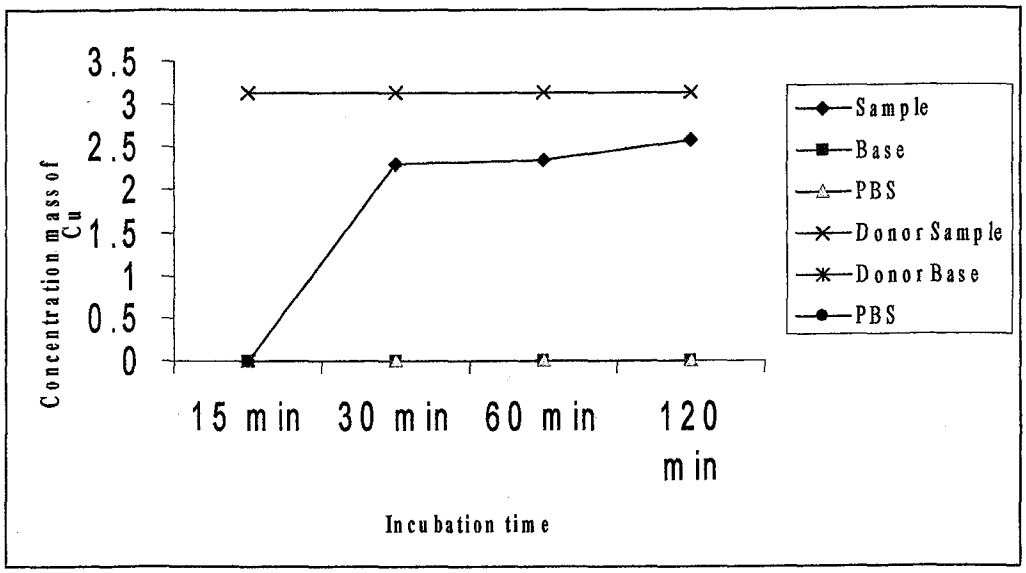


Figure 11. Concentration Mass of copper (Cu) in samples collected at four different time points. Samples were evaluated for elements by PIXI analysis. Donor sample at the concentration used had Cu at a concentration mass of 3.132 (straight line). Experimental samples (with **Collagen Biosynthesis compound**) 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.

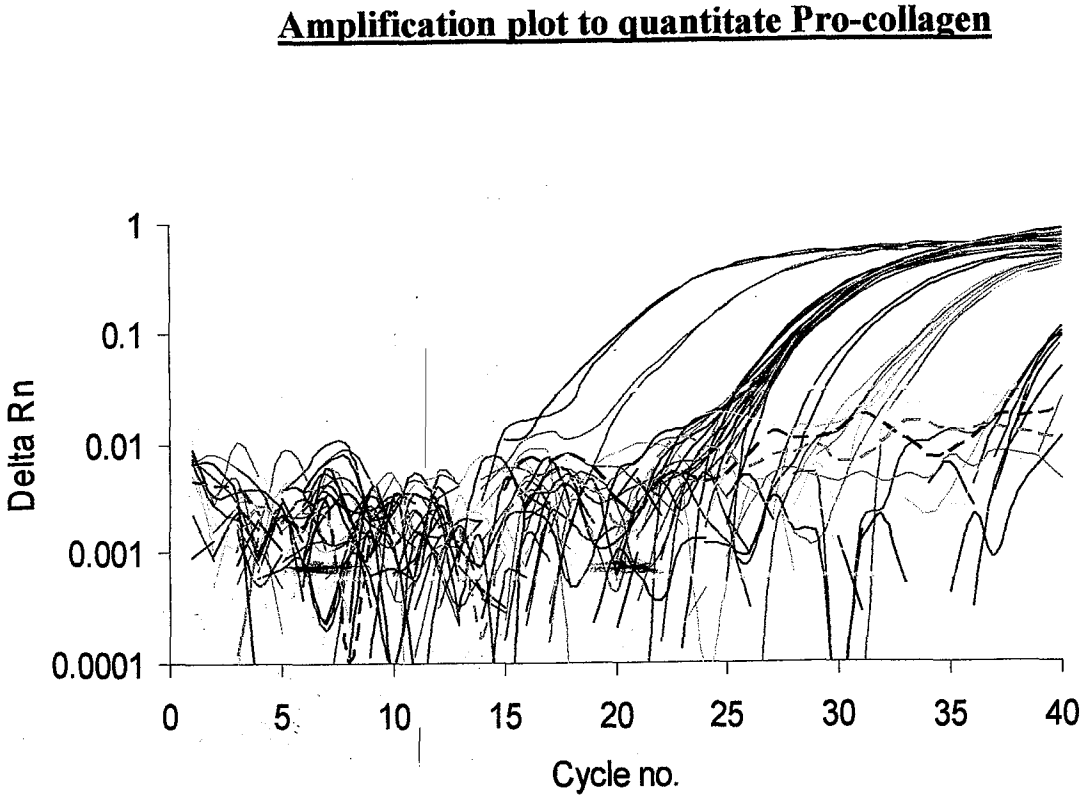


Figure 12. Amplification plot data using pro-collagen primers and probes. These results show that human dermal fibroblast cells began expressing pro-collagen within 30 min after exposure to **Collagen Biosynthesis compound** sample. Control samples exposed to base alone did not express pro-collagen at this time point.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/72239

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/00, A61K 38/03, A61K 38/39, A61P 17/02 (2015.01)

CPC - A61K 31/00, A61K 38/03, A61K 38/39, A61Q 17/00, A61Q 19/08

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)- A61K 31/00, A61K 38/03, A61K 38/39, A61P 17/02 (2015.01);
CPC- A61K 31/00, A61K 38/03, A61K 38/39, A61Q 17/00, A61Q 19/08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 424/78.03, 424/93.7, 424/642, 514/18.6, 514/300, 514/356, 514/783;
Patents and NPL (classification, keyword; search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Pub West (US EP JP WO), Pat Base (AU BE BR CA CH CN DE DK EP ES FI FR GB IN JP KR SE TH TW US WO), Google Patent, Google Scholar, Free Patents Online; search terms: topical, cream, location, cosmetic, skin, care, aging, antiaging, collagen, solar, sun, methionine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, histidine, arginine...

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 2013/0108603 A1 (BENNETT) 02 May 2013 (02.05.2013), para [0008], [0010], [0011], [0014], [0021], [0025], [0028], [0029], [0037], [0044], [0045]	1-3, 6, 10, 11, 16 ---------- 4, 5, 7-9, 12-15, 17-22, 38-47
Y	US 2006/0286406 A1 (HABER) 21 December 2006 (21.12.2006), para [0005], [0007], [0021], [0032], [0040], [0056], [0071], [0074]-[0079], [0109], [0113], [0126]	4, 5, 7-9, 12-15, 17-22, 38-47
Y	US 2009/0068255 A1 (YU et al.) 12 March 2009 (12.03.2009), para [0011]-[0247]	1-22, 38-47
Y	US 2009/0053290 A1 (SAND et al.) 26 February 2009 (26.02.2009), para [0019]-[0352]	1-22, 38-47
Y	US 2005/0287182 A1 (MONKS et al.) 29 December 2005 (29.12.2005), para [0019]-[0178]	1-22, 38-47

☐ 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
28 April 2015 (28.04.2015)	29 MAY 2015
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. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/72239

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.:
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:

-- Please See Extra Sheet --

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.:

1-22, 38-47

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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 14/72239

Continued from Box Number III, Observations where unity of invention is lacking,

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-22 and 38-47, drawn to a topical composition for the treatment of pre-mature solar aging.

Group II: Claims 23-37, drawn to a method for treating animal tissue.

Special Technical Features

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group II do not require a composition, comprising nutritionally balanced bioactive formulation to enhance the synthesis of collagen, elastin and other components of the dermal extracellular matrix, as required by Group I.

Group I do not require a method, comprising:
(a) providing an exposed surface area of animal tissue;
(b) applying an alkaline-penetration enhancing-solution to the exposed tissue surface area to form a tissue protein having a different net charge;
(c) providing a quantity of acylating compound powder, which has been milled to particle size of about 2 microns to about 10 microns, and
(d) applying an acylating /alkaline-penetration enhancing solution to the exposed surface area so that the acylating/alkaline-penetration enhancing solution penetrates the epidermis and reacts with the tissue protein to form a protein complex having a different charge than the net charge of the tissue protein formed in above step (b), as required by Group II.

Shared Common Features

The only feature shared by Groups I and II that would otherwise unify the groups is dermal epidermis tissue. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by US 2012/0046225 A1 to Prestrelski, et al. (hereinafter 'Prestrelski'). Prestrelski discloses a dermal epidermis tissue (para [0023], [0051]).

As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.