Cosmetic Ingredient Review
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Memorandum

To: CIR Expert Panel

From: Director, CIR

Subject: Human Umbilical Extract – new data

Date November 18, 2010

In 2002, CIR published its final safety assessment of ingredients derived from human and animal placentas and umbilical cords. These ingredients included Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract.

The available data were considered insufficient to support safety. Even while announcing this decision, the CIR Expert Panel did alert the industry that none of these ingredients as used in cosmetics should deliver any metabolic/endocrine activity and that any current or future use should be free of detectable pathogenic viruses or infectious agents.

Several of these insufficient data ingredients, including Human Umbilical Extract, have been placed on the "Insufficient Data – No current uses" list with the expectation that if there were to be usage in the future, sufficient data to support safety would be provided.

We have received correspondence from Cosmetikare Laboratories, LLC indicating that they intend to use Human Umbilical Extract in cosmetic products. They have provided a number of pieces of information to support the safety of this ingredient.

In the attached table, we have listed the data needs that led to the original insufficient data finding (along with the two additional caveats adopted by the Panel), and, where possible, have matched the pieces of data provided by the company.

Do these additional data provide a basis for reopening this safety assessment to change the conclusion for Human Umbilical Extract?

An element of potential confusion exists in that the INCI name "Human Umbilical Extract" (which has the circular definition of "extract from human umbilical cord") appears to be synonymous with "umbilical cord blood serum." Cord blood would appear to be commonly understood to come from the umbilical cord and serum is clearly a derivative of blood, but the question could be asked: Is blood serum what is usually considered under the single word: "extract?" It may be that this ingredient is not actually Human Umbilical Extract, but the circular definition in the *International Cosmetic Ingredient Dictionary and Handbook* offers little help in resolving the question.

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Data needs from original safety assessment	Data provided
Skin sensitization at concentration of use	L & L Cord Extract Blind Study (6 week clinical trial)
Gross pathology and histopathology in skin and other major organ systems associated with repeated exposures, and dermal reproductive and developmental toxicity data	1. 48 hour primary irritation testing (Consumer Product Testing) 2. Histogeometric Analysis of the Effects of Product A versus B on Human Skin "Other major organ systems" and dermal reproductive and developmental toxicity not addressed
Photosensitization data	Not provided
One genotoxicity assay in a mammalian system, if positive, a 2-year carcinogenicity study using NTP methods may be needed	Not provided
Ocular toxicity, if available	Not provided
*free of metabolic/endocrine activity	Not addressed
*free of pathogenic viruses or infectious agents	1. Cryobanks International SOP 2. Certificate of analysis
	Patent application for topical composition comprising umbilical cord blood serum
	Promotional material - UnderEye recovery cream with Cord Serum Complex - Anti Aging Face Cream with CSC

Final Report on the Safety Assessment of Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract[1]

Various proteins, lipids, or other extracts from human or other animal placentas are described as cosmetic ingredients. Human Placental Protein comprises protein derived from human placentas. Placental Protein is derived from animal placentas. Similarly, Human Placental Lipids and Placental Lipids are the lipid fractions from the same source materials. Hydrolyzed Human Placental Protein and Hydrolyzed Placental Protein are produced from the respective protein extracts by acid, enzyme, or other hydrolysis methods. Human Placental Enzymes and Placental Enzymes are enzymes obtained by aqueous extraction of human or other animal placental material. Human Umbilical Extract and Umbilical Extract are unspecified extracts of material from human or other animal umbilical cords. Different materials called Human Placental Extracts and Placental Extracts, assumed to contain estrogenic hormones or other biologically active substances, are not recognized as cosmetic ingredients, even though the use of these ingredients in cosmetics have been reported to the Food and Drug Administration (FDA). Human-derived ingredients are prohibited from use under the provisions of the European Union cosmetics directive based on concerns about transmission of human spongiform encephalopathies and viral diseases, for example, human immunodeficiency virus (HIV). Umbilical Extract has precedent for unrestricted use in Japan, except for certain products. Most of these ingredients are described as hair-conditioning agents and miscellaneous skin-conditioning agents, although the umbilical extracts function as biological additives in cosmetics. Of the human-derived ingredients, only Human Placental Protein is currently reported to be used. Animal-derived placental proteins, hydrolyzed proteins, lipids, and enzymes were all currently reported to be used. No current uses of the umbilical extracts were reported. Most of the available data relates to placental derivatives that appear to have estrogenic or other biological activity. The one clinical study that appears to utilize proteinaceous material only reported no irritant reaction. Clearly, the available data are insufficient to support safety of these ingredients in cosmetics. The additional data needed include (1) skin sensitization at concentration of use; (2) gross pathology and histopathology in skin and other major organ systems associated with repeated exposures, and dermal reproductive and developmental toxicity data; (3) photosensitization; (4) one genotoxicity assay in a mammalian system; if positive, then a 2-year dermal carcinogenicity study using National Toxicology Program (NTP) methods may be needed; (5) ocular toxicity, if available. Any studies should be done on all ingredients unless chemical analysis data show similarity among ingredients. Because there is confusion and concern about the use of substances with estrogenic or other biological activity in cosmetic formulations, it was concluded that none of these ingredients used in cosmetics should deliver any metabolic/endocrine activity. In addition, any current use of these ingredients should be free of detectable pathogenic viruses or infectious agents.

INTRODUCTION

This report is a compilation of data concerning Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract. Ingredients designated "human," for example Human Placental Protein, are derived from human sources. Ingredients not designated "human," for example Placental Protein, are derived from bovine and other animal sources.

The "Cosmetic Product-Related Regulatory Requirements and Health Hazard Issues" section of *FDA's Cosmetic Handbook* has stated the following regarding placental-derived ingredients (both human and animal):

> Products containing Estrogenic Hormones, Placental Extract or Vitamins
>
> In addition to being considered misbranded drugs, products claiming to contain placental extract may also be deemed to be misbranded

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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. Bindu Nair and Amy R. Elmore, former Cosmetic Ingredient Review staff members, prepared this report. Address correspondence to F. Alan Andresen, Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

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> cosmetics if the extract has been prepared from placentas from which the hormones and other biologically active substances have been removed and the extracted substance consists principally of protein. The FDA recommends that this substance be identified by a name other than "placental extract" which describes its composition more accurately because consumers associate the name "placental extract" with a therapeutic use or some biological activity (FDA 1994).

Human placental extract and placental extract are not recognized as cosmetic ingredients in the Cosmetic, Toiletry, and Fragrance Association (CTFA) *International Cosmetic Ingredient Dictionary and Handbook* (Wenninger and McEwen 1997). However, in January 1998 "human placental extract" and "placental extract" were reported to be used in 14 and 31 cosmetic formulations, respectively (see Use section).

The Cosmetic Ingredient Review (CIR) Expert Panel expects that cosmetic grade Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract do not contain hormones or other biologically active components. Similarly, the Panel expects that ingredients identified as human placental "extract" or placental "extract" are also free of biological activity.

The published literature contains numerous articles concerning human placental extract. Three preparation techniques are noted and are detailed in the Chemistry—Method of Production section of this report. Because limited information was found regarding the cosmetic ingredients, articles concerning presumed biologically active human placental extract and placental extract are contained in this report. How the composition of these tested extracts compares to those reported to be used in cosmetic formulations is not known.

CHEMISTRY

Definition

No definitions were found for cosmetic-grade human placental extract or placental extract.

Human Placental Protein

This ingredient is the protein derived from human placenta obtained from normal afterbirth (Wenninger and McEwen 1997). Younoszai and Haworth (1969) reported that term placentas are comprised of 12.0% ± 0.12% protein by wet weight, or 78.5% ± 0.85% by dry weight.

Hydrolyzed Human Placental Protein

This ingredient is the hydrolysate of Human Placental Protein (*q.v.*) derived by acid, enzyme, or other method of hydrolysis (Wenninger and McEwen 1997).

The CAS number 73049-73-7 refers to several hydrolyzed proteins in the CTFA *International Cosmetic Ingredient Handbook*. Further, the published literature identifies the CAS number as that of tryptone, which is defined as "a peptone produced by proteolytic digestion with trypsin" (Taylor 1988). Because it is not specific to Hydrolyzed Human Placental Protein, this CAS number was not used to obtain published articles.

Human Placental Enzymes

These ingredients are the enzymes derived from human placentas obtained from normal afterbirth (Wenninger and McEwen 1997).

Human Placental Lipids

These ingredients are the lipids derived from human placentas obtained from normal afterbirth (Wenninger and McEwen 1997).

Younoszai and Haworth (1969) reported that term placentas are comprised of ~0.4% lipids by wet weight, or ~2.9% by dry weight. The English abstract of a French article described the lipid content of blood-free placentas to have the following composition: 62% phospholipids, 13% to 18% free fatty acids, and 16% to 18% nonesterified cholesterol. Arachidonic acid accounted for 19.5% of total fatty acids (free and esterified). The 62% phospholipid content was itself composed of 40% diacyl phosphatidylcholine, 25% sphingomyelin, 10% ethanolamine plasmalogen, 7% diacyl phosphatidylethanolamine, 4% phosphatidylserine, 3% phosphatidylinositol, and 9% lysophospholipids. The investigators reported that the industrial process used to extract blood from the placenta did not induce either fatty acid oxidation or phospholipid hydrolysis (Chirouze, Entressangeles, and Helme 1987).

Human Umbilical Extract

This ingredient is an extract of human umbilical cord (Wenninger and McEwen 1997).

Placental Protein

Placental Protein is a mixture of proteins derived from animal placentas (Wenninger and McEwen 1997).

Hydrolyzed Placental Protein

This ingredient is the hydrolysate of Placental Protein (*q.v.*) derived by acid, enzyme, or other method of hydrolysis (Wenninger and McEwen 1997). Like its human-derived counterpart, Hydrolyzed Placental Protein is also identified by the CAS number 73049-73-7.

Placental Enzymes

Placental Enzymes is a mixture of enzymes obtained from an aqueous extraction of animal placentas (Wenninger and McEwen 1997).

Placental Lipids

Placental Lipids is a mixture of lipids derived from animal placentas (Wenninger and McEwen 1997).

Umbilical Extract

Umbilical Extract is an extract of animal umbilical cords (Wenninger and McEwen 1997).

Method of Preparation

Human Placental Extract

Two placental extracts (one human the other bovine) were described as used in "dermocosmetology." These extracts were obtained using the Filatov technique (not explained) under conditions "favoring the development of biogenic stimulins." The extracts were purified by filtration and were sterilized by autoclaving at 120°C. They were supplied as pale yellow liquids with a characteristic odor (CTFA 1998).

Several studies cited in this report tested human placental extract that was prepared in one of two ways. Fresh human placentas from normal deliveries were collected, washed to remove blood, homogenized with buffer, filtered until clear, and the preservative, benzyl alcohol added. Each milliliter of extract was derived from 0.1 g of fresh human placenta. Known constituents of the extract include human placental lactogen (HPL), corticotropin-releasing factor (CRF), fibrin-stabilizing factor (FSF), and lactoferrin (Banerjee, Bishayee, and Chatterjee 1993). Studies cited in this report that used this extraction method identified the human placental extract as HPE.

Other studies tested a human placental extract fraction (EAP). Placentas were collected at delivery and immediately frozen at −20°C. Pools of 500 to 600 placentas were mechanically ground, and then stirred until thawed in an 8% (*v/v*) ethanol/water solution. Placental blood was separated from the tissue by means of a press. The tissue pulp was extracted with acid and the extract was neutralized and precipitated with 15% ethanol. The supernatant was recovered by centrifugation, concentrated by ultrafiltration, and diafiltered against 0.9% NaCl solution with 10,000-Da cut-off membranes. One liter of the fraction "corresponded to" ~21 kg of placental tissue pulp (Klein, Chiodino, and Yamasaki 1991).

Contaminants

Human Placental Extract

One source reported that two Filatov-type placental extracts (human and bovine) used for "dermocosmetology" were devoid of estrogenic activity. The extracts were subcutaneously administered (20 ml/kg) to 11 female Sprague-Dawley rats (3 weeks old). Five hours after dosing, the rats were killed and the uterus was removed, weighed, dehydrated, and reweighed. The difference between fresh and dry weight was used to calculate the water content of the uterus. An increase in this parameter was evidence of estrogenic activity. No significant increase was noted compared to nontreated controls (CTFA 1998).

Beyssac, Martini, and Cotte (1986) detected estriol at a calculated maximum of 100 μg/l in various human placental extract preparations that were defined as "used in the cosmetics industry." In addition, a survey concerning use of hormone/placenta-containing hair preparations by children measured an estriol content of 1.9% (*w/w*) in one placenta-containing hair preparation (not distinguished as human or animal in origin). The investigator of the survey suggested that use of these products by children could cause sexual maturation at an earlier age (Tiwary 1997).

USE

Cosmetic

The human and animal placental-derived protein, hydrolyzed protein, enzyme, and lipid ingredients all function in cosmetic formulations as hair-conditioning agents and skin-conditioning agents—miscellaneous. Human Umbilical Extract and Umbilical Extract are used as biological additives (Wenninger and McEwen 1997).

As of January 1998, Human Placental Protein was reported used in 30 cosmetic formulations. Two uses of "human placental extract, liquid" and 12 uses of "human placental extract, lyophilized" were reported. Placental Protein (identified as animal or bovine) was used in five formulations; Hydrolyzed Placental Protein was used in seven formulations; Placental Enzymes was used in seven formulations; and Placental Lipids, bovine was used in one formulation. "Placental extract" was used in 31 formulations (FDA 1998) (Table 1). Where available, current concentration of use data (CTFA 1999) are also shown in Table 1. Historical concentration of use data are also given in Table 1.

One source recommended use of human and bovine placental extract at concentrations between 5% and 20% (CTFA 1998).

The European Community Directive prohibits the use of Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, and Human Umbilical Extract in cosmetics. The European Union designation, "cells, tissues or products of human origin," is listed in Annex II—"List Of Substances Which Must Not Form Part Of The Composition Of Cosmetic Products." The preamble to the 18th Commission Directive stated (Cosmetics Directive of the European Union 1995):

> Whereas cells, tissues or products of human origin are liable to transmit the Creutzfelt-Jakob disease, human spongiform encephalopathy, and certain virus diseases; whereas it is therefore necessary, given the current state of scientific knowledge, to prohibit their use in cosmetic products.

Umbilical Extract, identified as umbilical cord extract, is listed in the Japanese *Comprehensive Licensing Standards of Cosmetics by Category* (CLS) (Rempe and Santucci 1997). Umbilical Extract, which conforms to the specification of the *Japanese Cosmetic Ingredients Codex* (JCIC), has precedent for use without restriction in various CLS categories except eyeliner, lip, oral, or bath preparations for which there are no precedent for use.

GENERAL BIOLOGY

Anti-Inflammatory Activity

Human Placental Extract (presumed active, see Introduction)

Banerjee et al. (1990) reported a reduction in carrageenin-induced inflammation in rats that had been given an

TABLE 1
Frequency and concentration of use (FDA 1998)

Product category (no. formulations in category) (FDA 1998)	No. containing ingredient (FDA 1998)	Current concentration of use (CTFA 1999)	Historical concentration of use (FDA 1984)
	Human Placental Protein		
Hair conditioners (636)	3	—	—
Shampoos—noncoloring (860)	5	—	—
Tonics, dressings, and other hair-grooming aids (549)	4	—	—
Other hair preparations (276)	1	—	—
Hair rinses—coloring (33)	1	—	—
Douches (5)	1	—	—
Face and neck skin care—excluding shaving (263)	2	—	—
Body and hand skin care—excluding shaving (796)	2	—	—
Moisturizing (769)	4	—	—
Other skin care preparations (692)	7	—	—
1998 total for Human Placental Protein	**30**		
	Human Placental Extract		
Aftershave lotion[a] (216)	1	—	—
Face and neck skin care—excluding shaving[a] (263)	1	—	—
Hair conditioners[b] (636)	1	—	0%–0.1%
Rinses—noncoloring[b] (40)	1	—	0%–0.1%
Shampoos—noncoloring[b] (860)	2	—	0%–0.1%
Tonics, dressings, and other hair-grooming aids[b] (549)	2	—	0%–5%
Body and hand skin care—excluding shaving[b] (796)	1	—	0.1%–5%
Moisturizing[b] (769)	1	—	0%–0.1%
Night skin care[b] (188)	1	—	0.1%–5%
Other skin care preparations[b] (692)	3	—	—
1998 total for Human Placental Extract	**12**		
	Placental Protein (animal or bovine)		
Makeup bases (132)	1	—	—
Face and neck skin care—excluding shaving (796)	3	0.2%	—
Moisturizing (769)	—	0.2%	—
Night skin care (188)	—	0.2%	—
Skin fresheners (184)	1	—	—
1998 total for Placental Protein	**5**		
	Hydrolyzed Placental Protein		
Face and neck skin care—excluding shaving (263)	1	—	—
Body and hand skin care—excluding shaving (796)	1	—	—
Moisturizing (769)	2	—	—
Night skin care (188)	2	—	—
Other skin care preparations (692)	1	—	—
1998 total for Hydrolyzed Placental Protein	**7**		
	Placental Enzymes		
Shampoos—noncoloring (860)	1	—	—
Other hair preparations (276)	1	—	—
Face and neck skin care—excluding shaving (263)	1	5%	—
Moisturizing (769)	2	—	—
Other skin care preparations (692)	2	—	—
1998 total for Placenta Enzymes	**7**		

(Continued on next page)

TABLE 1
Frequency and concentration of use (FDA 1998) *(Continued)*

Product category (no. formulations in category) (FDA 1998)	No. containing ingredient (FDA 1998)	Current concentration of use (CTFA 1999)	Historical concentration of use (FDA 1984)
	Placental Lipids (bovine)		
Face and neck skin care—excluding shaving (263)	1	4%	—
1998 total for Placental Lipids	**1**		
	Placental Extract		
Permanent waves (192)	1	—	—
Shampoos—noncoloring (860)	1	—	0%–0.1%
Tonics, dressings, and other hair-grooming aids (549)	1	—	—
Hair shampoos—coloring (24)	1	—	—
Body and hand skin care—excluding shaving (796)	8	—	0%–>50%
Moisturizing (769)	11	—	0%–5%
Night skin care (188)	2	—	0.1%–5%
Paste masks—mud packs (255)	1	—	1%–5%
Other skin care preparations (692)	5	—	0.1%–1%
1998 total for Placental Extract	**31**		

[a]Ingredient used in liquid form in these product types.
[b]Ingredient used in lyophilized form in these product types.

intraperitoneal (IP) dose of HPE. Maximal suppression was noted with a dose of 0.3 ml/100 g body weight, given $^1/_2$ hour before or after carrageenin administration.

A dose-dependent inhibition of increased hepatic succinic dehydrogenase (SDH) activity (in reponse to carrageenin-induced edema) was noted in rats that had been pretreated with a subcutaneous (SC) dose of 1 to 5 ml/kg of HPE. The extract had little or no effect on the hepatic SDH activity of normal rats (Banerjee et al. 1994b).

A study that investigated the biochemical mechanism for the anti-inflammatory action reported a significant reduction in glucose-6-phosphate dehydrogenase activity in the liver, kidneys, and brain of rats following IP administration of a commercial HPE (0.4 ml/100 g body weight). The investigators cautioned that this enzyme was key in the production of NADPH and that inhibition can result in decreased amounts of reduced glutathione that was involved in free-radical scavenging. Enzyme inhibition also could alter steroid synthesis (Banerjee et al. 1992).

Clinical/Therapeutic Application

Vitiligo—Human Placental Extract (presumed active, see Introduction)

Several studies have reported the use of HPE in treatment of vitiligo (skin disorder marked by loss of pigmentation). "Remarkable improvement" in 20.6% and "moderate improvement" in 50% of 34 patients with vitiligo was noted after topical treatment (Sharma et al. 1988). Another study tested the repigmentation claims of a commercially available topical HPE. Following manufacturer's instructions, the extract was applied to affected areas three times a day, and the treated area was exposed to infrared light following the third application. Of 16 patients with vitiligo, 69% had no significant repigmentation, 19% had scattered repigmentation, and 12% had obvious repigmentation of some lesions. Five had complete repigmentation of some lesions within 3 to 16 months, and two (one a child, and the other a man who had been recently diagnosed) had an "obvious reduction in vitiliginous areas" (Suite and Quamina 1991).

Pal et al. (1995) conducted a guinea pig study to determine the chemical agent responsible for repigmentation. Human placentas were chopped, blended, extracted with ethanol, and then filtered. The 60% hydroalcoholic extract was topically applied around the nipples covering the areola zones of immature male white guinea pigs daily for 60 days followed by 15 minutes of infrared exposure. Clear pigmentation and hypertrophy was noted to varying degrees. The extract was chemically analyzed and glycosphingolipids, known modulators of B and T cells, were considered to induce melanocytes resulting in skin pigmentation.

Chorioretinal Dystrophy—Human Placental Extract (presumed active, see Introduction)

Thirty-four panelists with chorioretinal dystrophy (myopic or senile) of different degrees of anatomofunctional alteration received daily intramuscular doses of 3 ml human placental extract (equivalent to 1.80 g of fresh organ) for 20 days. The preparation method of the extract was not reported. Varying improvement was noted in visual acuity, the luminous sense, the visual field and the electrophysiological activity of the retina.

The investigators considered the "efficacy" of the extract was due to the "high proteic value, to polypeptide with a low molecular weight and to free amino-acids, particularly alanine, leucine, lysine, (and) valine" (Girotto and Malvinerni 1982).

Neurological Activity

Human Placental Extract (presumed active, see Introduction)

In response to claims that HPE increased the grasping, learning, and retention capacity of children of slow-learners, Banerjee, Bishayee, and Chatterjee (1995) investigated the effect of the extract on rat monoaminergic neurotransmitters and brain monoamine oxidase (MAO) activity. Subchronic IP administration (once daily for 5, 10, 15, or 20 days) of human placental extract (2 to 4 ml/kg/day) increased brain concentration of monoamines and decreased MAO activity in rats.

Cellular Effects

Human Placental Extract (presumed active, see Introduction)

Ikawa, Aida, and Saito (1975) reported that addition of 2% to 3% of a commercial human placental extract preparation to the clonal culture lines of Friend leukemia cells resulted in hemoglobin production after 4 to 6 days.

O'Keefe and Chiu (1988) reported that incubation with placental extract (100 to 200 μg) resulted in a 50-fold increase of thymidine incorporation by keratinocytes.

Kimoto et al. (1987) reported that a placental extract with demonstrated antimutagenic activity in the Ames assay "greatly diminished" adriamycin-mediated toxicity. The placental extract was prepared by homogenization of full-term human placentas, incubation with pronase, centrifugation, lyophilizing the supernatant, dissolving the resulting powder, and eluting fractions via a Sephadex column. Fractions were tested in the Ames assay. The fraction demonstrating antimutagenicity was used in in vitro studies with Adriamycin. These in vitro studies noted that the fraction diminished superoxide production by Adriamycin-incubated liver microsomes and reduced the effects of aeration on Adriamycin semiquinone radical generation.

ANIMAL TOXICOLOGY

Short-Term Oral Toxicity

Dried Human Placenta (presumed active, see Introduction)

A group of 10 male weanling rats were fed a diet in which the 10% protein allotment consisted of human placenta that had been dried and powdered. The control group was fed casein. Diets also had 15% fat and were complete with regard to vitamins and minerals. Rats were killed after either 4 or 8 weeks of feeding, and the liver and testes were removed. No changes were noted in the testes. Fatty changes in the liver of placenta-fed rats was noted at microscopic examination. The lesions consisted of diffuse cytoplasmic vacuolation of hepatocytes; the lesion was severe in cells of the periportal and adjacent mid-zones and mild in cells of the central zone. A "striking amount of stainable lipids" was noted when stained with oil red 0. The lesions were similar in rats killed at either 4 or 8 weeks of feeding. Mild-to-moderate vacuolation was also noted in controls rats, but little-to-no stainable fat was detected (Bamji and Krishnamurthi 1970).

In order to elucidate the cause of the lesions, a second study was conducted in which six male rats were fed a 20% placenta-protein diet. The diet contained 0.4 g cholesterol, 0.02 mg estradiol, and 0.5 mg progesterone/100 g diet and controls were fed a casein diet containing a comparable amount of one or more of these hormones. Animals were killed after 4 weeks, and the liver and testes were removed for microscopic examination. The liver was also analyzed for lipids and proteins. No changes were noted in the testes. Livers of test animals were mottled in appearance with no significant change in weight. Total lipids, triglycerides, and total cholesterol concentrations were markedly greater in the liver of rats fed the placenta diet compared to the control group. No changes in phospholipid and protein concentration were noted. Similar changes in hepatic lipids were noted in rats that were fed casein supplemented with cholesterol, but not in those that were fed casein supplemented with estrogen and/or progesterone. At microscopic examination, the livers of rats fed the placenta diet and those fed casein supplemented with cholesterol (with or without hormones) had mild cytoplasmic vacuolation of cells of the periportal and adjacent midzone lobules. In oil red 0–stained sections mild accumulation of fat as multiple small and fine round globules was observed in the cytoplasm without nuclear displacement (Bamji and Krishnamurthi 1970).

In an earlier study, 20 rats were fed ad libitum for 112 days 5.0% acetone-dried human placental powder or 0.30% Human Placental Lipids (identified as placental lipid extract). Hepatic malic oxidase (MO) activity ($p < .05$) and testicular oxygen uptake ($p < .01$) were significantly increased. Hepatic succinoxidase (SO) activity was comparable between treated and control rats. In the second part of the study, immature male rats were fed raw human placenta for 83 days. Significant increases in MO ($p < .05$) and SO ($p < .01$) activities, increased testicular oxygen uptake, and atrophy of the testes were noted. The atrophy was attributed to the sex hormones contained in the placenta, but the investigators did not consider hormones to have induced the increased oxygen uptake (Gershbein and Malik 1967).

Parenteral Toxicity—Acute

Human Placental Protein

Some published literature refers to human placental protein(s) that have hormone-like effects. These proteins are different from the cosmetic ingredient. Florini et al. (1966) reported a "human placental protein" that when injected into hypohysectomized rats and mice, had anabolic effects such as those noted after dosing with human growth hormone. The protein also reacted to antisera for human growth hormone.

Using the extraction technique of Florini et al. (1966), Riggi et al. (1966) reported that intramuscular (IM) administration of "purified human placental protein" into fasted rabbits (25 or 50 mg/kg), and monkeys (50 or 100 mg/kg) produced significant increases in plasma free fatty acid concentrations. Plasma lactescence associated with hypertriglyceridemia and hyperglycemia developed in rabbits following daily SC dosing with 75 mg/kg for 25 days. In mice, hepatic lipidosis developed after injections with 16 mg of Human Placental Protein daily for 7 days. All of the described effects were similar to those observed following porcine growth hormone administration.

Human Placental Extract (presumed active, see Introduction)

Banerjee, Bishayee, and Chatterjee (1993) reported that a single IP dose of HPE (4 ml/kg) to rats caused a significant enhancement of lipid peroxidation with a decline in both hepatic and blood glutathione (GSH) concentrations. A dose-dependent increase in glutathione *S*-transferase (GST) activity and dose-dependent inhibition of catalase, glutathione peroxidase, and glutathione reductase activities were noted. The extract was considered hepatotoxic because it increased serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, serum lactate dehydrogenase activities, and blood methemoglobin concentration. The magnitude of the increase of serum enzymes was "much less" than that induced by carbon tetrachloride.

In a subsequent study by Bishayee, Banerjee, and Chatterjee (1995), rats were given a single IP injection of HPE (4 ml/kg) and some were killed at 2, 6, 24, 45, 72, and 96 hours post treatment. Livers were removed, homogenized, and centrifuged, and the fractions were analyzed for cytochrome and enzymic activity. The vehicle control received 1.5% (*v/v*) Benzyl Alcohol in buffer. Maximal induction of hepatic microsomal cytochrome P-450 and cytochrome b_5 activities was noted beginning at 24 hours post dosing. Cytosolic GST activity was also significantly increased beginning at 48 hours post dosing. A reduction in microsomal UDP-glucuronyltransferase activity was also observed. All activity returned to zero-time values 96 hours after treatment.

Parenteral Toxicity—Short-Term

Human Placental Extract (presumed active, see Introduction)

Dose-dependent increases in hepatic cytochrome parameters and GST activity were noted in rats following repeated IP dosing (30 days) with 1, 2, or 4 ml/kg HPE. The cytochrome changes were significant with the 2 ml/kg dose ($p < .05$); more pronounced increases were noted in the 4 ml/kg dose group where the change in cytochrome P-450 activity was 130% ($p < .01$) and the increase in cytochrome b_5 activity was 88% ($p < .05$) greater than the control. Microsomal NADPH cytochrome c reductase activity was not affected by either acute or repeated treatment. The investigators cautioned that human placental extract had "substantial ability to alter the patterns of drugs metabolizing enzyme systems in mammals," and that prolonged administration could induce some forms of hepatic neoplasms (Bishayee, Banerjee, and Chatterjee 1995).

Similar findings were reported in an earlier study in which HPE (1 to 4 ml/kg) was injected IP into rats for 15 days. Significant increases were noted in the activities of serum glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, lactic dehydrogenase, alkaline phosphatase, glutamic dehydrogenase, and sorbitol dehydrogenase. Activities of other enzymes were also increased. A marked depletion of cytochrome P-450 and reduction of hepatic glycogen and protein concentrations were noted with a concurrent rise in hepatic lipid peroxides. The investigators considered that the active components of the extract, 19-hydroxyprogesterone and corticotropin-releasing factor, were responsible for the alterations (Banerjee et al. 1994a).

Dermal Irritation

Human and Animal Placental Extract (presumed active, see Introduction)

Two Filatov-type placental extracts (human and bovine) used for "dermocosmetology" were each applied (0.5 ml) under gauze to rabbit skin. Both extracts were nonirritating. No further details were given (CTFA 1998).

Ocular Irritation

Human and Animal Placental Extract (presumed active, see Introduction)

Two Filatov-type placental extracts (human and bovine) used for "dermocosmetology" were each instilled (0.5 ml) into one conjunctival sac of six rabbits. The human placental extract was "very slightly irritating" and the bovine placental extract was nonirritating. No further details were given (CTFA 1998).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In Vitro

Animal Placental Extract (presumed active, see Introduction)

Mammalian embryonic development was studied by Huxham et al. (1982) using a postimplantation rat-embryo culture. Wistar rat conceptuses were explanted on day 9.5 and cultured for 2 days with homogenate preparations from normal rat placenta or decidua. Conceptuses were examined for heart beat, vitteline circulation, yolk-sac diameter, and the achievement of allantoic fusion with the ectoplacental cone. Abnormal embryos (neural-tube defects, severe reduction in embryonic size) were produced with 2.5 to 4 mg/ml of the placental homogenate and 1.2 to 4 mg/ml of the decidual homogenate. Abnormalities were not induced by either solutions of bovine serum albumin or protein preparations of rat lung tissue.

GENOTOXICITY

Human Umbilical Extract

Immunizing mice with an extract of human umbilical cord significantly decreased the number of micronuclei in bone marrow cells for 5 days. The extract was described as having no clastogenic property. The investigators hypothesized that the antimutagenic effect was related to interferon induction by the extract (Mkrtchian and Nersessian 1993).

PROTECTIVE ACTIVITY

Human Placental Extract

Klein et al. (1991) reported that in in vitro studies, EAP inhibited growth of Ha-ras–transformed BALB/c 3Tc cells and human squamous lung carcinoma A-2182 cells. The fraction did not alter anchorage-dependent growth of these cells, but a slight mitogenic activity was noted in nontransformed cells. No significant cytotoxicity was noted. The fraction did contain transforming growth factor β, but the investigators did not consider that the growth factor was solely responsible for the observed growth suppression.

In a subsequent study, Klein, Chiodino, and Yamasaki (1993) reported that EAP suppressed growth of only the most highly tumorigenic cells in soft agar medium; growth of non- and low-tumorigenic counterparts was not affected or was stimulated, respectively, by the extract. Cells of both the colorectal and esophageal cell lines that had the greatest percentage of colonies in soft agar had their colony-forming efficiency decreased by the presence of 100 μg/ml EAP. In contrast, cells that did not give any colonies in soft agar did not grow in either the absence or presence of EAP. Growth of cells with an intermediate colony-forming efficiency was stimulated (by 150% in colorectal cells, and 200% in esophageal cells) in the presence of EAP. Similar findings were noted with murine BALB/c 3T3 1-1 cells that had been transfected or infected with various oncogenes. Further, "EAP did not significantly affect the doubling time of anchorage-dependent cell growth, suggesting that the extract specifically suppresses tumorigenic characteristics of cells such as their ability to grow in soft agar medium." Transforming growth factor β was most effective on less tumorigenic cells.

Human Placental Extract (presumed active, see Introduction)

Komura et al. (1983) conducted a chemical study on the antimutagenic action of human placental extract. A human placenta was washed, diced, and homogenized. The liquid was centrifuged; then the supernatant was boiled and recentrifuged, it was a pale pink liquid. The yield from one placenta was ~300 ml of "active juice." Two placentas were used to prepare HPE_1 and HPE_2. Bacteria (*Eschericheria coli*) were mutated by either radiation or incubation with a chemical mutagen N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and then combined with HPE and plated. A specific activity AD_{50} was defined as the dose that reduced the number of induced mutations by 50% without affecting cellular survival. The specific activities of HPE_1 against ultraviolet (UV)-, γ-ray–, and MNNG-induced mutations were 160, 50, and 200 μl/plate, respectively. The specific activities of HPE_2 against UV- and MNNG-induced mutations were 50 and 80 μl/plate, respectively. Cobalt(II) ions were considered essential for the antimutagenic action. However, the investigators noted studies using other mammal placentas and considered that "low molecular, nonproteic factors" could also have played a role.

Placental Extract (presumed active, see Introduction)

Mochizuki and Kada (1982) investigated the antimutagenic action of extracts prepared from the placentas of a human, monkey, dog, rat, and mouse. Placentas were washed with potassium chloride, homogenized without buffer, and centrifuged. The supernatant was treated with pronase followed by overnight dialysis in distilled water. Each solution was heated and loaded onto ion-exchange resin columns and eluted with water. Fractions were collected and evaporated under vacuum; the residue was dissolved in water and filtered using millipore filters. Bacteria (*E. coli* B/r WP2 trp^-) were mutated by either radiation or incubation with MNNG and then combined with an extract and plated. The extracts were also tested alone and were not mutagenic. The number of mutant colonies induced by UV irradiation, γ-ray, and MNNG were "decreased markedly in the presence of the placental extracts without significant effects on survival." The data were not analyzed for statistical significance.

Human Umbilical Extract

Vaccination of rats and mice with an extract of human umbilical cord resulted in a significant inhibition of growth, decreased tumor incidence, and partial resorption of ascitic fluid of transplantable tumors such as Ehrlich's ascites tumor, sarcoma 37, and Zajdel's hepatoma. The inhibition was not noted when sarcoma 180 was transplanted. Vaccination also interfered with dimethyl benzanthracene and benzo(a)pyrene-induced carcinogenesis by reducing tumor incidence and increasing the latent period and slowing cancer progression (Mkrtchyan et al. 1990).

CLINICAL ASSESSMENT OF SAFETY

Patch Testing

Human Placental Protein

A patch testing reference book by DeGroot (1994) noted that the published literature does not contain recommended test concentrations for Human Placental Protein. As a guide to the clinician, DeGroot reported the findings of an unpublished (and at the time, ongoing) study by members of the Dutch Contact Dermatitis Group. No irritant reaction was noted in 1 to 20 patients (exact number tested with ingredient not specified) suffering from or suspected to suffer from cosmetic product contact allergy after being patch tested with 30% Human Placental Protein aqua.

Animal Placental Extract (presumed active, see Introduction)

von den Driesch et al. (1993) reported contact dermatitis of the hand in a cosmetician up to 3 hours after external application of calf placenta extracts. She had previously worked as a hairdresser but developed a delayed-type allergy to *p*-phenylenediamine. The placenta extract contained mesodermin, collagen, and hyaluronic acid. The cosmetician and 10 healthy volunteers were tested with the extract and the solvent via the prick and scratch-chamber method. The cosmetician reacted in both tests. In the scratch-chamber test, the dissolved extract produced an eczematous reaction at day 1 and the undissolved extract caused a reaction after 2 days; the dissolved antigens were considered to have had greater penetration.

SUMMARY

The ingredients Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, and Human Placental Lipids are derived from human placentas obtained from normal afterbirth. Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, and Placental Lipids are mixtures derived from animal placentas. Human Umbilical Extract and Umbilical Extract are obtained from human and animal umbilical cords, respectively.

The human and animal placental-derived ingredients function in cosmetic formulations as hair- and skin-conditioning agents. The umbilical cord extracts are reported to function as biological additives.

As of January 1998, Human Placental Protein was reported to be used in 30 cosmetic formulations, Placental Protein (identified as animal or bovine) was used in five formulations, Hydrolyzed Placental Protein was used in seven formulations, Placental Enzymes was used in seven formulations, and Placental Lipids, bovine was used in one formulation. In addition, human placental "extract" and placental "extract" were reported to be used in 14 and 31 cosmetic formulations, respectively. These two extracts are not recognized as cosmetic ingredients. Virtually all of the available safety test data related to extracts. These extracts are presumed to be biologically active, for example containing hormones.

Animal and clinical studies testing biologically active human placental "extract" have reported anti-inflammatory activity, improvement in the treatment of vitiligo and chorioretinal dystrophy, and neurological and cellular effects.

Oral- and parenteral-dose rat studies tested biologically active extracts and protein preparations and found changes in hepatic enzyme activities. Placental "extracts" (both human and animal) were negative in dermal and ocular irritation studies using rabbits. In in vitro studies, placental "extracts" demonstrated antimutagenic action in bacteria and had anticarcinogenic activity against some tumor cell lines.

Human umbilical extract was negative for mutagenicity in a micronuclei assay, and inhibited growth of tumors transplanted in rats and mice.

Some studies stated that protein was tested. However, the study design appeared to test the effects of hormonal activity. The one exception is a clinical study that reported no irritant reaction to Human Placental Protein following patch testing of patients with cosmetic product contact allergy.

DISCUSSION

The CIR Expert Panel faced many issues with this group of ingredients. First was the reported use of substances identified as "human placental extract" and "placental extract." These names are not recognized in the CTFA *International Cosmetic Ingredient Dictionary and Handbook*. Further, FDA warned that cosmetics claiming to contain these ingredients may be misbranded and recommended using nomenclature other than "extract." The CIR Expert Panel advised industry that cosmetic formulations should not be identified as containing "human placental extract" or "placental extract" so as to comply with FDA guidelines.

The Expert Panel expected that the CTFA-recognized ingredients—Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract—will not deliver any metabolic/endocrine activity (e.g., hormones, growth factors).

The Panel was also concerned with the dangers inherent in using human or animal-derived ingredients, namely the transmission of infectious agents. The CIR Expert Panel stressed that these ingredients must be free of detectible pathogenic viruses or infectious agents (e.g., HIV, Bovine Spongiform Encephalopathy (BSE), or Creutzfeld-Jakob disease prions). Suppliers and users of these ingredients must accept responsibility for assuring that these ingredients are risk-free. Tests to assure the absence of a pathogenic agent in the ingredients, or controls to assure derivation from pathogen-free sources are two approaches that should be considered.

With the above conditions met, the CIR Expert Panel noted that additional data still were needed to assess the safety of the cosmetic ingredients. The vast majority of studies cited in this report tested biologically active "extracts" and other preparations. Thus, the Panel was unable to apply results of these studies to the safety assessment of the cosmetic-grade ingredients.

Section 1, paragraph (p) of the CIR Procedures states that "A lack of information about an ingredient shall not be sufficient to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the Procedures, the Expert Panel informed the public of its decision that the data on these ingredients were not sufficient for determination whether the ingredient, under relevant conditions of use, was either safe or unsafe. The Panel released a Notice of Insufficient Data on March 20, 1998, outlining the data needed to assess the safety of these ingredients. Comments concerning a human and bovine placental extract were received during the 90-day public comment period. However, additional data needed* to make a safety assessment are:

1. Skin sensitization at concentration of use
2. Gross pathology and histopathology in skin and other major organ systems associated with repeated exposures, and dermal reproductive and developmental toxicity data
3. Photosensitization
4. One genotoxicity assay in a mammalian system; if positive, then a 2-year dermal carcinogenicity study using NTP methods may be needed
5. Ocular toxicity, if available

(*To be done on all ingredients unless chemical analysis data shows similarity among ingredients.)

CONCLUSION

The CIR Expert Panel concludes that the available data are insufficient to support the safety of Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract for use in cosmetic products. If these ingredients are used, they should not deliver any metabolic/endocrine activity, and they must be free of detectable pathogenic viruses or infectious agents.

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October 11, 2010

F. Alan Andersen, PhD, Director, Cosmetic Ingredient Review, Members of The CIR Expert Panel.

Dr. Andersen, thank you and the Expert panel for the promptness of your reply.

On behalf of Rimar, LLC: Mark R. Engelman, M.D. is prepared to respond to questions and comments from the CIR Expert Panel. 602-840-6961
Dr. Engelman may also be identified on the search engine: Google.com

Re: for the attention of the CIR Expert Panel meeting December 13–14, 2010: the attached data/ documents/ information and the outline following, is presented in support of the use of "Human Umbilical Extract" in cosmetic products by Rimar, LLC, Lighter & Livelier, LLC and Cosmetikare Laboratories , LLC

Al Needleman BSc Pharm. Executive Director Lighter & Livelier, LLC, Las Vegas, NV Executive Director Cosmetikare Laboratories, LLC, Las Vegas , NV Executive Director Research and Development, Rimar, LLC, Scottsdale, AZ.

The following opinion: "Rational for utilizing umbilical plasma components as additives for skin treatments" was requested as a professional review of our proprietary research data, from Avi Treves, PhD Director of Research, Sheba Medical Center (Tel Hashomer) Israel, Cancer Research Center.

The encouraging opinion formed the basis for the eventual addition of Human Umbilical Extract (Cord Serum Complex) to our clinically successful topical cosmetics.

"During the adult life, skin regeneration is enabled by continuous development of its various layers and secretion of the extra cellular matrix components. Several types of progenitor cells are involved in this process, including fibroblasts, keratinocytes, hair pulp stern cells, melanoblasts and progenitors of sebaceous and sweat glands. The proliferative potential of the various skin stem and progenitor cells decrease with age and in several pathological conditions. Thus, maintaining the functional capacity and stimulating the secretory activity of the building blocks of skin layers, may enhance skin regeneration, viability and elasticity.

The umbilical cord plasma contains numerous growth factors, cytokines, hormones and chemokines that support and enhance the viability, function and proliferation of stem and progenitor cells. Cord blood is enriched in hematopoietic stern cells, as well as mesenchymal, endothelial and pluri-potent stern cells. Additional cells with regenerative capacity were also reported in cord blood, and their detailed lineage composition is still being studied. Thus, the sera and plasma of the umbilical cord blood are natural supportive nutritient environment for many types of stem, progenitor and regenerative cells. Indeed, cord blood serum was reported to replace fetal calf serum or other sources of sera in tissue culture media for ex vivo growing of several types of human cells, including fibroblasts and keratinocytes.
Taking together, although the direct interaction between skin stem cells or skin tissue with cord plasma components was not studied yet, it is suggested that such interaction may lead to similar stimulatory effect, and eventually to enhanced skin

Regeneration*. The combination of umbilical cord plasma components together with other known skin care reagents, may have a synergistic effect and further improve the regenerative activity of the combined preparation**.

Note: * and ** proven in the human invasive clinical trial (biopsies) attachment: “Histogeometric Analysis of the effects of Product A versus Product B on Human Skin”

Human Umbilical Extract, source, protocol, safety, efficacy: Cryobanks Laboratories, Lifeforce Cryobanks a division of Lifeforce Cryobank Sciences, Inc., 270 Northlake Blvd., Suite 1000, Altamonte Springs, FL 32701 was retained to generate the Pooled* Plasma Product (Human Umbilical Extract) from Human Umbilical Cord Blood. The following parameters were requested: “cellular depleted Umbilical Cord Blood plasma treated with Hydroxyethyl starch and centrifuged to remove cellular components… resulting in the Umbilical Cord Blood Plasma (Human Umbilical Extract). A detailed SOP is attached. *Pooled plasma product: 50ml aliquots.

Additional information regarding the intense safety procedures instituted for the collection, storage, shipment and use in our cosmetic products…

Al,

We have the clonogenic assays in hand and can look at the CFU activity of the plasma + or – exogenous cytokines. Storage of the TNC in LN2 is not a problem.

Test specifics:

Aerobic and fungi culture
Anaerobic culture

HBs Ag
HCV Ag
Anti HBc
Anti HTLV I/II (Human T cell Lymphotropic Virus)
Anti HIV 1&2
Anti HCV
Syphilis Test (Treponema Pallidum)
Anti CMV IgG and IgM
HIV antigen p 24

As far as estimating residual reagent, it would be very dependent upon the collection volume and hematacrit of the CBU. The bag sets are designed to accommodate a 150mL cord blood volume. Anything below this volume (which would be the majority of samples) would have more PrepaCyte remaining. Please let me know if you have any available time during your evenings over the next several days. BioE is located in Minneapolis which is one hour behind us here in Florida. I will arrange a call based on your and BioE's availability.

Best regards,

Donald L. Hudspeth, BSCLS, MT(ASCP)

General Manager and

International Projects Manager

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Lab Fax 407-331-3965

Website: www.cryo-intl.com

Additional sample tests prior to the shipment of processed and tested Human Umbilical Extract (Cord Serum Complex)

2034377	(CB-001) no bacterial growth observed
2036083	(CB-002) no bacterial growth observed
2039027 – CMV positive	(CB-003) no bacterial growth observed
2036079	(CB-004) no bacterial growth observed
2012531	(CB-005) no bacterial growth observed
2034240	(CB-006) no bacterial growth observed
2036033 – CMV positive	(CB-007) Not done (sample lost in transit)

Skin sensitization at concentration of use: Attached Clinical Trial, 48 hours occluded patch test, 50 Participants. Results: "under the conditions of this study, test material, Anti Aging Face Cream with Cord Serum Complex (Human Umbilical Extract) NBP0073, Batch 0013 did not indicate a potential for dermal irritation". Clinical Trial performed by Consumer Product Testing Company, Inc., Fairfield, N.J. (973) 808-7111. Clinical trial attached.

Conclusive evidence of safety and efficacy for the use of Human Umbilical Extract in Rimar cosmetics: Robert M. Lavker, PhD, Northwestern University, department of dermatology, was tasked with generating a clinical trial protocol based on participant tissue biopsies of skin areas following the application of the cosmetic product containing Human Umbilical Extract (Cord Serum Complex) versus the cosmetic product without Human Umbilical Extract. The detailed study, including histological slides and focused conclusions of this 26 day clinical trial are attached.

A detailed document outlining the phamacodynamics of the ingredients used in the various cosmetic products, and separate human ingredient studies are included in the document: "Anti Aging with Cord Serum Complex Development Document" attached and "UnderEye Recovery Cream with Cord Serum Complex" document attached.

Julio Garcia, MD
Cosmetic & Plastic Surgeon
Engaged to perform a study on 30 participants, a power point presentation, photos and his comments are attached. Dr Garcia is a well known cosmetic surgeon with extensive practices in the U.S. (Las Vegas, NV) and in India as a teaching and practicing cosmetic surgeon.

The attached patent application has been accepted by the USPTO and is presented here for your review. Please note the first sale date of cosmetics containing Human Umbilical Extract is within the body of the patent*.

UNITED STATES PATENT APPLICATION FOR:

TOPICAL COMPOSITION COMPRISING
UMBILICAL CORD BLOOD SERUM

INVENTORS:

Alvin Needleman

Attorney Docket No.: 838611-0002

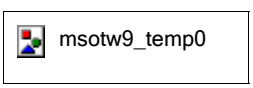
* Note product sales to consumers started without Human Umbilical Extract (Cord Serum Complex) January 2006. Cosmetics including Human Umbilical extract sales beginning October 2008. Via an internet website www.youngerlookingyou.com (no longer in use) a merchant account with Wells Fargo Bank, product fulfillment by Las Vegas Fulfillment, Craig Rd, North Las Vegas, NV. and manufacturing by an FDA-GMP licensed facility: ASI manufacturing, Commerce St, Las Vegas, NV

There are no reports of adverse reactions to any of the cosmetic products, no products have been returned for credit as a result of an irritability (merchant account charge records are available for review). A list of trial participants can be provided for your review.

All the information and data can be supplied in hard copy, as needed.

Thank you for the opportunity to present supportive documentation for the use of “Human Umbilical Extract”

Regards



Al Needleman
Cosmetikare Laboratories, LLC
1001 Meadowleah Street
Las Vegas, NV 89145-8642

From: Alan Andersen [mailto:andersena@cir-safety.org]
Sent: Friday, October 01, 2010 8:23 AM
To: Al Needleman
Cc: Halyna Breslawec
Subject: Re: Human Umbilical Extract

Mr. Needleman - thank you for letting us know that Cosmetikare Laboratories, LLC intends to include human umbilical extract in several cosmetic formulas. We would appreciate receiving the data from the clinical testing you described and the experiential findings from the field trials.

The CIR Expert Panel next meets on December 13-14. We could include a discussion of new data for human umbilical extract at that meeting if we received information before October 25th. Otherwise, the next meeting would be March 3-4, 2011 and we would need a submission by January 17th.

Let me remind you that the CIR Expert Panel had asked for data regarding 1) skin sensitization at concentration of use, 2) gross pathology and histopathology in skin and other major organ systems associated with repeated exposures, and dermal reproductive and developmental toxicity data, 3) photosensitization, 4) one genotoxicity assay in a mammalian system; if positive, then a 2-year dermal carcinogenicity study using NTP methods may be needed, and 5) Ocular toxicity, if available. It is not immediately clear how the data you have would address those issues, but that may be clear once we see the data.

In addition, I am certain the CIR Expert Panel would readdress the issue of function of human umbilical extract in cosmetics as it is currently "not reported" in the *International Cosmetic Ingredient Dictionary and Handbook*. The Panel has a low tolerance for uncertainty in the data when the reason for putting the ingredient in cosmetics isn't clear.

You mentioned that FDA standards for blood collection are followed. You may need to be more specific in terms of the waiting period between use of the extract and any test done to determine HIV infection in a donor in which sufficient antibody has not manifested to be detectable, if antibody tests are being used by Cryobanks Laboratories.

If you have any questions, let me know.

F. Alan Andersen, PhD
Director, Cosmetic Ingredient Review
1101 17th Street, NW, Suite 412
Washington, DC 20036
ph 202.331.0651
fax 202.331.0088
andersena@cir-safety.org

>>> "Al Needleman" <alneedleman@cox.net> 9/28/2010 6:36 PM >>>
CIR, Hello,

A recent review of the "Zero use ingredients with insufficient Data" listed an ingredient we expect to include in several cosmetic formulas: (2) Human Umbilical Extract.

The safety and function of the ingredient was proven in 2010, in an (invasive) clinical trial at Northwestern University, the department of dermatology. Punch biopsies were taken from several participants in three areas of application: (1) the product containing the human umbilical extract ingredient (2) the product without the human umbilical extract ingredient and (3) an area where no cosmetics were applied. The results of the trial proved the validity of the ingredient. The data is available for review. Products containing the ingredient have been in field trials for 36 months with approximately 2500 users, there are no reports of irritability or interaction.

The ingredient is produced using applicable FDA standards for blood collection, processing and testing by Cryobanks

laboratories, Tampa, Fl.

I look forward to your reply as soon as possible.
Thank you

Al Needleman
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Mobile: 702-203-2196
Fax: 702-838-7424

Lighter & Livelier, LLC

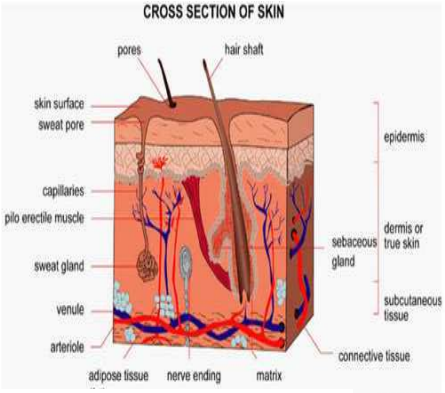
L&L Product # NBP-0073

Anti Aging Face Cream with Cord Serum Complex™, Formula Composition, Efficacy, Safety, Pharmacodynamics, Rational and Clinical studies of participants aged 39 to 74. Anti-wrinkling and lifting efficacy assessed by profilometry and photography T-30 as compared to T-0. Effect on muscle relaxation using in vitro nerve muscle co-culture.

Skin is made up of three main layers: the epidermis, the dermis and the hypodermis. The epidermis is the only layer we can see with our eyes and as we age, remarkable changes occur which are hidden from our view. For instance, the skin gradually thins over time, especially around the eyes. Elastin and collagen, located in the dermis keep the skin resilient and moist, but with ageing these fibers break down to create lines and wrinkles. Exposure to ultraviolet radiation accelerates this process. The best way to reduce fine lines and wrinkles is to limit our exposure to the sun and ultraviolet radiation. Regular, twice daily, application of the Anti aging face cream with cord serum complex restores collagen, elastin, and nutritional communication between cells at the DEJ (Dermal Epidermal Junction) modulates muscle neural sensitivity and raises HA.

The Anti Aging Face Cream: is the future in botox-like (non-invasive) skin care. Formulated to restore "age-lost adhesion" between the dermis and the epidermis… reducing fine lines, wrinkles and sagging.

The Anti Aging formulas are (site) focused on successful repair-restructure & enhancement of the physical, neural and chemical properties of the 3 main skin layers, sub-dermal musculature & the DEJ



Keywords: DEJ; elastin; collagen; profilometry; lamina; co-culture; charge coupled; bio-peptides, HA (Hyaluronic acid)

The anti aging formulas generate a high percentage of active energy at the DEJ, a prime area of “separation”; restoring smoothness, elasticity, adhesion and reducing fine lines and wrinkles (up to 45%) in 30 days or less.

General overview: Anti Aging actives: Composed of charge coupled Bio-peptides, marine micro algae, antioxidants, neurotransmitter modulators, sub dermal muscle control , tissue lubricant technology, damaged DNA replacement , topical-cellular immune system stimulation, accelerated active absorption and molecules from cord serum complex (Human Umbilical extract); comprising enzymes, hormones, proteins, vitamins, antioxidants, amino acids and minerals.

Collagen molecules and soft Keratin are responsible for skin strength and elasticity. Collagen degradation leads to wrinkles that accompany aging.

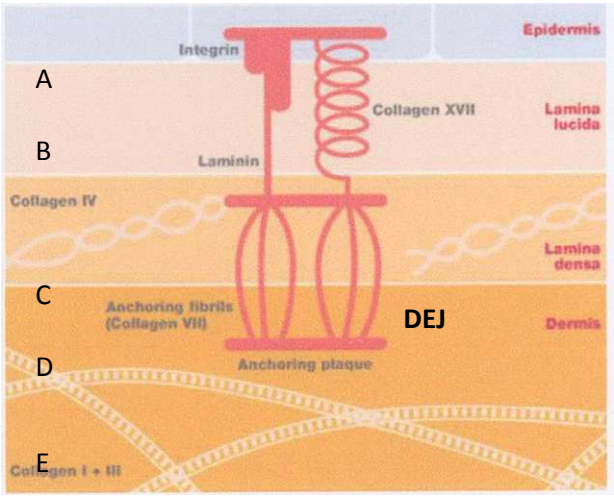
Anti Aging Formula Ingredients effecting ABC and ABCD repair damage and restore collagen.

Support for the Lamina Layers (B & C) and their protein network contributes toward cell attachment and differentiation, cell shape and movement, maintenance of tissue phenotype, and promotion of tissue survival.
.

Ingredients supporting B & C maintain and restore lamina layer integrity.

Integrins (A) play an important role in cell signaling by connecting to the extracellular matrix molecules (E) causing a signal to be relayed into the cell through protein kinases attached to the intracellular end of the Integrin molecule (B).

Ingredients supporting A & B maintain the functional properties of the Integrins. (Cell surface receptors, interact and mediate various intracellular signals)



Summary of Clinically validated ingredient results: 26% increase in skin smoothness in 26 days. 17% increase in skin tone. 55% decrease in skin fatigue. 30% reduction in the depth of wrinkles after 30 days. 44.9% reduction in surface occupied deep wrinkles. 18.5% reduction in main wrinkle average volume. 14.4% reduction in skin roughness. 16.6% reduction in skin complexity. 19.5% increase in elasticity. 2.6 times increase in sensitivity to neurotransmitter signaled muscle contraction.

Formula ingredients, not in the order of prominance (Patent pending): Acetyl Hexapeptide-3, Palmitoyl Oligopeptide, Palmitoyl Tetrapeptide-7, Lipopeptide (NATAH Ester), Undaria pinatifida, Stevia rebaudiana-Bertoni, Juglans regia leaf extract, Juglans regia shell extract, Centella asiatica extract, Pyrus germanica extract, Cord serum complex, Myristamidopropyl

(cont.) PG Dimonium Cl Phosphate, Dimethyl Isosorbide, Lecithin, Purified water, emulsion matrix.

Clinical validation:

Peptide (short polymers formed from the linking of alpha amino acids) reinforced cosmeceuticals in the Anti Aging formulas.

Peptide cosmeceuticals are one of the new popular options to treat aging skin. There are three main categories of cosmeceutical peptides: signal peptides, neurotransmitter-affecting peptides and carrier peptides. The evidence to support their use has been scientifically well validated enhancing their use in Anti aging cosmetics and their practical use in dermatology.

Botulinum neurotoxins (BoNTs) represent a revolution in cosmetic science because of their remarkable and long-lasting anti-wrinkle activity. However, their high neurotoxicity seriously limits their use. Thus, there is a need to design and validate non-toxic molecules that mimic the action of BoNTs.

Peptide: Affecting Neurotransmitters

Acetyl Hexapeptide-3 (revised as -8) was identified as a result of a rational design program. *Noteworthy, skin topography analysis of an oil/water (O/W) emulsion of this hexapeptide solution on healthy women volunteers reduced wrinkle's depth up to 30% upon 30 days treatment.*

Analysis of the mechanism of action showed that the hexapeptide (Acetyl Hexapeptide-3) **significantly inhibited neurotransmitter release** with a potency similar to that of BoNT Although, as expected, it displayed much lower efficacy than the injected neurotoxin. Inhibition of neurotransmitter release was due to the interference of the Hexapeptide with the formation and/or stability of the protein complex that is required to drive Ca^{2+} -dependent exocytosis,(cellular direction of secretory vesicles) namely the vesicular fusion (known as SNARE=soluble NSF attachment receptor) complex.

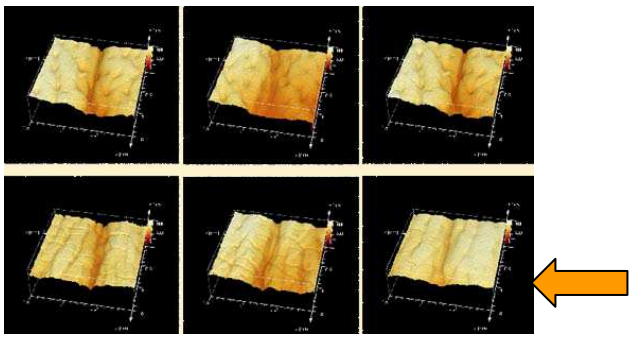
Notably, the hexapeptide did not exhibit in vivo oral toxicity or primary irritation at high doses.

Taken together, these findings demonstrate that this hexapeptide is a non-toxic, anti-wrinkle molecule that emulates the action of currently used BoNTs. *Therefore, the inclusion of this hexapeptide represents a biosafe alternative to BoNTs in cosmetics.*

Clinical results of skin topography using silicon impressions taken from the lateral preorbital area in healthy women volunteers and analyzed by confocal microscopy.

Reduction of wrinkle depth (below)

The top row illustrates the use of a placebo cream, while the bottom row illustrates the use of the molecule at the inclusion rate. The three periods from left to right illustrates the measurements taken at day 0, day 15 and day 30.



Peptide: messengers of cutaneous restructuring and repair.

Palmitoyl Oligopeptide & Palmitoyl Tetrapeptide-7.

The peptides are two matrikines (extracellular matrix-derived peptides which regulate cell activity) they act in synergy to restore and maintain the skin's youthful appearance. Activating the neosynthesis of extracellular matrix molecules providing visible anti wrinkle efficacy. As messenger molecules the matrikines are capable of regulating cell activities. They interact with specific receptors to activate certain genes involved in the extracellular matrix renewal and cell proliferation. (Since, with age these mechanisms become progressively weaker).

Clinical study: two panels of twenty three volunteers aged 39 to 74 applied a cream containing Palmitoyl Oligopeptide and Palmitoyl Tetrapeptide-7 to one half of their face against a placebo on the other half…twice a day for 30 days. Anti wrinkling and lifting efficacy was assessed by profilometry and photography.

Results:

Visible and measurable proof of Anti-wrinkle efficacy.

Surface occupied by deep wrinkles (>200µm) – reduced 44.9%.

Main wrinkle density – reduced by 37%

Main wrinkle average depth – reduced by 15.1%

Main wrinkle average volume – reduced by 18.5%

Roughness – reduced by 14.4%

Lifting effect (complexity) - reduced by 16.6%

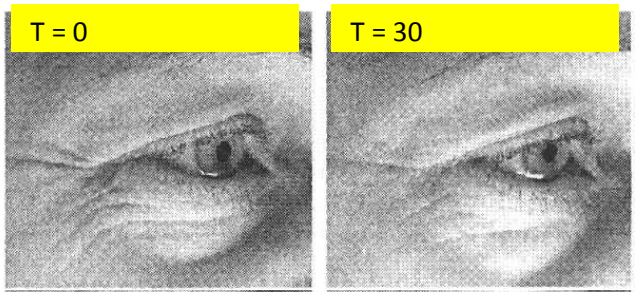
Elasticity – increased by 9%

Tone – increased by 19.5%

Gene activation (+): Collagen-1 (50%), Fibronectin (60%), Hyaluronic Acid (45%)

The synthesis of matrix molecules: In order to determine the ability of fibroblasts incubated for 72 hours with the bio-peptides, at 3 strengths, to identify the levels of stimulation of extracellular matrix components. Check validity

Photographic results below at ideal concentration.



Lipo-dipeptide, a messenger of tranquility and muscle relaxation.

Relieves skin/muscle tension to help prevent the onset of wrinkles and expression lines.

N-Acetyl-Tyrosyl-Arginyl-Hexadecyl Ester helps promote the release of pro-endorphins to provide a tranquilizing effect on the skin; relieves tension and inhibits the muscle contractions responsible for the appearance of wrinkles and expression lines.

In Vitro validation:
NEUROTRANSMITTERS FOR

RELAXATION; ß-endorphin and met-enkephalin are neuropeptides involved in the "down-regulation" of nerve and muscle activity… human keratinocytes were incubated for 24 hours. The RT-PCR technique was used to measure the increase in gene expression for Proopiomelanocortin (the precursor for ß-endorphin and metenkephalin). Results: 63% increase in muscle relaxation.
NEUROTRANSMITTER FOR CONTRACTION; calcitonin gene related peptide (CGRP) is a neuropeptide involved in the stimulation of muscle activity and sensitization. Human nerve culture was incubated in reagents and the amount of CGRP released was measured Results: a decrease in contraction of 3.1% as measured against standardized capsaicin. EFFECT ON MUSCLE RELAXATION; using an in vitro model of nerve-muscle co-culture containing axons and a neuromuscular junction. Results: the lipo-peptide progressively decreased muscle contraction frequency and within two hours totally inhibited contractions.

Botanicals:

Stevia rebaudiana Bertoni (SrB); moisturizing, smoothing, fine line reduction, anti-oxidant, tonifying. Related activities of active molecules: Diterpenglycosides = moisturizing, smoothing, conditioning. Flavonoids = anti-irritant, free radical scavenger. *Clinical Test; SKIN MOISTURIZER AND SMOOTHNESS: Two groups of twenty participants each. Twenty participants applied Stevia rebaudiana (SrB) Bertoni (1% aqueous gel) twice daily on the forearm for* 28 days. Second group – *of twenty participants applied a, physically and visually similar, placebo in the same manner. Results: skin hydration increased 20% after 28 days. Skin smoothness increased 24% after 14 days. Skin smoothness increased to 26% after 28 days. SrB supports the skin's moisture barrier, with a moisturizing effect 3 times greater than glycerin.*

(a) Juglans Regia Leaf Extract, (b) Juglans Regia Shell Extract, (c) Centella Asiatica Extract, (d) Pyrus Germanica Extract. In order to increase safety and efficacy, this botanical combination is based on 2 facts: the synergy between plants, the whole is more than the sum of its parts. Each plant imparts its own special qualities to the RMI formula, each one necessary for the firming effect.

(a) and (b): firming, free radical scavenging, tonifying. (c) Anti-stress, strengthening, stimulation of collagen synthesis. (d) Bio-protective, astringent, smoothing, restore skin moisture. (Tightens sagging skin). *Clinical study: TONIFYING AND ANTI-FATIGUE EFFECT; two groups of participants 10 volunteers per group. Twice daily application of an active cream to the neck for 28 days. Placebo group,inactive cream same application for 28 days. Results: immediate reaction 17% increase in tonicity. 28 days: 55% reduction in Cutaneous fatigue vs. the placebo.(untreated skin). FIRMING EFFECT; two groups of participants 10 volunteers per group. Twice daily application of an active cream to the neck and forearms' for 28 days. Placebo group inactive cream application for 28 days. Results: forearms – Elasticity (reversible skin deformability) increased 7%, forearms – Viscoelasticity (elastic/plastic ratio during suction) decreased 10%. Firmness-neck (elastic deformity ratio) increased 12%.*

Algae:

Undaria pinnatifida L.maintains skin firmness, elasticity and smoothness, supports skin regeneration, defends against environmental stress. Active molecules; sulphated polyfucose = Anti-hyaluronidase/anti-elastase effect, moisturizing, anti-oxidant, stimulation of fibroblasts. Proteins = conditioning, Oligoements = skin moisture balance, Vitamins = cell protection and regeneration, anti-oxidant. *Clinical study: volunteers participated in a dose related study of hyaluronidase inhibition. At 2% Undaria p. demonstrated 36% inhibition of hyaluronidase at 5% more than 50% inhibition. Inhibition of Hyaluronidase prevents deterioration of the extra cellular matrix. This is a dominant factor in defending skin against aging and the diminution of dermal thickness. Sulphated polyfucose (polysaccharide) protects both the algae body walls and human tissues from losing integrity and stability.*

PHOSPHOLIPID COMPLEX:

Myristamidopropyl PG-Dimonium Chloride Phosphate; reduces reliance or preservatives, provides sensorial benefit (feel), substantive conditioning, co-emulsifier.
Clinical and safety trials: Eye irritation None, using Red Blood Hemolysis assay. Eye irritation (2) None, using Isolated Eye test to screen for eye irritation. Skin irritation None, using Skin Integrity Function Test. Skin irritation (2) None, using 48 hour occluded skin patch tests.

DERMAL ABSORPTION, SITE DELIVERY:

Dimethyl Isosorbide (DMI), focuses the delivery of active ingredients where they are most needed. Formula benefits; enhances formulation and API (Active Pharmaceutical Ingredients) stability, improves formulation aesthetics, reduces cellular irritation, stable to hydrolysis, reduces gelatin cross linking, improves spreading. Clinical and safety studies conclusion: non-irritating as provided by testing under National Industrial Chemicals Notification and Assessment Scheme-Full Public Report. File # STD/1052, 05.12.2004. Super refined DMI (Dimethyl Isosorbide) is produced to the highest purity standards for advanced performance. Super Refining is a proprietary refining process that removes polar impurities, preventing adverse interactions with Active Pharmaceutical Ingredients (APIs). By minimizing peroxide and formaldehyde levels.

Absorption Ingredient summary: enhances formulation and API stability, improves epidermal penetration, Improves formulation aesthetics, Reduces cellular irritation, Stable to hydrolysis, Reduces gelatin cross linking.

CORD SERUM COMPLEX:

*The Energy of Creation™

Cord Serum Complex (FDA compliant, “Human Umbilical extract”): a proprietary composition containing enzymes, hormones, antioxidants, antigens, vitamins, amino acids, proteins and minerals. Derived from pooled cord blood plasma, obtained by regulatory approved methods of umbilical cord blood processing at accredited cord blood banks.

The Human Umbilical extract (plasma) is obtained following removal of all cellular components and further processed and freeze stored.

The Human Umbilical extract is pre-tested for microbial and fungal contamination and the mother's blood is pre-tested for

infectious viral contaminants as required for regular Umbilical extract and cord blood storing for clinical utilization.

Cord blood (Umbilical extract) is known to contain higher numbers of more clinically effective stem cells… for clinical indications and regenerative treatments.

The serum components (Human Umbilical extract) supports the viability and function of these stem cells. Cord serum components have been identified in research as potential promoters of the proliferation of skin progenitor cells such as keratinocytes and fibroblasts, and stimulate their function in regenerating skin tissue. Cord Serum Complex provides robust elasticity, smoothness, environmental and free radical damage repair, and enhances the product functional longevity.

Additional clinical evaluations: 48 hour occluded patch test. Consumer Product Testing, Fairfield, New Jersey. Six week clinical trials, Las Vegas, NV, under the direction of Dr. Julio Garcia

Note: Second generation RMI research and product development includes the new area of stem cell exudates (Paracrines and Paracrine signaling).

Al Needleman

LIGHTER & LIVELIER, LLC
1001 Meadowleah Street, Las Vegas, NV 89145

702-243-7423 Fax: 702-838-7424

Certificate of Analysis
The name printed at the end of this document is an electronic signature

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Customer details

Customer Ref.
Inspection Lot: (CB) 007, 007, 009, 0010, 0011

C of A printed	12.04.2008
RMI Order No.	
RMI Del No.	
Quantity	250
QA Contact	RMI, Inc.
Fax No	702-838-7424.

Batch Details

Product name:	Human Umbilical Extract	Review Conclusion "I"
Product Code:	RMI-CSC98	
Cust. Product Name:	Cord Serum Complex	
Cust. Product Code:		
Batch No.	0002 (2036068, 2036122, 2015901, 2086157)	
Date of Test:	09.10.2008	
Specification:	IJT 21(S1):81-91, 2002	
Manufactured at:	Cryobanks Laboratories, Inc. Altamonte Springs, Fl. Morphogenesis laboratories, St Jos Hsp, Tampa, FL	
Date of Manufacture:	09.03.2008	

Tests performed

HBs Ag	None
HCV Ag	None
Anti HBc	None
Anti HTLV I/II (Human T cell Lymphocyte Virus)	None
Anti HIV 1&2	None
Anti HCV	None
Syphilis Test (Treponema Pallidum)	None
Anti CMV IgG and IgM	None
HIV Antigen p24	None
Bacterial Growth (five day blood agar)	None
Aerobic and fungi culture	None
Anaerobic culture	None

Consumer Product Testing Co.

EST. 1975

FINAL REPORT

CLIENT:	Lighter & Livelier, LLC...RMI, Inc. 1001 Meadowleah Street Las Vegas, NV 89145
ATTENTION:	Al Needleman
TEST:	48 Hour Patch Test Protocol No.: 1.02
TEST MATERIAL:	Anti Aging Face Cream with Cord Serum Complex NBP0073 Batch 0013
EXPERIMENT REFERENCE NUMBER:	C08-5584.01

Reviewed by: Richard R. Eisenberg, M.D.
Medical Director
Board Certified Dermatologist

Approved by: Joy Frank, R.N.
Executive Vice President, Clinical Evaluations

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.
EST. 1975

QUALITY ASSURANCE UNIT STATEMENT

Study No.: C08-5584.01

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of clinical laboratory studies. These studies have been performed with adherence to the applicable ICH Guideline E6 for Good Clinical Practice and requirements provided for in 21 CFR parts 50 and 56 and in accordance to standard operating procedures and applicable protocols. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study. All data pertinent to this study will be stored in the Consumer Product Testing Company archive, unless specified otherwise, in writing by the Sponsor.

Quality Assurance personnel involved:

Quality Assurance — Date: 12/12/08

The representative signature of the Quality Assurance Unit signifies that this study has been performed in accordance with standard operating procedures and the applicable study protocol as well as any government regulations regarding such procedures and protocols.

Objective: To determine by epidermal contact the primary irritation potential of a test material.

Participants: Fifty-four (54) subjects, male and female, ranging in age from 20 to 79 years, who qualified were selected for this evaluation. Fifty-three (53) subjects completed this study. The remaining subject discontinued her participation for personal reasons unrelated to the use of the test material.

Inclusion Criteria:

a. Male and female subjects, age 16[a] and over.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History form and the understanding and signing of an Informed Consent form.
e. Considered reliable and capable of following directions.

Exclusion Criteria:

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

Test Material: Anti Aging Face Cream with Cord Serum Complex NBP0073 Batch 0013

Study Schedule:

Panel #	Initiation Date	Completion Date
20080489	December 2, 2008	December 5, 2008

Methodology: Approximately 0.2 ml of the test material, or an amount sufficient to cover the contact surface, was applied to the 3/4" x 3/4" absorbent pad portion of an adhesive dressing. When secured to the appropriate treatment site, this dressing formed an occlusive patch.

[a]With parental or guardian consent

Methodology (continued):

The test material remained in contact with the skin for a total of forty-eight hours. This site was then evaluated for gross changes. Absence of any visible skin change was assigned a zero value. The test site was re-evaluated at seventy-two hours.

Evaluation Criteria (Erythema and additional Dermal Sequelae):

0	=	No visible skin reaction	E	=	Edema
0.5 / +	=	Barely perceptible	D	=	Dryness
1	=	Mild	S	=	Staining
2	=	Moderate	P	=	Papules
3	=	Marked	V	=	Vesicles
4	=	Severe	B	=	Bullae
			U	=	Ulceration
			Sp	=	Spreading

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Results:

The results of each participant are appended (Table 1).

Observations remained negative throughout the test interval.

Subject demographics are presented in Table 2.

Summary:

Under the conditions of this study, test material, Anti Aging Face Cream with Cord Serum Complex NBP0073 Batch 0013, did not indicate a potential for dermal irritation.

Table 1
Panel #20080489

Individual Results

Anti Aging Face Cream with Cord Serum Complex NBP0073 Batch 0013

Subject Number	Observations 48 Hours	72 Hours
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0
16	0	0
17	0	0
18	0	0
19	0	0
20	0	0
21	0	0
22	0	0
23	0	0
24	0	0
25	0	0
26	0	0
27	0	0

Table 1
(continued)
Panel #20080489

Individual Results

Anti Aging Face Cream with Cord Serum Complex NBP0073 Batch 0013

Subject Number	Observations 48 Hours	72 Hours
28	0	0
29	0	0
30	0	0
31	0	0
32	0	0
33	0	0
34	0	0
35	0	0
36	0	0
37	0	0
38	0	0
39	0	0
40	0	0
41	0	0
42	0	0
43	0	0
44	0	0
45	0	0
46	0	0
47	0	0
48	0	0
49	0	0
50	---DID N	OMPLETE STUDY---
51	0	0
52	0	0
53	0	0
54	0	0

Table 1
Panel #20080489

Subject Data

Subject Number	Initials	Age	Sex
	JO	63	F
2	MV	58	F
3	EM	49	F
4	AF	73	F
5	JG	56	F
6	VR	73	F
7	CD	38	F
8	AM	57	F
9	CG	79	F
10	EG	79	M
11	GT	75	F
12	ET	69	F
13	DM	36	F
14	PD	71	F
15	FS	70	F
16	CE	65	F
17	MP	70	F
18	EV	74	F
19	AC	66	F
20	RT	72	M
21	DL	41	F
22	JF	76	F
23	CD	77	F
24	CG	76	F
25	RD	70	F
26	AG	58	F
27	VR	40	M

Table 2
(continued)
Panel #20080489

Subject Data

Subject Number	Initials	Age	Sex
28	DL	48	F
29	NM	41	F
30	JP	72	M
31	DD	42	F
32	DC	44	F
33	JC	57	M
34	MK	51	F
35	LB	62	F
36	MJ	51	M
37	AN	69	F
38	AN	72	M
39	VS	66	M
40	BP	65	F
41	SN	76	M
42	DI	67	F
43	RG	65	F
44	TP	75	M
45	LS	49	F
46	BW	69	F
47	NB	55	F
48	MK	22	F
49	VJ	49	F
50	KM	31	F
51	KG	20	F
52	LR	53	F
53	MU	60	F
54	AP	67	F

L&L Cord Extract Blind Study

Study Design

- No artificial damage allowed to skin prior to study
- No Botox or laser/chemical peel for 3 months prior
- Only products allowed were blind creams and previous facial skin cleanser
- All other products ceased

Study Design

- 6 week trial
- Photos taken before and at completion
- Evaluation by a plastic surgeon and random female in same office with no formal medical training
- All photos were rated prior to code being broken

Study Design

- Investigator given blinded samples of product, control and cord extract
- Ingredients of creams were also not divulged to investigator
- Patient chose which container would be used on which side
- 30 patients identified to participate

Study Design

- Rating System of wrinkles (static only)
- No change
- 25% reduction
- 50% reduction
- 75% reduction
- Complete effacement of wrinkle

Demographics

- Age range 29-73
- Avg. Age 56.6
- Female:Male ratio 27:2

Clinical results

- Plastic Surgeon (PS) found 53% of participants showed a response on either side
- PS found 47% did not respond
- Office member (OM) found 45% of participants showed a response on either side
- OM found 55% did not respond

Clinical Results

- PS found 28% showed results on control side
- OM found 10% showed results on control side

Clinical Results

- PS found 35% of patients responded on cord extract side
- OM found 35% of patients responded on cord extract side

Clinical Results

- Of those patients that showed a visible response
- PS saw a 31% degree of wrinkle reduction in the controls and a 43% degree of reduction on the cord extract side
- OM saw a 33% degree of reduction in the controls and a 40% reduction on the cord extract side

Clinical Results

- One participant did not complete the study (moved)
- No adverse or allergic reactions

Summary

- Positive response rate in 35% of participants using cord extract
- Positive response in 19% of participants using control
- Of those that responded, the control side showed a 32% improvement and the cord extract side showed a 41% improvement

Summary

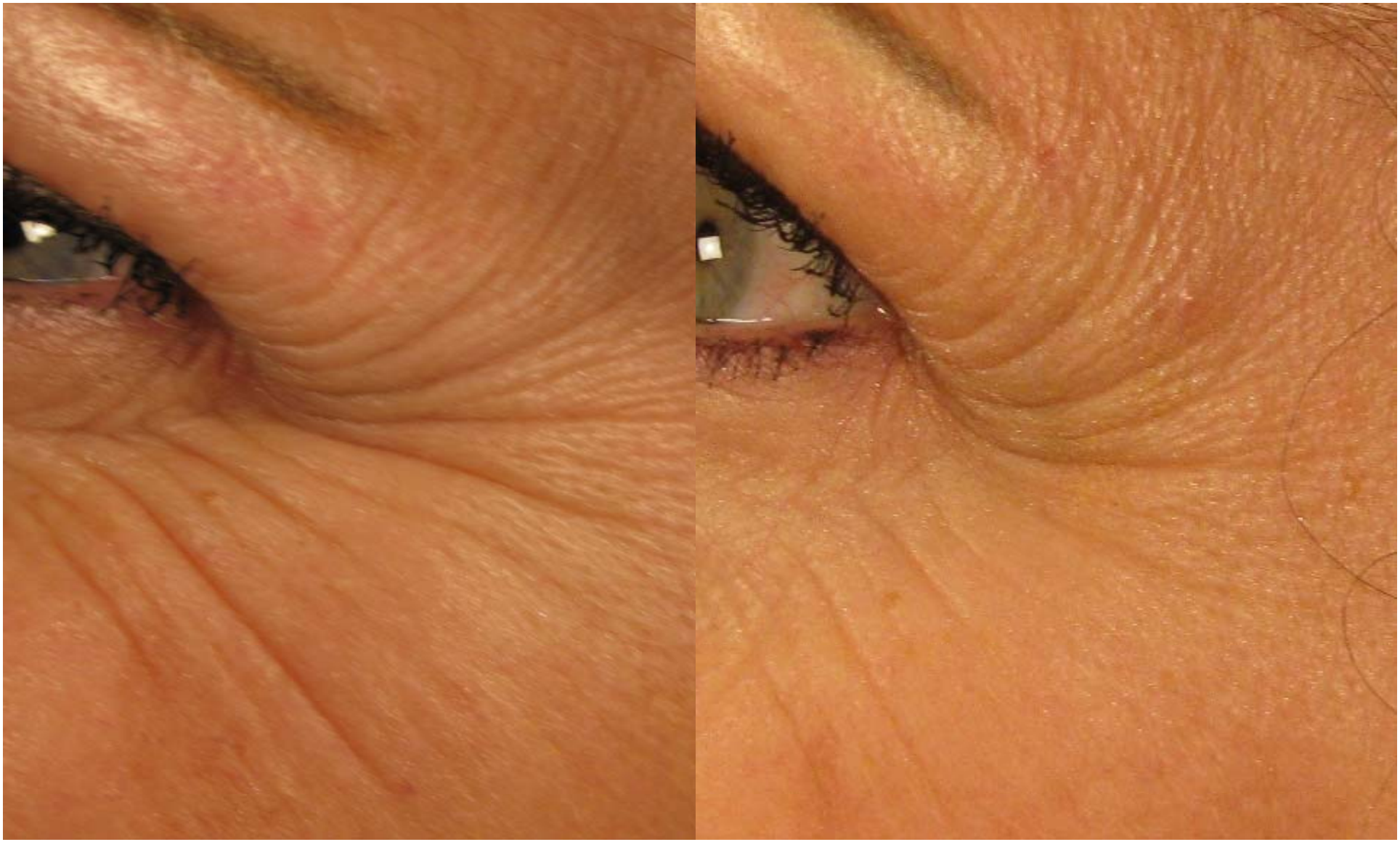
- Unsolicited comments on patient perception made during last photo session
- “Skin feels smoother”
- “Skin feels milky soft”
- “The surface seems softer”

Summary

- Solicited question
- 10 of 29 said they would buy the cream
- 16 of 29 said they would probably buy the cream
- 3 said they would not buy the cream
- Patients not shown their pictures until study completed
- Patient perception of change not evaluated as had been done in previous study

Demonstrative Slides

- Show before on left and after on right
- All photos show side that received cord extract



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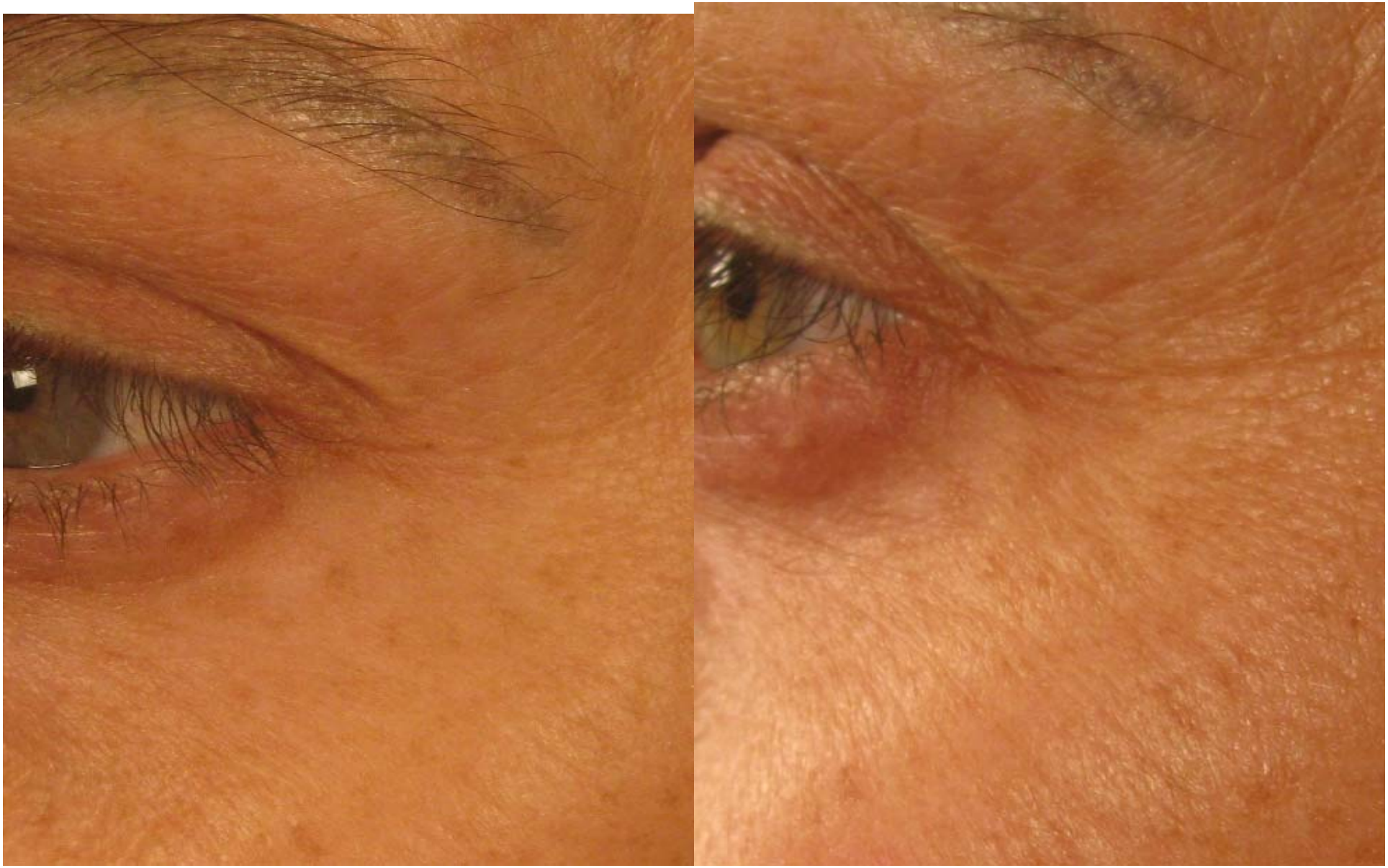

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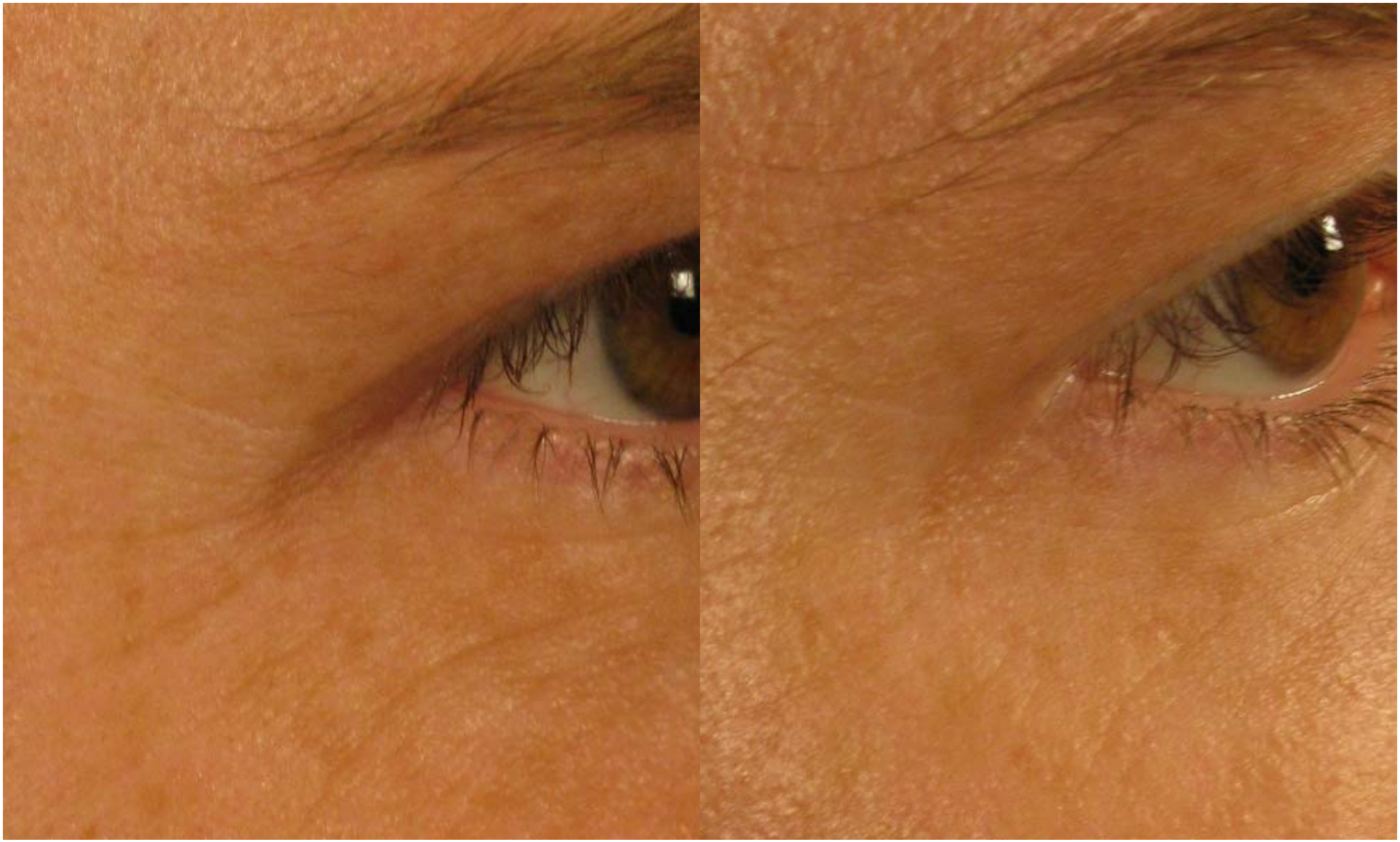

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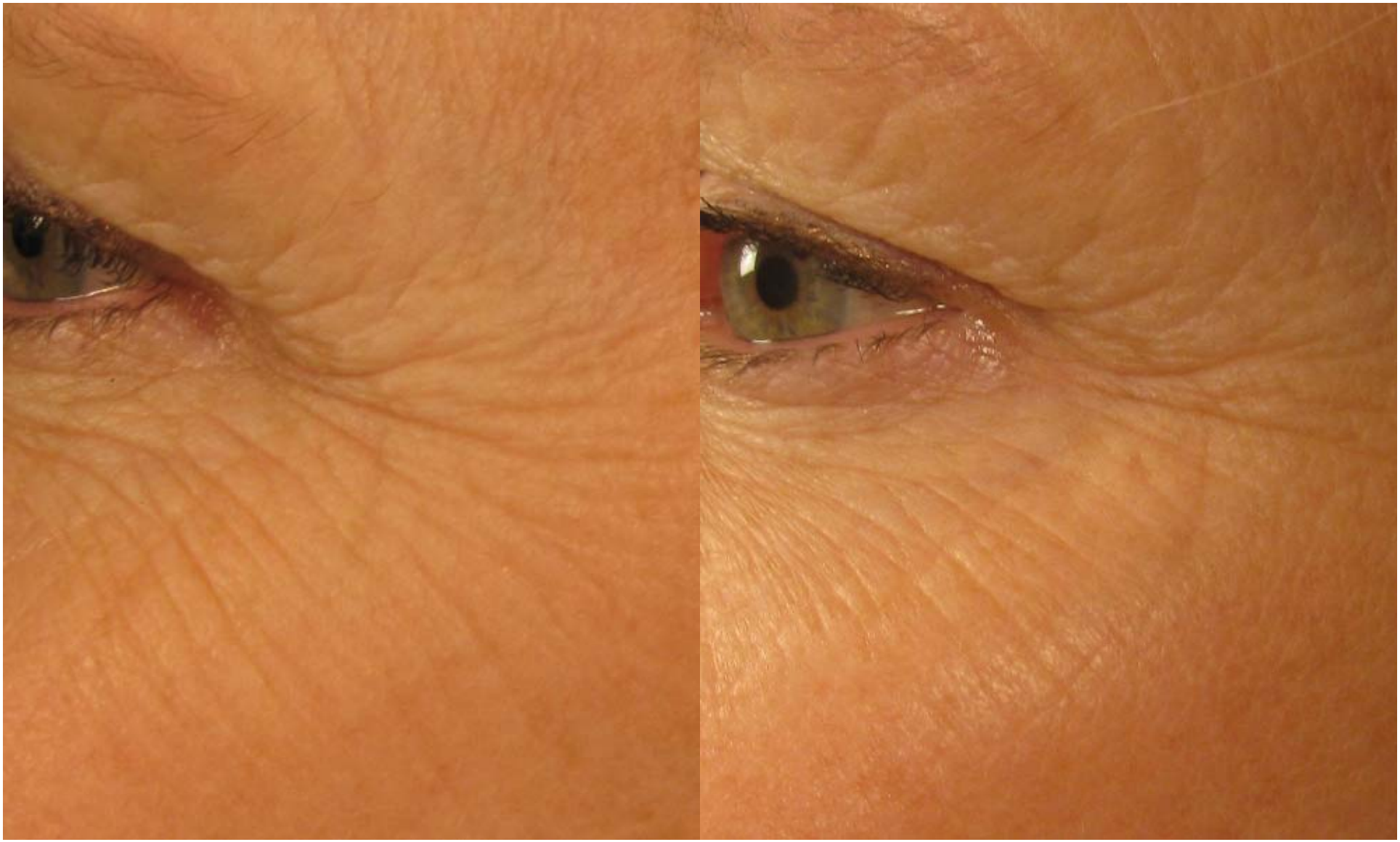

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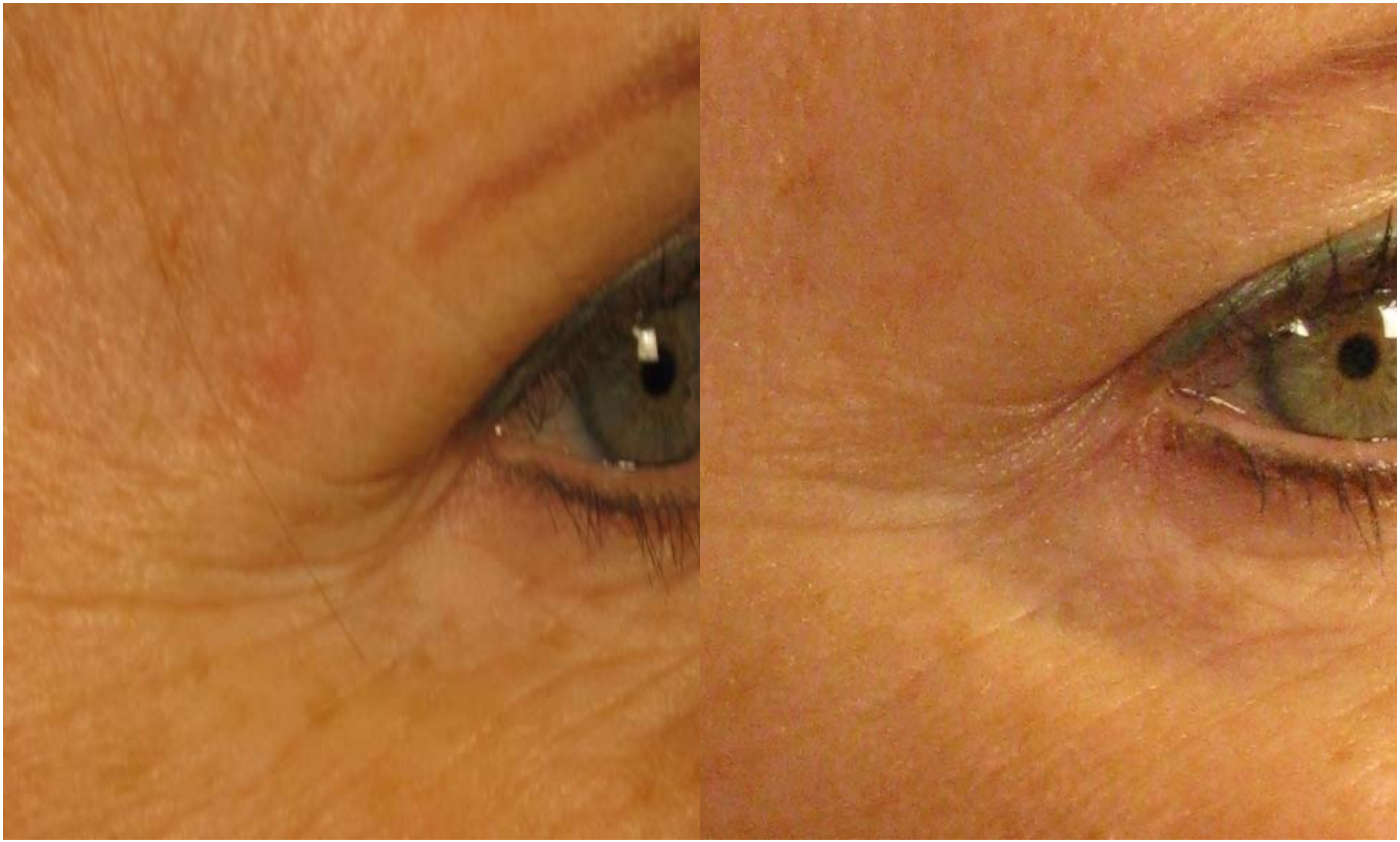

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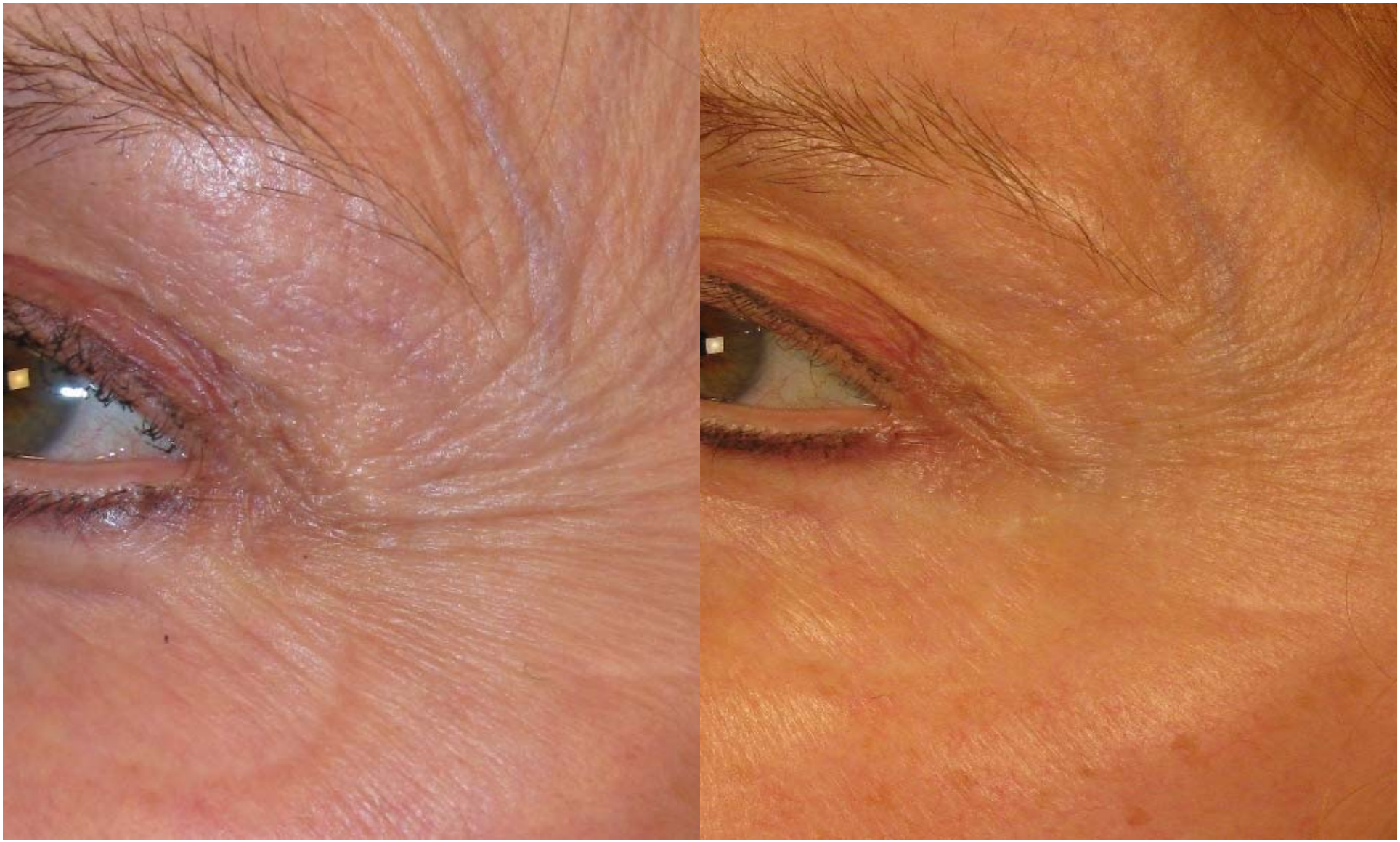

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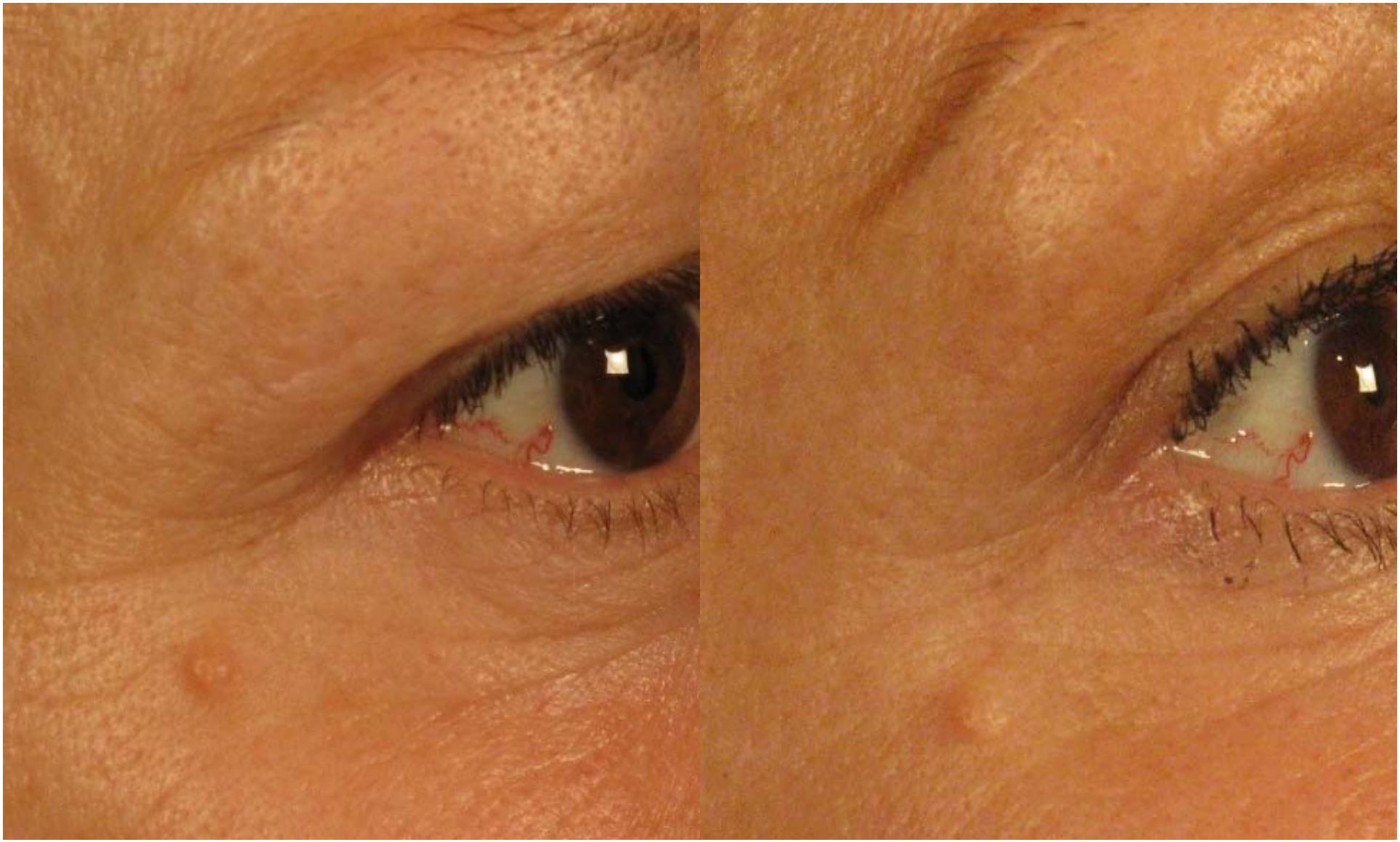

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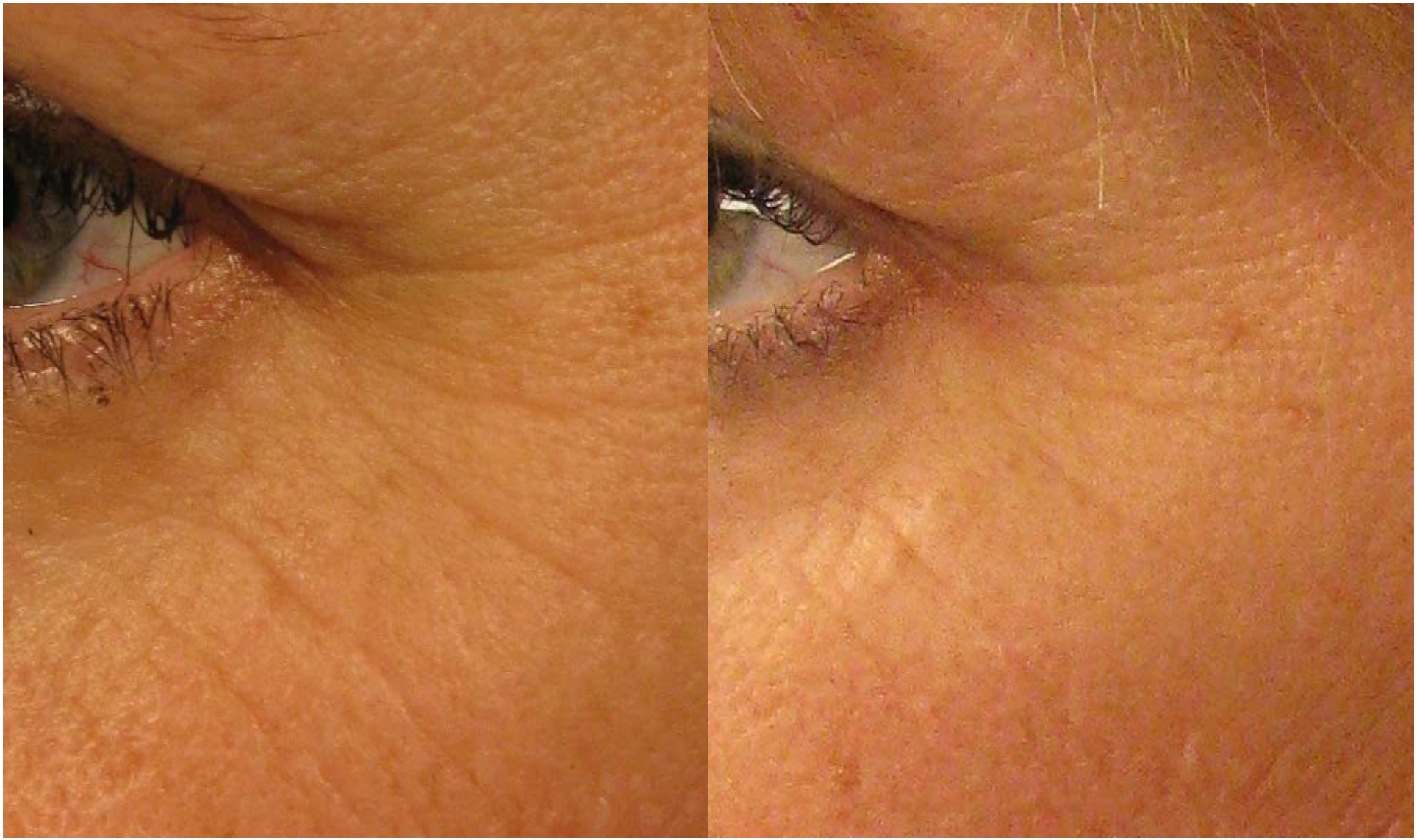

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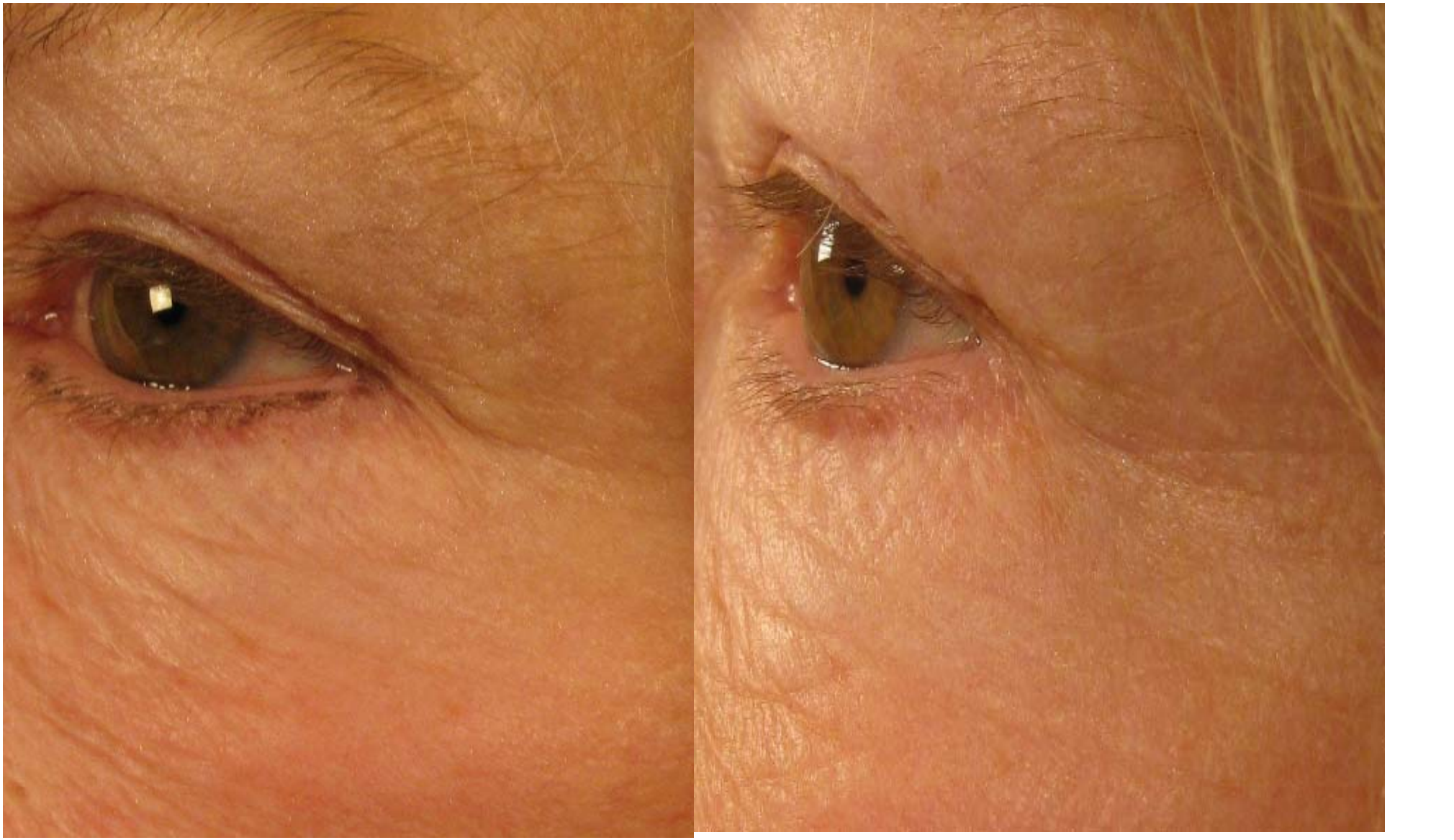

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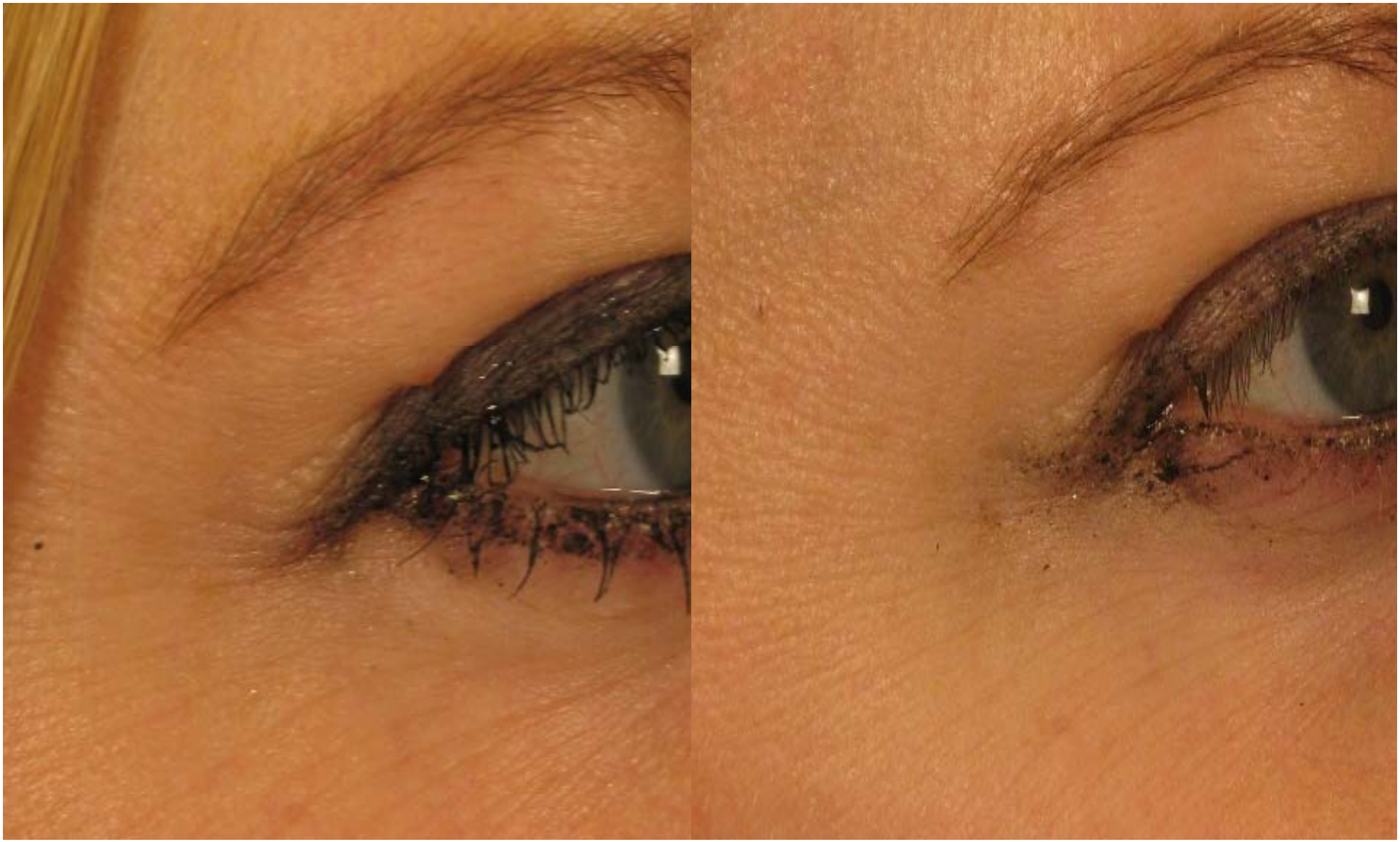

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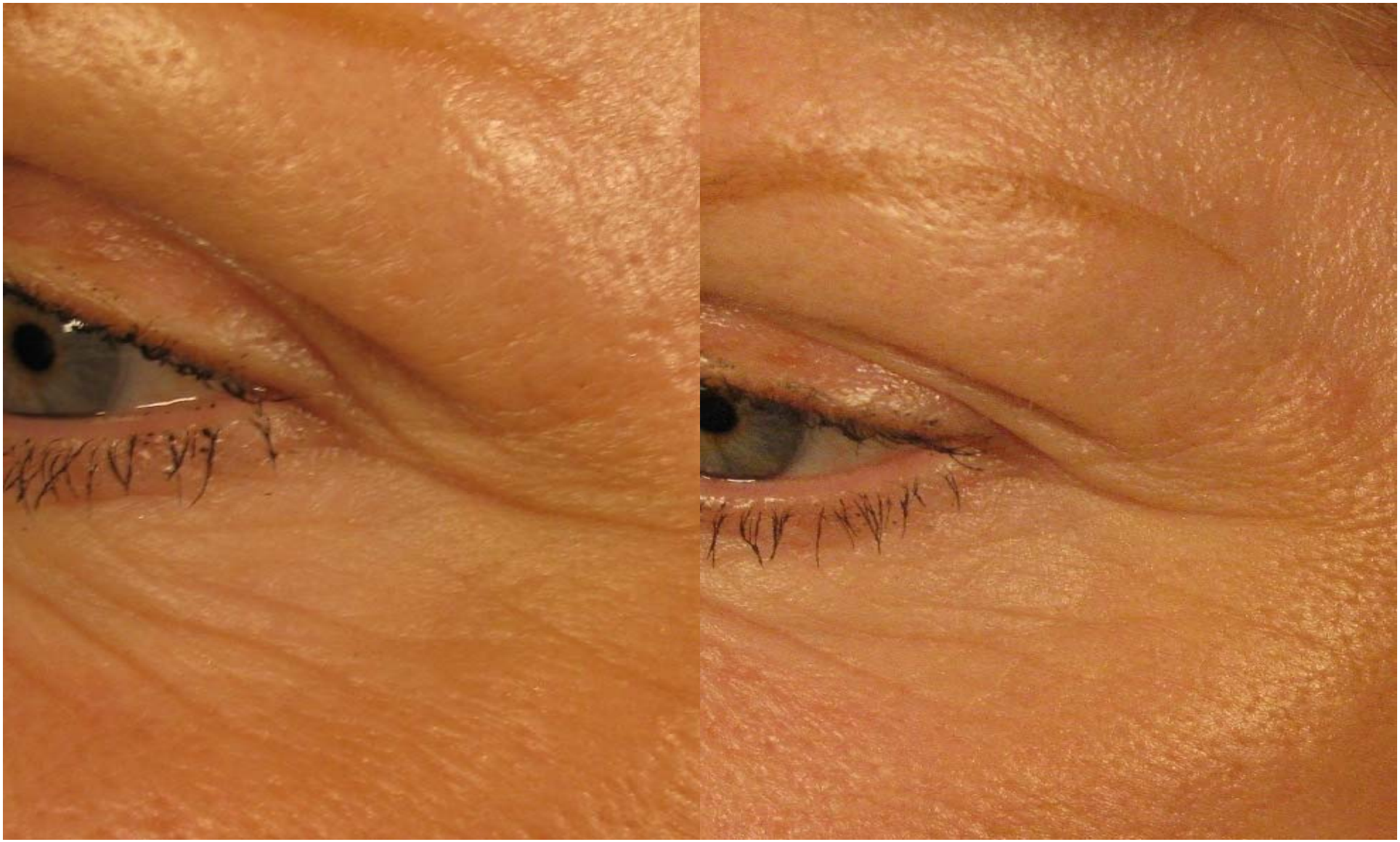

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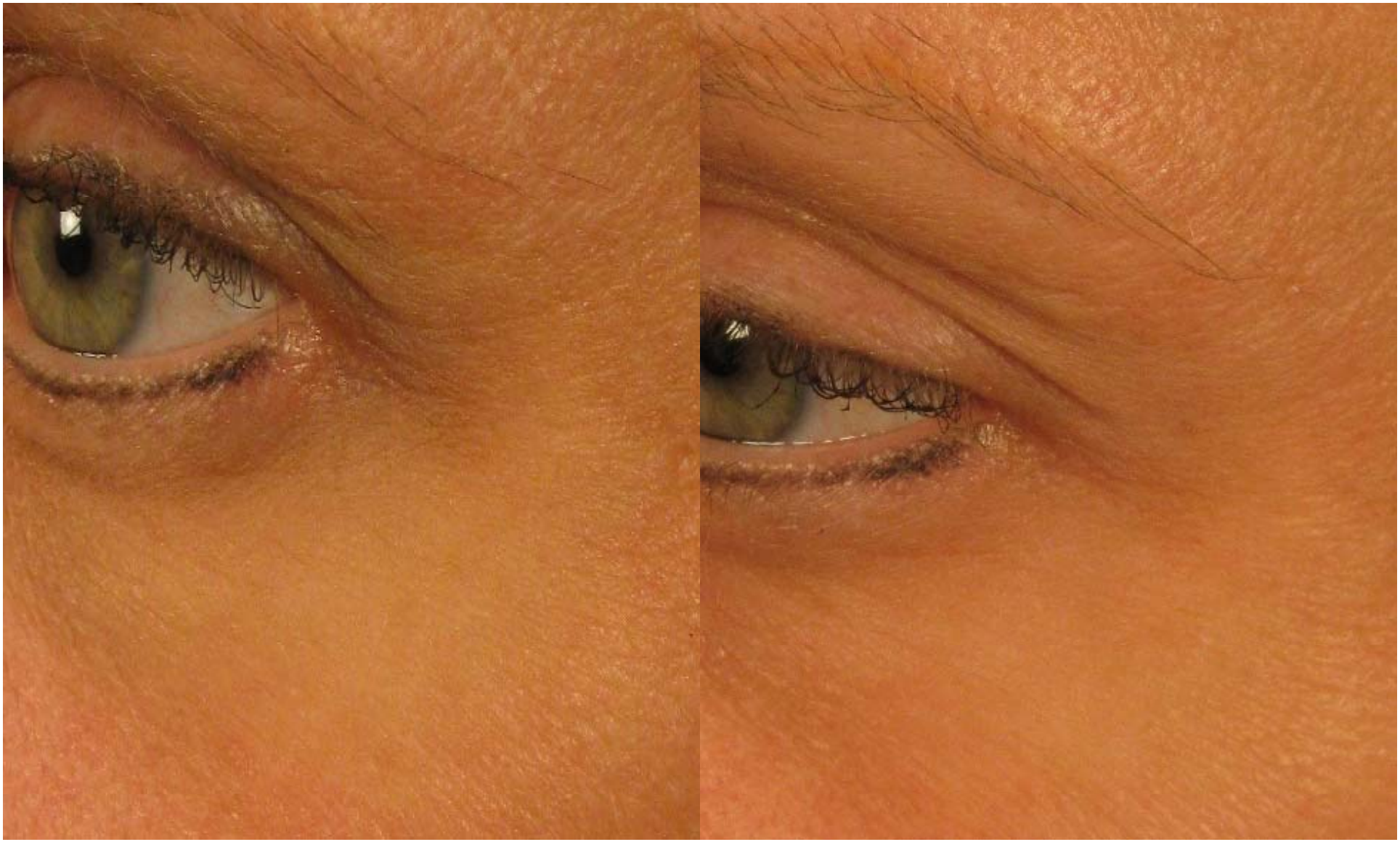


LM

Histogeometric Analysis of the Effects of Product A versus B on Human Skin

Introduction

The marketplace is filled with products that purport to have “anti-aging” or “skin rejuvenation” properties. Most of these claims are supported by non-invasive measurements such as clinical photography, and skin surface evaluation using replicas to assess the depth of fine-line wrinkles and other surface phenomena. Some products make claims based on in vitro studies where keratinocytes or fibroblasts are cultured in the presence of the “active” ingredients, and various parameters (i.e., proliferation, migration, protein synthesis) are assayed. These types of studies can provide valuable insights into the mechanism(s) by which certain agents might be working; however, in vitro findings often do not translate into the in vivo situation. A more stringent test is to apply the product to human skin in a manner similar to its anticipated use and evaluate its effects on the epidermis and dermis using a combination of morphology, histochemistry and immunohistochemistry.

The present study was designed to test the effects that a topical cream containing cord serum complex, had on human skin. As a control, the topical cream minus the cord serum complex was also evaluated and both products were compared with an untreated site.

Methods

Six healthy volunteers were enrolled in the study. Product A was applied to the right aspect of the upper inner arm (R site), product B was applied to the left aspect of the upper inner arm (L site) and the volar forearm was chosen as the untreated site. Both products were applied daily for 26 days. The volar forearm received no product but was rubbed gently, daily, in an effort to simulate the manner in which both products were applied to the skin.

At the end of the treatment period, a 2-3 mm punch biopsy specimen was obtained from each treated site and the untreated site. Each specimen was immediately placed in 10% buffered formalin and processed for paraffin sectioning. All sections were five-micrometers in thickness and all slides were stained simultaneously as a group for each histochemical determination. The entire biopsy specimen was photographed with a high resolution digital camera system (Axiocam, Zeiss Corporation) mounted on a Zeiss Axioplan 2 light microscope at a magnification of 20x. All slides were photographed under the identical white balance light settings and exposure time to insure consistency in micrographs. Micrographs were subsequently analyzed using computer assisted image analysis software (Axiovision, Zeiss Corporation). All measurements were made from at least four areas of the biopsy specimen, except for two biopsies, which had smaller amounts of tissues and thus three measurements were made.

Five-micrometer paraffin sections were stained with hematoxylin-eosin (H&E) for overall morphologic evaluation and viable epidermal thickness determinations (VET; Fig. 1; Table 1). For estimation of viable epidermal thickness, care was taken to cut the sections perpendicular to the surface. The VET includes the area from the dermoepidermal interface to the lowermost

portion of the stratum corneum (seen as a bright red zone; Fig. 1). For estimation of elastic fibers, van Geison's stain was employed, which stains elastic fibers blue-black to black, collagen pale red, other tissue elements yellow, and nuclei blue to black (Fig. 2). For pro-collagen, anti-type I collagen (EMD Bioscience Inc.) antibody was used. This antibody to type I collagen was made against the triple helical portion and it is able to stain procollagen I. Immunohistochemical analysis of the paraffin sections was carried out using the DAB kit, which produces a brown reaction product (Fig. 3). For glycosaminoglycans (GAGs), Hale's colloidal iron was used since Hale's stainable material (blue) represents, for the most part, GAGs and is commonly used as an indicator of changes in ground substance (Fig. 4).

The quantification of stainable material was determined using a custom designed software program, integrated into the Axiovision image analysis system (Zeiss Corporation). The analysis is conducted in the following manner: (i) the reaction product (i.e., blue-black – elastin; brown – pro-collagen; blue – GAGs) is detected from a histogram and only objects with that color are outlined on the micrograph. The total area occupied by the outlined areas is measured; (ii) the entire area of the dermis is outlined and measured; and (iii) area of reaction product divided by total area = the percentage of material deposited.

It should be noted that all photomicrographs were taken and analyses performed in a double blind manner, and only after the data was tabulated was the investigator informed about the identity of the R and L sites.

Results

Morphology

The epidermis did not appear to be morphologically altered in any of the subjects at the two treatment as well as the untreated site (Fig. 1). In most instances, the undulating nature of the dermoepidermal interface was maintained. The granular layer was prominent in all specimens and there was little evidence of apoptosis (sunburn cells) within the epidermis. The "basketweave" architecture of the stratum cornea, characteristic of formalin-fixed human skin was maintained in all subjects in all sites.

For the most part, the fibrous components of the dermis (i.e., collagen, elastin) did not appear altered on the H&E sections from any of the treatment regimens or the untreated sites of the six subjects (Fig.1). In some cases, the dermis from the untreated site appeared more compact (Fig. 1). In a few subjects, occasional areas of blue-gray staining material, usually associated with elastosis, were noted; however, frank signs of photodamage were not seen in any of the subjects. Importantly, there did not appear to be an unusual amount of inflammatory cells in biopsies from any of the treatment sites or in the untreated site from any of the subjects. Some increased cellularity was noted around portions of hair follicles present in some of the sections but this was not deemed significant. Vascular profiles appeared normal and there was no evidence of increased vascularity, vasodilatation and/or extravasation of red blood cells.

Viable Epidermal Thickness (VET)

There was no consistent trend seen in the VET measurements (Tables 1 and 2). Subjects 1, 2, and 6 had similar VET values for the untreated, R and L sites. Subject 3 had a thinner VET measurement for the untreated site compared with the R and L sites. Subject 4 had a thinner VET for the R site compared with the untreated and L site, whereas Subject 5 had a thinner VET for the L site when compared with the R and untreated sites. Given the lack of inflammation, which usually is responsible for epidermal thickening, it is not surprising that VET was not affected by either R or L treatment.

Elastin

The overall area of the dermis occupied by elastin appeared to be greater in the untreated sites from all six subjects when compared with either the R or L treatment site (Fig. 2, Tables 1 and 2). This finding in no way implies that either of the treatments had a negative impact on elastin fiber deposition, synthesis and/or destruction. It most likely represents inherent differences in elastin content between the volar forearm and the upper inner arm.

With respect to treatment sites, there was no obvious trend (Tables 1 and 2). Subjects 1 and 4 had significantly more elastin-stained material in the L site when compared with the R site. In contrast, subject 3 had significantly more elastin-stained material in the R site compared with the L site. There was no significant difference in elastin-stained material when the R and L sites were compared in Subjects 2, 5, and 6. When all six subjects were compared there was no change in elastin-stained material (Table 1). This is not surprising since elastin is one of the more stable components of the dermis with an extremely long turnover time. New elastin deposition is most often seen during tissue regeneration following a wound. Thus the lack of evidence for skin perturbation due to either of the treatments (Fig. 1) could account for the failure to detect a change in elastin.

Procollagen

Five of the six subjects showed an increase in the immunostaining for procollagen when the R site was compared with the L site (Fig. 3, Tables 1 and 2). Of these 5 subjects, one (#4) was significant at the $P<0.05$ level and two (#2 and #3) were highly significant ($P<0.01$ level). While an overall increase in procollagen immunostained material was detected for the R site versus the L site in Subjects 1 and 5, the difference was not statistically significant. The R and L treatment did not affect the procollagen-stainable material in Subject 6. When all six subjects were combined there was a greater amount of immunostaining for procollagen in the R site versus the L site; however due to subject to subject variability, this difference was not significant.

With respect to the untreated site, 4 subjects had less immunostained material corresponding to procollagen when compared with the R site; two subjects had more immunostained material. Due to potential differences in the dermis between the treated and untreated sites it is difficult to meaningfully interpret these changes.

GAGs

Four of the six subjects had significantly ($P<0.01$) increased Hale's-stainable material in the R site when compared with the L site (Fig. 4, Tables 1 and 2). Subjects 4 and 6 had increases in Hale's-stainable material in the L site versus the R site; however, this difference was not statistically significant. When all six subjects were combined there was a greater amount of Hale's stainable material in the R site versus the L site; however due to subject to subject variability, this difference was not significant.

The untreated site showed the greatest subject to subject variability in Hale's-stainable material. Nevertheless 4 subjects had greater Hale's-stainable material in the R site when compared to the untreated site. As mentioned previously, due to regional differences it is difficult to draw meaningful comparisons between the treated and untreated sites.

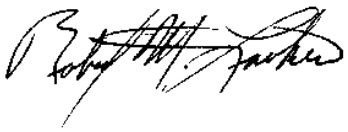
Conclusions

There are several conclusions to be drawn from this small pilot study. The active and vehicle-only formulations did not have any deleterious effects on the skin that were discernable at the light microscopic level. This is important because any changes seen in the other parameters were not confounded by and/or secondary to an inflammatory response. The assumption is that any changes are the result of the cord serum complex. The R site, which received the cord serum complex daily for 26 days, had increased amounts of stained material corresponding to procollagen and GAGs compared to the vehicle-treated (L) site. Cord serum complex had no discernable effect on VET or elastin.

Since ground substance (GAGs) is the dermal component that is most rapidly turned over, it is not surprising that changes were seen. Furthermore, ground substance is well known for its water-holding capacity, and it is this increase in water binding that could be partially responsible for the disappearance of fine-line wrinkling after use of this product. The increase in procollagen-stained material was also significant. In contrast to the GAGs, collagen is slowly turned over. This suggests that some of the changes in skin quality that have been reported following use of the cord serum complex facial cream may be somewhat more long lasting than the GAG-induced changes because collagen fibers are a more stabile dermal component than GAGs. Taken together these findings suggest that facial cream with serum cord complex stimulates the synthesis of GAGs and procollagen, which in part is responsible for the clinical changes seen after use of this product.

It is important to remember that conclusions drawn from histochemical staining experiments need to be tempered by the fact that despite precautions taken to eliminate and/or minimize the subtle differences in section thickness and staining inconsistency, such events can occur, which will confound the results. The conclusions drawn thus far can be markedly strengthened by determining the change in levels of these proteins biochemically using immunoblotting techniques. For example, following biopsy, the epidermis can be separated from the dermis and a dermal protein lysate can be obtained. This lysate can be analyzed for the amount of procollagen and certain GAG components (e.g., hyaluronate, heparin sulfate) by Western blot techniques. If increases in the levels of procollagen and hyaluronate are observed, this would

dramatically strengthen the histochemical data. Alternatively, the study can be repeated with a larger cohort of subjects. If this is study were to be conducted, the procollagen and GAG components should be analyzed. H&E sections should also be obtained for an overall morphological assessment.

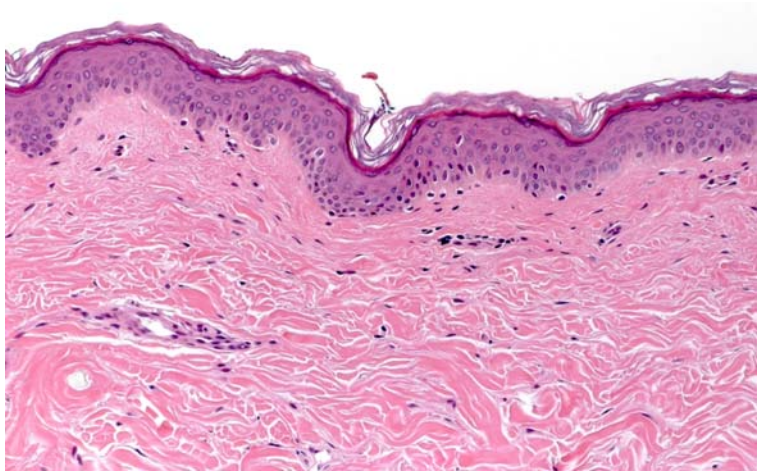


Robert M. Lavker, Ph.D.

June 14, 2010

H&E

NT



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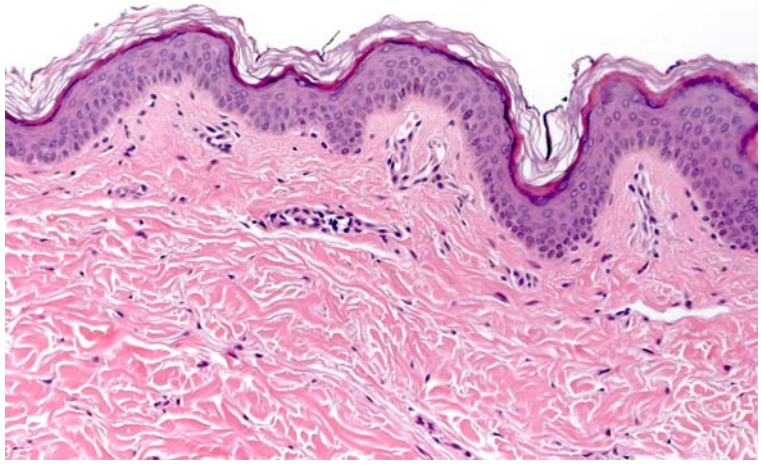

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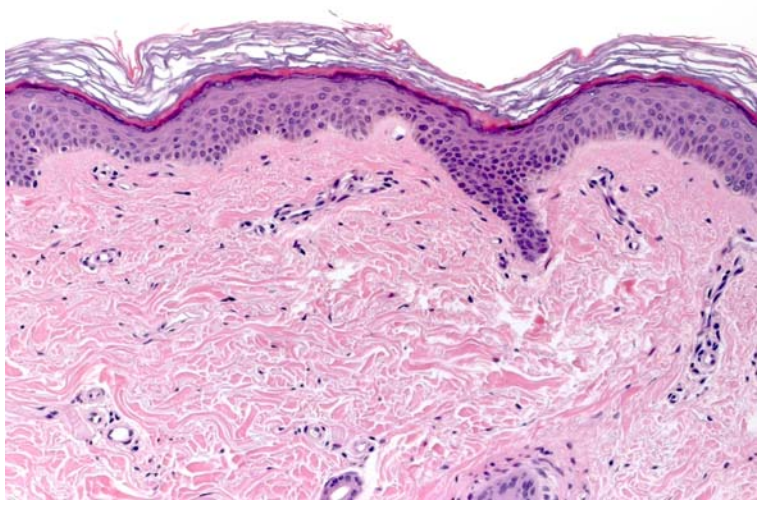
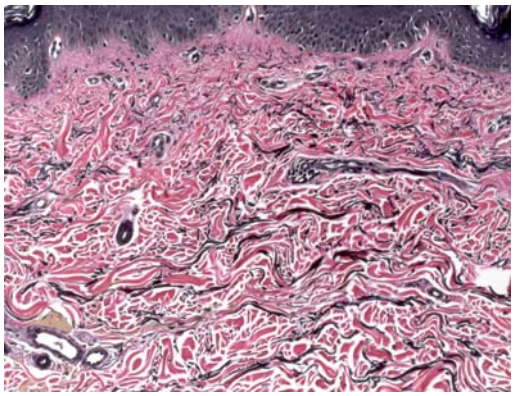


Figure 1

elastin - Van Geison’s stain

NT



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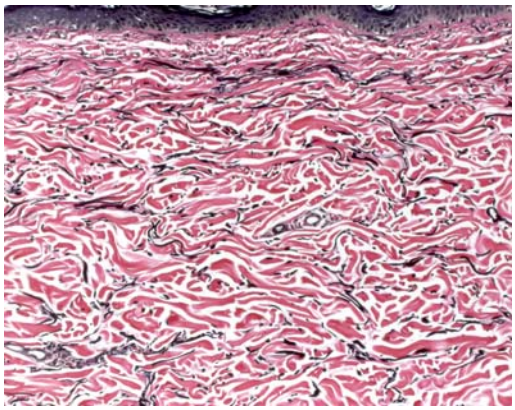

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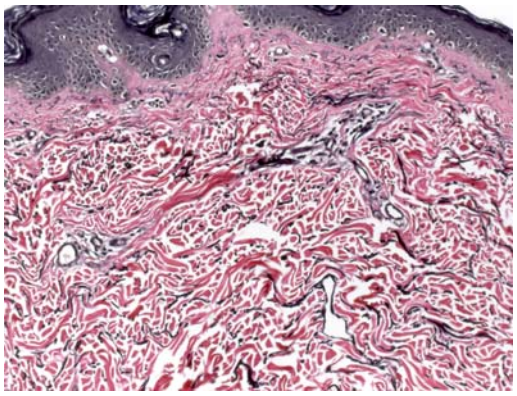


Figure 2

Pro-collagen - Type 1 collagen stain

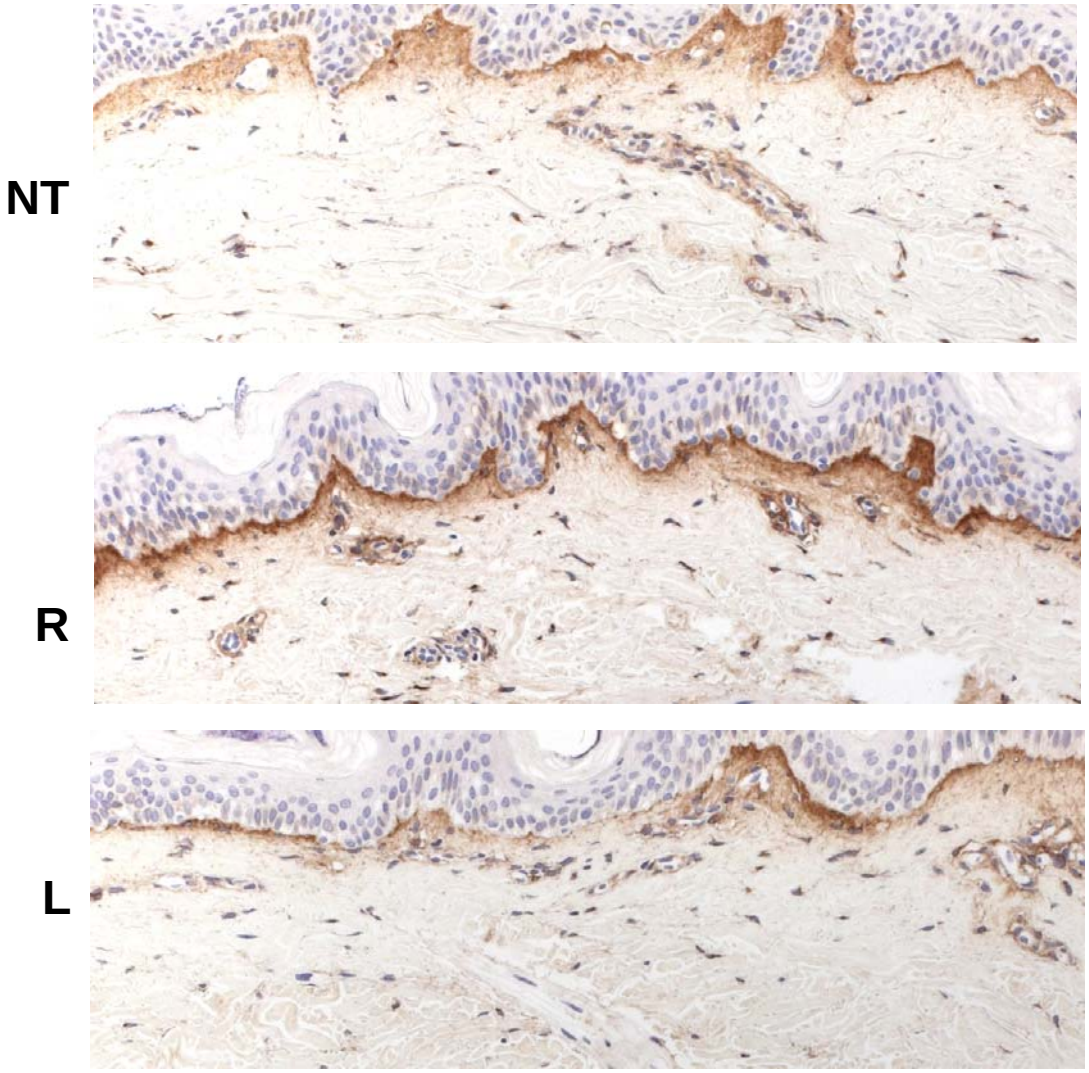
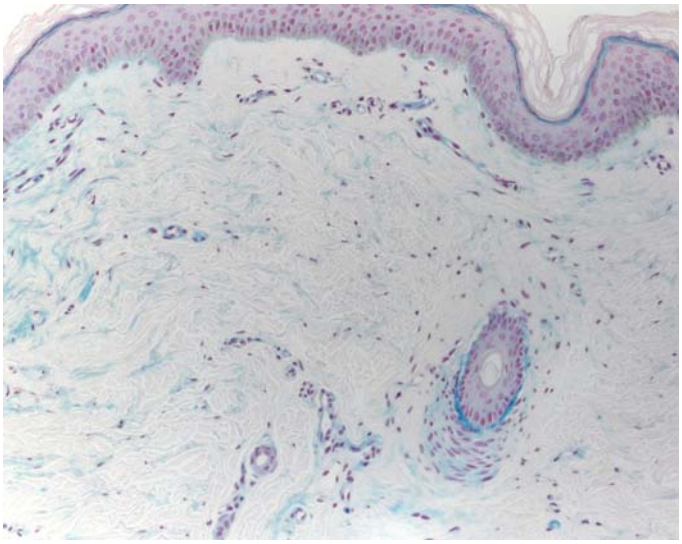


Figure 3

GAGs - Hales Colloidal Iron stain

NT



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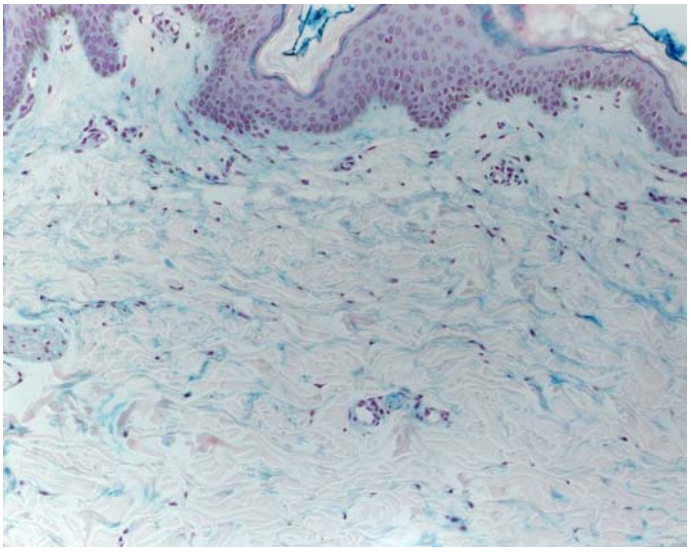

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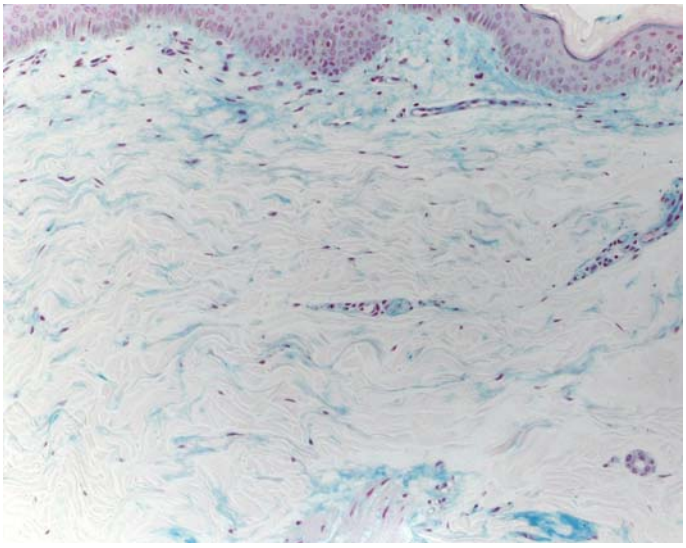

Figure 4

Table 1 - Histogeometric Analyses

VET (microns)	1	2	3	4	5	6	∑
NT	126 ± 7	99 ± 7	98 ± 8	115 ± 4	128 ± 16	114 ± 3	113 ± 12
R	132 ± 7	94 ± 4	120 ± 15	91 ± 2	118 ± 20	119 ± 3	96 ± 37
L	126 ± 5	91 ± 8	115 ± 10	108 ± 4	87 ± 9	113 ± 5	107 ± 14
Elastin (% dermis)							
NT	14.0 ± 1	10.4 ± 1.1	12.5 ± 0.7	12.2 ± 2.4	13.0 ± 2.0	7.6 ± 2.0	11.6 ± 2.0
R	8.0 ± 1.5	9.8 ± 0.3	8.3 ± 1.6**	5.8 ± 0.4	8.2 ± 1.1	5.0 ± 0.4	7.5 ± 1.6
L	11.8 ± 0.7**	8.0 ± 1.7	3.3 ± 0.3	8.6 ± 0.8**	9.5 ± 1.1	5.3 ± 1.1	7.8 ± 2.8
Procollagen (% upper dermis)							
NT	8.1 ± 0.6	9.9 ± 1.7	4.7 ± 1.8	16.2 ± 1.8	9.1 ± 1.5	16.4 ± 2.9	10.7 ± 4.3
R	9.2 ± 0.6	13.3 ± 1.6**	9.9 ± 1.7**	12.3 ± 2.3*	19.2 ± 5.4	10.0 ± 2.4	12.3 ± 3.4
L	9.1 ± 1.1	7.8 ± 1.3	5.2 ± 2.1	7.9 ± 1.8	15.8 ± 3.4	11.5 ± 1.4	9.6 ± 3.4
GAGs (% dermis)							
NT	1.8 ± 0.8	24.3 ± 2.8	22.6 ± 2.4	13 ± 3.8	14.7 ± 2.2	5.2 ± 0.9	13.6 ± 8
R	22 ± 5.0**	11.4 ± 0.9**	20.1 ± 2.2**	15 ± 2.7	23 ± 3.0**	10.8 ± 3.9	17.4 ± 4.9
L	4.4 ± 2.3	6.5 ± 1.5	8.3 ± 2.7	18 ± 5.5	14.3 ± 1.5	13.9 ± 3.9	10.9 ± 4.8

*P <0.05; **P< 0.01

Table 2 - Percentage Change

VET	1	2	3	4	5	6
R vs L	4.5 ↑	3 ↑	4.2 ↑	15.7 ↓	26.3 ↑	5.0 ↑
R vs NT	4.5 ↑	5.1 ↓	18.3 ↑	20.8 ↓	7.8 ↓	4.2 ↓
L vs NT	0	8.1 ↓	14.8 ↑	6.1 ↓	32.0 ↓	0.9 ↓
elastin						
R vs L	32.2 ↓	18.4 ↑	60.2 ↑	32.6 ↓	13.7 ↓	5.7 ↓
R vs NT	42.9 ↓	5.8 ↓	33.6 ↓	52.5 ↓	36.9 ↓	34.2 ↓
L vs NT	15.7 ↓	23.1 ↓	73.6 ↓	29.5 ↓	26.9 ↓	30.3 ↓
procollagen						
R vs L	1.1 ↑	41.4 ↑	47.5 ↑	35.8 ↑	17.7 ↑	13.0 ↓
R vs NT	12.0 ↑	25.6 ↑	52.5 ↑	24.0 ↓	52.6 ↑	39.0 ↓
L vs NT	11.0 ↑	21.2 ↓	9.6 ↑	51.2 ↓	42.4 ↑	6.1 ↓
GAGs						
R vs L	80.0 ↑	43.0 ↑	58.7 ↑	16.7 ↓	37.8 ↑	22.3 ↑
R vs NT	91.8 ↑	53.1 ↓	11.1 ↓	13.3 ↑	36.0 ↑	51.9 ↑
L vs NT	59.0 ↑	73.3 ↓	63.3 ↓	27.8 ↑	2.7 ↓	62.6 ↑

UNITED STATES PATENT APPLICATION FOR:

TOPICAL COMPOSITION COMPRISING UMBILICAL CORD BLOOD SERUM

INVENTORS:

Alvin Needleman

Attorney Docket No.: 838611-0002

TOPICAL COMPOSITION COMPRISING UMBILICAL CORD BLOOD SERUM

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is based on and claims priority to U.S. Provisional Application Serial No. 61/278,040, filed on October 2, 2009 which is incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not applicable.

BACKGROUND OF THE INVENTION

[0003] 1. FIELD OF THE INVENTION

[0004] The present invention relates to the field of cosmetics. It relates more particularly to novel cosmetic compositions comprising umbilical cord blood serum, and to novel uses of such compositions in the field of cosmetics, especially as an anti-aging and anti-wrinkle formulation.

[0005] 2. DESCRIPTION OF RELATED ART

[0006] The gradual development of facial wrinkles, whether fine surface lines or deeper creases and folds, is an early sign of accumulated skin damage and skin aging, which may be intrinsic and/or caused or accelerated by external factors. For example, premature aging and wrinkling of the skin may be accelerated by excessive exposure to the sun and other damaging elements, overactive facial expression muscles, frequent use of tobacco products, poor nutrition, or skin disorders. Fine surface wrinkles that progress to deeper creases, deepening facial expression due to repeated skin folding, and deep folds which develop with one's maturity are visible changes which may combine to portray a less desirable appearance.

[0007] Various attempts at anti-aging skin care compositions have used botanicals, antioxidants, and biopeptides, among other things. Several invasive techniques are available in which substances are injected or implanted in the area of the skin which either temporarily weaken the muscles or act as skin volume fillers. However, invasive techniques are often risky and require the supervision or assistance of a physician, which can be inconvenient and costly, and non-invasive treatments have historically met with only minimal success. Regardless of the cause of facial creases or folds, safe and effective treatments for reduction or elimination of these problems have been exceedingly difficult to achieve. Thus, there remains a need for new and improved topical skin care compositions that are useful as an anti-aging composition.

BRIEF SUMMARY OF THE INVENTION

[0008] The present invention is directed to novel skin care composition comprising an effective amount of umbilical cord blood serum for topical application to the human skin. The compositions are useful for imparting an so-called "anti-aging" benefits to the skin.

[0009] In one aspect, the umbilical cord blood serum is present in an amount from about 0.0001 wt% to about 90 wt% of the composition, more preferably between about 0.01 wt% to about 25 wt%, and still more preferably about 0.01 wt % to about 10 wt%.

[0010] In still another aspect, the composition is formulated with other cosmetic actives and excipients. For example, in one exemplary aspect, the skin care composition comprises a peptide selected from the group consisting of the tyr-arg, acetyl hexapeptide-3, palmitoyl oligopeptide, palmitoyl tetrapeptide-7, and mixtures thereof. In another exemplary aspect, the skin care composition comprises a biological additive selected from the group consisting of *Juglans regia*, *Centella asiatica*, *Pyrus germanica* extract, and mixtures thereof. In another exemplary aspect, the skin care composition comprises a biological additive selected from the

group consisting of *Undaria pinnatifida* and *Stevia rebaudiana Bertoni*, and mixtures thereof. In still another exemplary aspect, the skin care composition comprises one or more phosphospholipids, such as one selected from the group consisting of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, diphosphatidyl glycerol, and mixtures thereof. in still another exemplary aspect, the skin care composition comprises one or more preservatives, such as methyl and/or propyl paraben. In yet another exemplary aspect, the skin care composition comprises one or more penetration enhancers, such as dimethyl isosorbide and diethyl-glycol-monoethylether. In still another exemplary aspect, the skin care composition comprises one or more neutralizing agents, such as triethanolamine. In yet another exemplary aspect, the skin care composition comprises one or more hyaluronans. In still a further exemplary embodiment, the skin care composition comprises one or more skin-conditioning emollients, such as those selected from the group jojoba oil, almond oil, capric/caprylic triglyceride and mixtures thereof. In yet another exemplary aspect, the skin care composition comprises one or more surfactants, such as myristamidopropyl PG-dimonium chloride phosphate. In yet another aspect, the skin care composition comprises one or more spreading agents, such as PPG-3 benzyl ether myristate is used as a spreading agent. In another aspect, the skin care composition comprises one or more gelling agents, such as carbomer.

[0011] In another aspect, the skin care composition comprises about 0.1 to about 0.35 wt% umbilical cord blood serum, about 3 to 5 wt% palmitoyl oligopeptide, about 3 to about 5 wt% palmitoyl tetrapeptide-7. In still yet another aspect, the skin care composition comprises about 2 to about 3 wt% of the dipeptide tyr-arg and about 0.5 to 1.5 wt% acetyl hexapeptide-3. Yet in another aspect, he skin care composition about 0.5 to about 1.2 wt% phosphatidyl choline, about 0.5 to about 1.5 wt % hyaluronans, and 0.01 to 0.085 of a preservative selected from the

group consisting of methyl paraben and propyl paraben, or mixtures thereof. In yet another aspect, the skin care composition comprises *juglans regia*, *centella asiatica*, *pyrus germanica*, *Undaria pinnatifida*, and *Stevia rebaudiana Bertoni*.

[0012] The present invention is also directed to a method for imparting an anti-aging benefit to human skin comprising: topically applying to the skin of an individual in need thereof any of the foregoing compositions. In a preferred aspect, application of the composition to the skin results in improved procollagen and glycosaminoglycans content.

[0013] Additional aspects of the invention, together with the advantages and novel features appurtenant thereto, will be set forth in part in the description which follows, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned from the practice of the invention. The objects and advantages of the invention may be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENT

[0014] The present invention is directed to novel skin care compositions for topical application to the human skin comprising an effective amount of umbilical cord blood serum. The umbilical cord blood serum is present in an effective amount to treat, reverse, ameliorate, and/or repair signs of skin damage or skin aging. Such benefits may include without limitation, the following: (a) treatment, reduction, and/or prevention of fine lines or wrinkles, (b) improvement in skin thickness, plumpness, and/or tautness; (c) improvement in skin suppleness and/or softness; (d) improvement in skin tone, radiance, and/or clarity; (e) improvement in procollagen and/or collagen production; (f) improvement in maintenance and remodeling of elastin; (g) improvement in skin texture and/or promotion of retexturization; (h) improvement in

skin barrier repair and/or function; (i) improvement in appearance of skin contours; (j) restoration of skin luster and/or brightness; (k) replenishment of essential nutrients and/or constituents in the skin; (l) improvement of skin appearance decreased by aging; (m) improvement in skin moisturization and/or hydration; (n) increase in and/or preventing loss of skin elasticity and/or resiliency; (o) treatment, reduction, and/or prevention of skin sagging; and/or (p) treatment, reduction, and/or prevention of discoloration of skin.

[0015] In practice, the compositions of the invention are applied to skin in need of treatment. That is, the composition is applied to skin which suffers from a deficiency or loss in any of the foregoing attributes or which would otherwise benefit from improvement in any of the foregoing skin attributes. As such, in certain preferred embodiments the compositions and methods of the invention are directed to the prevention, treatment, and/or amelioration of fine lines and/or wrinkles in the skin. In one exemplary preferred case, the compositions are applied to skin in need of treatment, by which is meant skin having wrinkles and/or fine lines. Preferably, the compositions are applied directly to the fine lines and/or wrinkles (which may be the entire face and/or neck area, or a portion thereof). The compositions and methods are suitable for treating fine lines and/or wrinkles on any surface of the skin, including without limitation, the skin of the face, neck, and/or hands.

[0016] The compositions of the present invention may be applied as needed to the skin. The composition can be applied periodically, e.g., daily, twice daily, weekly, or several times a week. The composition is generally applied for a duration of one week to indefinitely, such often will be applied for a period of 1, 2, 3, 4, 5, 6 or more months. The duration of application can also be applied for an indefinite time period, if desired. It will be appreciated that the results discussed herein will depend upon the amount frequency, and duration of application, with

highest amounts and more frequent applications providing accordingly faster results. The skin-care compositions daily preferably for at least four weeks, and more preferably at least eight weeks, by which an effect upon the appearance of skin should be observed. Application may be continued as long as desired to maintain the condition of the skin.

[0017] In addition, it is also contemplated that the compositions of the present invention may be applied to normal healthy skin, and may improve the brilliance, smoothness, radiance, and/or elasticity of the normal skin. Thus, in another aspect, the compositions are applied to the skin of the face, neck, and or hands of a patient having normal skin. It is anticipated that the surface characteristics of the unwrinkled, unsagging skin may be improved.

[0018] The compositions according to the invention can be formulated in a variety of forms for topical application. Typically, the compositions will comprise from about 0.0001 wt% to about 90 wt% of umbilical cord blood serum, and preferably will comprise from about 0.001 wt% to about 25 wt% , more preferably from about 0.01 wt% to about 10 wt%, and still more preferably about 0.05 wt% to about 5 wt% of umbilical cord blood serum. Within the more preferred range, the composition may comprise umbilical cord blood serum within a 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, or 1.0 wt% of the total composition. As an example, the compositions will comprise an effective amount of umbilical cord blood serum, by which is meant an amount sufficient improve procollagen and/or glycosaminoglycans, for example as discussed in the Examples. An "effective amount" in the context of umbilical cord blood serum includes one that provides a particular anti-aging benefit to the skin and refers to the amount required to provide a clinically measurable improvement in the particular manifestation of skin aging when applied for a time sufficient to provide a clinically measurable improvement in the particular manifestation of skin aging.

[0019] The composition of the present invention may be formulated in a variety of product forms, such as, for example, a lotion, cream, serum, spray, aerosol, cake, ointment, essence, emulsion, gel, paste, patch, pencil, towelette, mask, stick, foam, concentrate, and the like, particularly for topical administration. Preferably the composition is formulated as a lotion, cream, ointment, serum, or gel.

[0020] The compositions of the present invention may include a cosmetically acceptable vehicle. Such vehicles may take the form of any known in the art suitable for application to skin and may include water (e.g., deionized water); vegetable oils; mineral oils; esters such as octal palmitate, isopropyl myristate and isopropyl palmitate; ethers such as dicapryl ether and dimethyl isosorbide; alcohols such as ethanol and isopropanol; fatty alcohols such as cetyl alcohol, cetearyl alcohol, stearyl alcohol and biphenyl alcohol; isoparaffins such as isooctane, isododecane and is hexadecane; silicone oils such as cyclomethicone, dimethicone, dimethicone cross-polymer, polysiloxanes and their derivatives, preferably organomodified derivatives; hydrocarbon oils such as mineral oil, petrolatum, isoeicosane and polyisobutene; polyols such as propylene glycol, glycerin, butylene glycol, pentylene glycol and hexylene glycol; waxes such as beeswax and botanical waxes; or any combinations or mixtures of the foregoing.

[0021] The composition may optionally comprise other cosmetic actives and excipients, obvious to those skilled in the art including, but not limited to, fillers, emulsifying agents, antioxidants, surfactants, film formers, chelating agents, gelling agents, thickeners, emollients, humectants, moisturizers, vitamins, minerals, viscosity and/or rheology modifiers, sunscreens, keratolytics, depigmenting agents, retinoids, hormonal compounds, alpha-hydroxy acids, alpha-keto acids, anti-mycobacterial agents, antifungal agents, antimicrobials, antivirals, analgesics, lipidic compounds, anti-allergenic agents, H1 or H2 antihistamines, anti-inflammatory agents,

anti-irritants, antineoplastics, immune system boosting agents, immune system suppressing agents, anti-acne agents, anesthetics, antiseptics, insect repellents, skin cooling compounds, skin protectants, skin penetration enhancers, exfollients, lubricants, fragrances, colorants, depigmenting agents, hypopigmenting agents, preservatives, stabilizers, pharmaceutical agents, photostabilizing agents, neutralizers, and mixtures thereof. In addition to the foregoing, the cosmetic compositions of the invention may contain any other compound for the treatment of skin disorders.

[0022] As used herein, "topical application" means directly laying on or spreading on outer skin.

[0023] As used herein, "cosmetically acceptable" means that drugs, medicaments, botanicals, chemicals, or inert ingredients which the term describes are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, incompatibility, instability, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio. Cosmetically acceptable vehicles must be of sufficiently high purity and sufficiently low toxicity to render them suitable for administration to the human or lower animal being treated.

[0024] As used herein, "comprising" means that other steps and other ingredients which do not affect the end result can be added. This term encompasses the terms "consisting of" and "consisting essentially of."

[0025] The term "sagging" as used herein means the laxity, slackness, or the like condition of skin that occurs as a result of loss of, damage to, alterations to, and/or abnormalities in dermal elastin and includes the age-related loss of adhesive plaque at the dermal-epidermal junction.

[0026] The terms "smoothing" and "softening" as used herein mean altering the surface of the keratinous tissue such that its tactile feel is improved. "Signs of skin aging" include, but are not limited to, all outwardly visible or tactilely perceptible manifestations as well as any other macro or micro effects due to skin aging. Such signs may be induced or caused by intrinsic factors or extrinsic factors, e.g., chronological aging and/or environmental damage. These signs may result from processes that include, but are not limited to, the development of textural discontinuities such as wrinkles and coarse deep wrinkles, skin lines, crevices, bumps, large pores (e.g., associated with adnexal structures such as sweat gland ducts, sebaceous glands, or hair follicles), or unevenness or roughness, loss of skin elasticity (loss and/or inactivation of functional skin elastin), sagging (including puffiness in the eye area and jowls), loss of skin firmness, loss of skin tightness, loss of skin recoil from deformation, discoloration (including under eye circles), blotching, sallowness, hyperpigmented skin regions such as age spots and freckles, keratoses, abnormal differentiation, hyperkeratinization, elastosis, collagen breakdown, and other histological changes in the stratum corneum, dermis, epidermis, the skin vascular system (e.g., telangiectasia or spider vessels), and underlying tissues, especially those proximate to the skin.

[0027] The term "umbilical cord blood" or "cord blood" generally refers to blood obtained from a neonate or fetus, most preferably a neonate and preferably refers to blood which is obtained from the umbilical cord or placenta of newborns. The use of cord or placental blood is advantageous because it can be obtained relatively easily and without trauma to the donor. Cord blood is preferably obtained by direct drainage from the umbilical vein of a discarded placenta.

[0028] The term "umbilical cord blood serum" or "cord serum" generally refers to umbilical cord blood in which the cells have been removed so that the cord serum is substantially free of whole cells.

[0029] The term "wrinkle" or "wrinkling" includes both fine wrinkling and coarse wrinkling. Fine wrinkling or fine lines refers to superficial lines and wrinkles on the skin surface. Coarse wrinkling refers to deep furrows, particularly deep lines/wrinkles on the face and around the eyes, including of expression lines such as frown lines and wrinkles, forehead lines and wrinkles, crow's feet lines and wrinkles, nasolabial fold and marionette lines and wrinkles. Forehead lines and wrinkles refer to superficial lines and/or deep furrows on skin of the forehead. Crow's feet lines and wrinkles refer to superficial lines and/or deep furrows on skin around the eye area. Marionette lines and wrinkles refer to superficial lines and/or deep furrows on skin around the mouth. Wrinkles can be assessed for number, length, and depth of the lines.

[0030] Umbilical cord blood serum

[0031] The umbilical cord blood serum used in the compositions of the present invention is typically prepared in the following manner. First, umbilical cord blood is collected at the time to birth from pre-screened mothers for infectious disease causing organisms, such as HIV 1 and 2, Hbs and HCV and sexually transmitted diseases. The collection is made after the baby is separated from the clamped cord, and therefore there is no harm to the baby. Blood is collected from an umbilical vein using the conventional blood bag containing no anticoagulants. The needle of the bag is inserted into the vein and blood is allowed to flow into the blood bag. A good collection can average 40 ml and may exceed 100 ml. This blood is now allowed to clot at room temperature and transported to the processing area, which is a cGMP clean room. The

clotting process is allowed to take place from 8-16 hours. The blood is then centrifuged at 1000 g in a blood bag centrifuge and the clear serum is collected into sterile containers. The cord serum is tested for sterility by microbiological assays for aerobic or anaerobic microorganisms. The complement is inactivated by keeping the cord serum at about 56 °C for 1/2 hour. The serum is then aliquoted into 50 ml sterile vials and capped and frozen at about -70 °C for future use in the compositions of the present invention. Suitable cord serum is commercially available from Cryobanks Laboratories (Allamonte Springs, FL).

[0032] Other Optional Components

[0033] The cosmetic compositions of the present invention preferably include one or more bioactive peptides, including but not limited to, dipeptides, tripeptides, tetrapeptides, pentapeptides, and hexapeptides, and derivatives thereof. The peptides are provided in the compositions of the present invention in amounts that are safe and effective. As used herein, "peptides" refers to both the naturally occurring peptides and synthesized peptides. Also useful herein are naturally occurring and commercially available compositions that contain peptides. The peptides used in the present invention may include neuropeptides as well as so-called charge-coupled peptides.

[0034] Suitable dipeptides for use herein include carnosine (beta-ala-his) and tyr-arg. Suitable tripeptides for use herein include gly-his-lys, arg-lys-arg, and his-gly-gly. Preferred tripeptides and derivatives thereof include palmitoyl oligopeptide (palmitoyl-gly-his-lys); Peptide CK (arg-lys-arg); Peptide CK+ (ac-arg-lys-arg-NH_2); and a copper derivative of his-gly-gly sold commercially as lamin, from Sigma (St. Louis, Mo.). Suitable tetrapeptides for use herein include Peptide E, arg-ser-arg-lys (SEQ ID NO: 1); palmitoyl tetrapeptide-3/7 (palmitoyl-gly-gln-pro-arg (SEQ ID NO: 2)). Suitable pentapeptides for use herein include lys-thr-thr-lys-

ser (SEQ ID NO: 3), palmitoyl-lys-thr-thr-lys-ser (SEQ ID NO: 4). Suitable hexapeptides include acetyl hexapeptide-3 (Ac-Glu-Glu-Met-Gln-Arg-Arg (SEQ ID NO: 5)). When included in the present compositions, peptides are typically present in amounts of from about 0.01 wt% to about 10 wt%, or from about 0.1 wt% to about 6.0 wt%, or from about 1.0 to about 0.5%, by weight of the composition.

[0035] In one aspect, the cosmetic compositions of the present invention include one or more dipeptides. The preferred dipeptide comprises tyr-arg. In a preferred aspect, the tyrosine-arginine dipeptide is acetylated to make it more lipophilic, more stable, and bio-available on a cutaneous level. Acetyl tyrosine-arginine-1 cetyl ester stimulates the synthesis of the messenger neuropeptides of muscular relaxation and inhibits the synthesis of the messenger mediators of muscular contraction. The compositions of the invention preferably comprise about 0.001 to 5.0 wt% of the dipeptide, especially tyr-arg. In a preferred aspect, the compositions comprise about 2.0 to about 3.0 wt% of the dipeptide.

[0036] In one aspect, the cosmetic composition of the present invention includes one or more tripeptides and tetrapeptides. In a preferred embodiment, the cosmetic composition includes the commercially available product known as MATRIXL 3000® (Sederma Corp., France), which includes both palmitoyl oligopeptide and palmitoyl tetrapeptide-7. The compositions of the invention preferably comprise about palmitoyl oligopeptide and palmitoyl tetrapeptide-7 in a combined amount of about 0.001 to about 10 wt%. In a preferred aspect, the compositions comprise about 3.0 to about 5.0 wt% palmitoyl oligopeptide and palmitoyl tetrapeptide-7.

[0037] In one aspect, the cosmetic compositions of the present invention include one or more hexapeptides. The preferred hexapeptide used in the compositions of the invention is

acetyl hexapeptide-3. The peptide may be purchased from Lipotec under the tradename Argireline® in either the powder or solution form. The powder form appears as a white to off-white powder. The compositions of the invention preferably comprise about 0.001 to about 5.0 wt% acetyl hexapeptide-3. In a preferred aspect, the compositions comprise about 0.5 to about 1.5 wt% acetyl hexapeptide-3.

[0038] In one aspect, the cosmetic compositions of the present invention include one or more biological additives, such as botanicals or herbals. As used herein, the term "biological additive" indicates any compound obtained from a natural source, including plants, animals, bacteria and yeast, which has a medicinal or otherwise beneficial effect when topically applied to the skin. Examples of biological additives include oil of *Melaleuca alternifolia*, oil of *Lavandula angustifolia*, *Carica papaya* extract, *Echinacea angustifolia* extract, Mimosa tenuiflora extract, *Hydrocotyl (centella) asiatica* extract, *gingko biloba* extract, oil of *Melaleuca alternifolia* (tea tree oil), *Matricaria chamomila* (chamomile) extract, *Hypericum perforatum* extract, *Aloe barbedensis* extract, and the like. The biological sources for "biological additive" may also include, but are not limited to the following: Aloe Vera, *Aloe Barbedensis*; Arnica, *Arnica Montana*; Bladderwrack (seaweed), *Fucus Vesciculosis*; Seaweed, *Undaria pinnatifida;* Birch, *Betula Alba (Pendula)*; Chamomile, *Matricaria Chamomila (Chamomila Recutita*); Marsh Mallow, *Althea Officinalis*; Meadow Sweet, Spirea Ulmaria (*Filipendula*); Mint/Lemon Balm, *Melissa Officinalis*; Mimosa, *Mimosa Tenuiflora*; Myrrh Tincture, *Commiphor Myrrha*; Neem, Melia Azadirachta; Nettle (stinging), *Urtica Dioica*; Papaya, *Carica Papaya*; Propolis (bee glue), *Propolis Cera*; Raspberry, *Rubis Idaeus*; Red Poppy, *Papaver Rhoeas*; Rose Hip (dog rose), *Rosa Carima*; Rosemary, *Rosemarinus Officinalis*; Sage, *Salvia Officinalis*; St. Johns Wort, *Hypericum Perforatum*; Strawberry, *Fragaria Vesca*; Thea Sinensis (green tea), *Camelia*

Sinensis; Walnut, *Juglans Regia*; Witchhazel (dist/extr), *Hamamelis Virginiana*; Yarrow, *Achillea Millefolium*; Wild Yam, *Dioscorea Villosa*; Hawthorn, *Crataegus Monogina/Oxyantha*; Herma (black/rod), *Lawsoma Ehemus*; Hops, *Humulus Lupulus*; Horse Chestnut, *Aesculus Hippocastanum*; Horse Tail, *Equisitum Arvense*; Ivy, *Hedera Helix*; Linden/Lime Tree Blossoms, *Tilia Argentea Cordata*; Madder, *Rubia Tinctorum*; Marigold, *Calendula Officinalis*; Centella Asiatica, *Centella Asiatica Urban (hydrocotyl Asiatica)*; Carrot (roots), *Daucus Carota*; Comfrey (Allantoine), *Symphytum Officinale*; Coneflower (Echinacea), *Echinacea Angustifolia*; Cucumber, *Cucumis Sativus (Frucus Cucumis)*; Fenugreek, *Trigonella Foenum Greacum*; Gingko, *Gingko Biloba*; Ginseng, *Panax Ginseng*; Great Burdock, *Radix Bardanea/Arctium Lappa*; Tea Tree Oil, Oil of *Melaleuca Alternifolia*; Colts Foot, *Tussilago Farfara*; Clover, Trifolium *Pratense*; Speedwell, *Veronica Officinalis;* Medlar, *Pyrus Germanica*.

[0039] In another aspect, the biological additive may be those selected from the group consisting of plants such as *Angelica keiskei*, adzuki bean, avocado, hydrangea, *Gynostemma pentaphyllum*, Aruteka, arnica, almond, aloe, apricot, nettle, iris, fennel, turmeric, Eijitsu, Scutellariae radix, Amur cork tree, goldthread, barley, gumbo, Saint-John's-wort, dead nettle, ononisu, watercress, persimmon, the root of kudzu, *Valeriana fauriei*, birch, cattail, chamomile, chamomilla, oats, licorice, raspberry, kiwi, cucumber, apricot, coconut, cape jasmine, *Sasa albo-marginata*, a walnut, cinnamon, mulberry, gunjo, gentian, cranesbill, burdock, sesame, wheat, rice, *Camellia sasangua*, saffron, hawthorn, Japanese pepper tree, mushroom, *Rehmannia clutinosa*, prop root, beefsteak plant, Japanese linden, *Filipendula multijuga*, peony, ginger, calamus, white birch, Japanese honeysuckle, field horsetail, *Stevia rebaudiana Bertoni*, western ivy, western hawthorn, elder, needle juniper, milfoil, mint, sage, common mallow, *Cnidium officinale*, Japanese green gentian, soybean, daiso, thyme, tea plant, clove, dried orange peel,

evening primrose, camellia, *Centella asiatica*, English walnut, *Angelica acutiloba*, pot marigold, ginseng, orange peel, corn, *Houttuynia cordata*, tomato, carrot, garlic, wild rose, malt, parsley, rye, adlay, Japanese mint, papaya, hamamelis, rose, white cedar, sunflower, loquat, butterbur, dandelion, grape, placenta, hazelnut, dishcloth gourd, safflower, bo tree, peony, hop, macadamia nut, pine, horse chestnut, melissa, melilot, peach, malt, Rodger's bronze leaf, palm, eucalyptus, creeping saxifrage, lily, Yokuninin, mugwort, rye, peanut, lavender, apple, litchi, lettuce, lemon, Chinese milk vetch, rosemary, camomile, agrimony, Japanese catalpa, hiba arborvitae, Horutso, *Isodon japonicus Hara*, jijitsu, senkishi, chickweed, duckweed, mugwort, ginkgo, Chinese bellflower, chrysanthemum, soapberry and weeping golden bell.

[0040] In one exemplary aspect, the biological additive comprises PHYTOTAL FM (Croda Singapore) in an amount of about 0.5 to about 6.0 wt% of the composition, preferably about 3.5 to about 5.5 wt% of the composition. PHYTOTAL FM comprises glycerin, butylene glycol, *juglans regia* leaf extract, *Juglans regia* shell extract, *Centella asiatica* extract, *Pyrus germanica* extract, lecithin. In another exemplary aspect, the cosmetic compositions of the present invention comprise about 0.1 to about 6.0 wt%, preferably about 2.0 to about 4.0 wt % seaweed, *Undaria pinnatifida.* In still another exemplary aspect, the cosmetic compositions of the present invention comprise *Stevia rebaudiana Bertoni* in an amount of about 0.1 to about 6.0 wt%, with about 2.0 to about 4.0 wt% being most preferred.

[0041] In general, these extracts can be obtained by grinding the whole of the respective plants or one or more of their parts (hereinafter referred to as "stocks" such as leaves, bark, roots, branches, seeds or fruits or nuts, and flowers or blossoms after drying them or without drying them, and then extracting them either with a solvent or by means of an extractor such as a Soxhlet's extractor at ordinary temperature or an elevated temperature. No particular limitation

is imposed on the solvent used here. However, examples thereof include known solvents, such as water, primary alcohols such as methyl alcohol and ethyl alcohol, liquid polyhydric alcohols such as propylene glycol and 1,3-butylene glycol, lower alkyl esters such as ethyl acetate, hydrocarbons such as benzene and hexane, ethyl ether, and acetone. These solvents may be used either singly or in any combination thereof.

[0042] The cosmetic compositions also preferably includes one or more lipids, preferably one or more phosphospholipids. Examples of three classes of phospholipids are phosphoglycerides, lysophosphoglycerides, and and sphingomyelins. Examples of phosphoglycerides include phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, diphosphatidyl glycerol, and mixtures thereof. The most preferred phosphoglycerides include phosphatidyl choline and lecithin, particularly soybean lecithin, which comprises a mixture of some of the above examples of specific phosphoglycerides. Examples of lysophosphoglycerides includes: lysophosphatidyl choline, lysophosphatidyl ethanolamine, lysophosphatidyl serine, lysophosphatidyl inositol, and mixtures thereof. The lipids (e.g. phosphadityl choline) preferably comprise about 0.1 to about 6.0 wt% of the cosmetic composition, with about 0.5 to about 1.2 wt% being most preferred.

[0043] The cosmetic compositions of the present invention may also comprise one or more preservatives. Suitable traditional preservatives for compositions of this invention are alkyl esters of para-hydroxybenzoic acid. Other preservatives include hydantoin derivatives such as 1,3-bis(hydroxymethyl)-5,5-dimethylhydantoin, propionate salts, and a variety of quaternary ammonium compounds such as benzalkonium chloride, quaternium 15, benzethonium chloride, and methylbenzethonium chloride. Cosmetic chemists are familiar with appropriate preservatives and routinely choose them to satisfy the preservative challenge test and to provide

product stability. Particularly preferred preservatives are disodium EDTA, phenoxyethanol, methyl paraben, propyl paraben, imidazolidinyl urea, sodium dehydroacetate and benzyl alcohol. The preservatives should be selected having regard for the use of the composition and possible incompatibilities between the preservatives and other ingredients in the emulsion. The preservatives preferably are employed in amounts ranging from about 0.001 wt% to about 5 wt%, more preferably from about 0.01 wt% to about 2.5 wt%, and most preferably from about 0.01 wt% to about 1 wt%, by weight of the composition.

[0044] The cosmetic compositions of the present invention may also comprise one or more penetration enhancers. As used herein, a penetration enhancer is a material capable of aiding the penetration of the active agents into the skin. Examples of penetration enhancers include, but are not limited to, dimethyl isosorbide and diethyl-glycol-monoethylether. The penetration enhances typically comprise about 0.5 to about 5 wt% of the composition, preferably about 1.0 to 3.0 wt %.

[0045] In still another aspect, the compositions of the present invention may further comprise one or more neutralizing gents or pH adjusters, which may be used to adjust the pH of the compositions. The term "neutralizing agent," as used herein, refers to a material that may be used to modify the pH of the present compositions, for example, from an acidic pH to a more basic pH, or from a basic pH to a more acidic pH. Components of the present compositions, such as certain of the thickening agents, may be acidic, and may be preferably neutralized to achieve the desired thickening effect. Accordingly, the neutralizing agents are preferably those materials which may be used to modify the pH of the present compositions from an acidic pH to a more basic pH.

[0046] A wide variety of neutralizing agents are known to those skilled in the art and may be used in the practice of the present invention. Exemplary neutralizing agents include, for example, ammonium hydroxide, arginine, 2-amino-2-methyl-1-propanol (AMP-95 (Angus)), dimethanolamine, dibutanolamine, diisobutanolamine, tributanolamine, triisobutanolamine, tri-sec-butanolamine, tripropylamine, ethanolamine, diethanolamine, triethanolamine, PEG-15 cocamine, diisopropanolamine, methylethanolamine, diisopropylamine, dipropylenetriamine, tromethamine, isopropylamine ethylene diamine, triisopropanolamine, tetrahydroxypropyl ethylenediamine, trimethamine, 2-aminobutanol, aminoethyl propanediol, aminomethyl propanediol, aminomethyl propanol, sodium hydroxide, potassium hydroxide and mixtures thereof. Most preferably, the preferably, the neutralizing agent triethanolamine. The amount of neutralizing agent in the cosmetic composition is preferably about 1.0 to about 6.0 wt% with about 0.75 to about 1.5 wt% being most preferred. .

[0047] In one aspect, the cosmetic compositions of the present invention include one or more hyaluronans. Preferably, the hyaluronan is present in the form of hyaluronic acid or salt thereof or a homologue, analogue, derivative, complex, ester, fragment and subunit of hyaluronic acid. More preferably, the hyaluronic acid in the form of sodium hyaluronate and is is of medical grade and has an average molecular weight of about 700 kilodaltons. **[Al -- Please check the MW??].** The compositions of the invention preferably comprise about 0.01 to about 4.0 wt% hyaluronan (in the form of sodium hyaluronate). In a preferred aspect, the compositions comprise about 0.5 to about 1.5 wt% hyaluronan (in the form of sodium hyaluronate).

[0048] In one aspect, the cosmetic compositions of the present invention include one or more skin-conditioning emollients. The emollient functions as a softener to help the composition give a desirable feel on the skin. Useful emollients include, but are not limited to fatty bodies

liquid at ambient temperature, such as esters, mineral oils, animal oils, vegetable oil, synthetic oils, and silicone oils. Examples of useful esters include, but are not limited to, isononyl isononanoate, octyl palmitate, cetyl lactate, pentaerythrityl tetraoctanoate, tridecyl octanoate, tridecyl behenate, isopropyl jojobate and jojoba alcohols, butyloctyl salicylate, polyglyceryl-3 diisostearate, squalane, tridecyl trimellitate, tridecyl stearate, and neopentylglycol dicaprylate/dicaprate. Examples of useful oils include, but are not limited to, petrolatum oil, liquid lanolin, arara oil, sesame oil, macadamia oil, almond oil, jojoba oil, silicone oils such as phenyl trimethicone and dimethicone, and synthetic triglycerides such as capric/caprylic triglyceride and hydrogenated cocoglycerides. The emollient(s) can be present in the present invention in an amount about 0.1 wt% to about 6 wt%. In an exemplary aspect, the cosmetic composition comprises about 0.1 to about 6 wt%, and more preferably about 0.75 to 1.5 capric/caprylic triglycerides. In another aspect, the cosmetic composition comprises about 0.1 to 6 wt%, and more preferably about 0.3 wt% to about 0.7 wt% almond oil. In an another aspect, the cosmetic composition comprises about 0.1 to about 6 wt%, and more preferably about 0.75 wt% to about 1.5 wt% jojoba oil.

[0049] In still another aspect, the cosmetic compositions may contain one or more surfactants. Exemplary surfactants are disclosed in Harrison et al. U.S. Patent No. 6,642,194, which is incorporated by reference. A preferred surfactant is myristamidopropyl PG-dimonium chloride phosphate and it has cationic properties and is also a preservative booster. Another preferred surfactant are the betaines, preferably alkylamidoalkylbetaines, such cocoamidopropylbetaine.

[0050] Other agents that may form part of the cosmetically acceptable vehicle of the cosmetic formulation include carbomers, propylene glycol, butylene glycol, dipropylene glycol,

glycerin, glycereth-18 ethylhexanoate, glycereth-18, betaine, diglycerin, glycol, inositol, meadowfoamamidopropyl betaine, ethyl alcohol, isopropyl alcohol, polyethylene glycol with varied molecular weights, sorbitol, xylitol, urea, tripropylene glycol, sodium PCA, glycereth-7 glycolate, diglycereth-7 malate, 2,3-butanediol, propanediol, xylose, almond oil PEG-6 esters, apricot kernel oil PEG-6 esters, argan oil PEG-8 esters, argan oil polyglyceryl-6 esters. Other vehicle agents include PEG-3 dimethicone, PEG/PPG-20/23 dimethicone;, PEG-8 dimethicone, cyclomethicone, dimethicone, cetyl dimethicone, caprylyl methicone, ethyl trisiloxane, trimethylsiloxyamodimethicone, stearyl dimethicone, silicones with polypropylene glycol functionality such as PPG-12 dimethicone, silicones with polyethylene glycol functionality such as PEG-8 trisiloxane, PEG-10 dimethicone and silicones which combine both functionalities in varying ratios such as PEG/PPG-5/3 trisiloxane, PEG/PPG-8/26 dimethicone, PEG/PPG-20/15 dimethicone, bis-PEG-4 dimethicone, bis-PEG-12 dimethicone, bis-PEG/PPG-14/14 dimethicone, bis-PEG/PPG-18/6 dimethicone, bis-PEG/PPG-20/20 dimethicone, butylene glycol behenate, butylene glycol diisononanoate, butylene glycol laurate, butylene glycol myristate, butylene glycol oleate, butylene glycol palmitate, butylene glycol stearate, butyl isostearate, butyl myristate, butyloctyl behenate, butyloctyl benzoate, butyloctyl cetearate, butyloctyl palmitate, butyl oleate, butyl stearate C14-15 alcohols, C18-28 alkyl acetate, C12-15 alkyl benzoate, C16-17 alkyl benzoate, C30-45 alkyl cetearyl dimethicone crosspolymer, C32 alkyl dimethicone, C30-45 alkyl dimethicone/polycyclohexene oxide crosspolymer, C12-13 alkyl ethylhexanoate, C12-15 alkyl ethylhexanoate, C14-18 alkyl ethylhexanoate, C12-13 alkyl lactate, C12-15 alkyl lactate, C20-24 alkyl methicone, C24-28 alkyl methicone, calodendrum capense nut oil, calophyllum tacamahaca seed oil, cetearyl dimethicone/vinyl dimethicone crosspolymer, cetearyl ethylhexanoate, cetearyl isononanoate, cetearyl nonanoate, cetearyl

palmitate, cetrimonium laureth-12 succinate, cetyl acetate, cetyl caprylate, cetyl C12-15 pareth-8 carboxylate, cetyl dimethicone, cetyl dimethicone/bis-vinyldimethicone crosspolymer, cetyl dimethyloctanoate, cetyl esters, cetyl ethylhexanoate, cetyl glyceryl ether, cetyl glycol, cetyl glycol isostearate, cetyl isononanoate, cetyl lactate, cetyl laurate, cetyl oleate, cetyloxy dimethicone, C12-15 pareth-3 benzoate, C 12-15 pareth-9 hydrogenated tallowate, C11-15 pareth-3 oleate, C 12-15 pareth-12 oleate, C 11-15 pareth-3 stearate, C11-15 pareth-12 stearate, dibutyl adipate, dibutyldecyl IPDI, dibutyloctyl IPDI, dibutyloctyl malate, dibutyloctyl sebacate, dibutyl sebacate, Ddi-C12-15 alkyl adipate, di-C12-15 alkyl fumarate, di-C12-13 alkyl malate, di-C12-15 alkyl maleate, di-C12-13 alkyl tartrate, -C14-15 alkyl tartrate, dicaprylyl carbonate, dicaprylyl ether, dicaprylyl maleate, dicetyl adipate, dicocoyl pentaerythrityl distearyl citrate, diethyl adipate, isobutyl myristate, isobutyl palmitate, isobutyl pelargonate, isobutyl stearate, isobutyl tallowate, isocetyl alcohol, isocetyl ethylhexanoate, isocetyl isodecanoate, isocetyl isostearate, isocetyl laurate, isocetyl linoleoyl stearate, isocetyl palmitate, isocetyl stearate, lanolin, lanolin oil, lanolin wax, lauryl lactate, neopentyl glycol diheptanoate, neopentyl glycol diisononanoate, neopentyl glycol dilaurate, octyldodecyl ethylhexanoate, octyldodecyl lactate, octyldodecyl neodecanoate, octyldodecyl neopentanoate, PPG-3 benzyl ether myristate, PPG-1 -ceteth-1, PPG- 1 -ceteth-5, PPG-1-ceteth-10, PPG-1-ceteth-20, sunflower oil, safflower oil, mineral oil, almond oil, and jojoba oil diisoamyl malate, diethylhexyl malate, dibutyloctyl malate, dimethyl capramide, diethylhexyl 2,6 napthalate, N,N-dimethyldesamide, diisopropyl adipate, phenethyl benzoate, octocrylene, PEG-7 methyl ester, and combinations thereof In one aspect, PPG-3 benzyl ether myristate is used as a spreading agent. In another aspect, a carbomer is used as a gelling agent or rheology modifier.

[0051] The invention provides a method for treating aging skin by topically applying a cosmetic composition comprising umbilical cord blood serum, preferably in a cosmetically acceptable vehicle, over the affected area for a period of time sufficient to reduce, ameliorate, reverse, or prevent dermatological signs of aging. This method is particularly useful for treating signs of skin photoaging and intrinsic aging.

[0052] Generally, the improvement in the condition and/or aesthetic appearance involves the regulation of wrinkles and/or surface enhancement, such as radiance and glow. In one aspect, improvement in the condition and/or aesthetic appearance involves is selected from the group consisting of: reducing dermatological signs of chronological aging, photo-aging, hormonal aging, and/or actinic aging; preventing and/or reducing the appearance of lines and/or wrinkles; reducing the noticeability of facial lines and wrinkles, facial wrinkles on the cheeks, forehead, perpendicular wrinkles between the eyes, horizontal wrinkles above the eyes, and around the mouth, marionette lines, and particularly deep wrinkles or creases; preventing, reducing, and/or diminishing the appearance and/or depth of lines and/or wrinkles; improving the appearance of suborbital lines and/or periorbital lines; reducing the appearance of crow's feet; rejuvenating and/or revitalizing skin, particularly aging skin; reducing skin fragility; preventing and/or reversing of loss of glycosaminoglycans and/or collagen; ameliorating the effects of estrogen imbalance; preventing skin atrophy; preventing, reducing, and/or treating hyperpigmentation; minimizing skin discoloration; improving skin tone, radiance, clarity and/or tautness; preventing, reducing, and/or ameliorating skin sagging; improving skin firmness, plumpness, suppleness and/or softness; improving procollagen and/or collagen production; improving skin texture and/or promoting retexturization; improving skin barrier repair and/or function; improving the appearance of skin contours; restoring skin luster and/or brightness;

minimizing dermatological signs of fatigue and/or stress; resisting environmental stress; replenishing ingredients in the skin decreased by aging and/or menopause; improving communication among skin cells; increasing cell proliferation and/or multiplication; increasing skin cell metabolism decreased by aging and/or menopause; retarding cellular aging; improving skin moisturization; enhancing skin thickness; increasing skin elasticity and/or resiliency; enhancing exfoliation; improving microcirculation; decreasing and/or preventing cellulite formation; and any combinations thereof.

[0053] The composition will typically be applied to the skin one, two, or three times daily for as long as is necessary to achieve desired anti-aging results. The treatment regiment may comprise daily application for at least one week, at least two weeks, at least four weeks, at least eight weeks, or at least twelve weeks. Chronic treatment regimens are also contemplated.

[0054] A composition comprising umbilical cord blood serum is topically applied to an "individual in need thereof," by which is meant an individual that stands to benefits from reducing visible signs of skin damage or aging. In a specific embodiment, the umbilical cord blood serum is provided in a pharmaceutically, physiologically, cosmetically, and dermatologically-acceptable vehicle, diluent, or carrier, where the composition is topically applied to an affected area of skin and left to remain on the affected area in an amount effective for improving the condition and aesthetic appearance of skin.

[0055] In one embodiment, methods for treating fine lines and wrinkles comprise topically applying the inventive compositions comprising umbilical cord blood serum to the skin of an individual in need thereof, e.g., topically application directly to the fine line and/or wrinkle in an amount and for a time sufficient to reduce the severity of the fine lines and/or wrinkles or to prevent or inhibit the formation of new fine lines and/or wrinkles. The effect of a composition

on the formation or appearance of fine lines and wrinkles can be evaluated qualitatively, e.g., by visual inspection, or quantitatively, e.g., by microscopic or computer assisted measurements of wrinkle morphology (e.g., the number, depth, length, area, volume and/or width of wrinkles per unit area of skin). This embodiment includes treatment of wrinkles on the skin of the hands, arms, legs, neck, chest, and face, including the forehead.

[0056] It is also contemplated that the compositions of the invention will be useful for treating thin skin by topically applying the composition to thin skin of an individual in need thereof. "Thin skin" is intended to include skin that is thinned due to chronological aging, menopause, or photo-damage. In some embodiments, the treatment is for thin skin in men, whereas other embodiments treat thin skin in women, pre-menopausal or post-menopausal, as it is believed that skin thins differently with age in men and women, and in particular in women at different stages of life.

[0057] The method of the invention may be employed prophylactically to forestall aging including in patients that have not manifested signs of skin aging, most commonly in individuals under 25 years of age. The method may also reverse or treat signs of aging once manifested as is common in patients over 25 years of age.

[0058] The following examples are directed to various exemplary embodiments of the skin care compositions and their use in accordance with the present invention.

Example 1: Cosmetic composition

[0059] In this example, an exemplary cosmetic composition was prepared in accordance with the present invention. The cosmetic composition is ideally suited to be used as an anti-aging cream. Table 1 provides a list of the various ingredients.

Table 1: Formulation Containing Cord Serum

Formulation Components	**Range %**	**Phase**
Acetyl Hexapeptide-3 (Argireline)	0.5-1.5	A
Dipeptide Tyrosil Arginine (Calmomensine, Hydroxyethylcellulose, Laureth-3 Acetyl Dipeptide, Cetyl Ester)	2.0-3.0	A
Human Umbilical Cord Serum	0.1-0.35	A
Juglans Regia leaves & shell extract, Centella leaves, Pyrus Germanica leaves, Lecithin (PHYTOTAL FM)	3.5-5.5	A
Palmitoyl Oligopeptide and Palmitoyl Tetrapeptide-7 (MATRIXL 3000)	3.0-5.0	A
Phosphatidyl choline	0.5-1.2	A
Stevia Rebaudiana Bertoni (Phytessence Stevia)	1.0-2.5	A
Undaria Pinnatifidia (Phytessence Wakami)	2.0-4.0	A
Deionized water qs	35-45ml	B
Dimethyl Isosorbide (Arlasolve DMI-PC)	1.0-3.0	B
Methyl Paraben	0.01-0.085	B
Propyl Paraben	0.01-0.085	B
Triathanolamine	0.75-1.5	B
Hyaluronic Acid (Sodium hyaluronate)	0.5-1.5	C
Myristamidopropyl PG-Dimonium Chloride Phosphate (Arlasilk Phospholipis PTM)	0.3-0.6	C
PPG-3 Benzyl Ether Myristate (Crodamol STS)	2.0-3.8	C
Almond Oil (Cropure Almond)	0.3-0.7	D
Caprylic/Capri triglycerides (Crodamol GTCC)	0.75-1.5	D
Jojoba Oil	0.75-1.5	D
Carbomer 924 (Optasense G-34)	0.75-1.5	D

[0060] The components of Part A were mixed together and heated to about 70 to 80 °C. The mixture was allowed to cool to about 50 °C. The Part A mixture was then homogenized at increasing rates. In this example, the mixtures was homogenized for about 60 seconds at about 11,000 RPM, about 60 seconds at about 13,000 RPM, about 60 seconds at about 19,00 RPM, about 60 seconds at about 22,000 RPM, and about 60 seconds at about 24,000 RPM.

[0061] Separately, the components of Part B were mixed together and heated to about 70 to 80 °C. After cooling to about 50 °C, the mixture from part A was added to Part B with continuous mixing. The Part A/B mixture was then allowed to cool to about 40 C.

[0062] Separately, the components of Part C were mixed together with sufficient deionized water to solubilize the components of Part C.

[0063] Lastly, the Part A/B mixture, Part C mixture, and Part D components were all mixed together at about 75 to 80 C. The mixing was continued to provide a uniform texture as the product cooled. In the laboratory, an IKA Turrax homogenizer T-25 Basic S1 equipped with an IKA S25N-10G dispersing tool was used. The dispersing tool is immersed in the completed formula, and the homogenizer is operated for about 5 to 8 minutes at about 24,000 rpm. The intense shearing reduces particles size to about 50 to 1000 nanometers.

Example 2: Histogeometric Analysis

[0064] This example was designed to test the effects that the topical cream containing cord serum of Example 1 had on human skin. As a control, the topical cream minus the cord serum was also evaluated and both products were compared with an untreated site.

[0065] Methods

[0066] Six healthy volunteers were enrolled in the study. Product A was applied to the right aspect of the upper inner arm (R site), product B was applied to the left aspect of the upper inner arm (L site) and the volar forearm was chosen as the untreated site. Both products were applied daily for 26 days. The volar forearm received no product but was rubbed gently, daily, in an effort to simulate the manner in which both products were applied to the skin.

[0067] At the end of the treatment period, a 2-3 mm punch biopsy specimen was obtained from each treated site and the untreated site. Each specimen was immediately placed in 10% buffered formalin and processed for paraffin sectioning. All sections were five-micrometers in thickness and all slides were stained simultaneously as a group for each histochemical determination. The entire biopsy specimen was photographed with a high

resolution digital camera system (Axiocam, Zeiss Corporation) mounted on a Zeiss Axioplan 2 light microscope at a magnification of 20x. All slides were photographed under the identical white balance light settings and exposure time to insure consistency in micrographs. Micrographs were subsequently analyzed using computer assisted image analysis software (Axiovision, Zeiss Corporation). All measurements were made from at least four areas of the biopsy specimen, except for two biopsies, which had smaller amounts of tissues and thus three measurements were made.

[0068] Five-micrometer paraffin sections were stained with hematoxylin-eosin ("H&E") for overall morphologic evaluation and viable epidermal thickness determinations (VET; Table 2). For estimation of viable epidermal thickness, care was taken to cut the sections perpendicular to the surface. The VET includes the area from the dermoepidermal interface to the lowermost portion of the stratum corneum. For estimation of elastic fibers, van Geison's stain was employed, which stains elastic fibers blue-black to black, collagen pale red, other tissue elements yellow, and nuclei blue to black. For pro-collagen, anti-type I collagen (EMD Bioscience Inc.) antibody was used. This antibody to type I collagen was made against the triple helical portion and it is able to stain procollagen I. Immunohistochemical analysis of the paraffin sections was carried out using the DAB kit, which produces a brown reaction product. For glycosaminoglycans ("GAGs"), Hale's colloidal iron was used since Hale's stainable material (blue) represents, for the most part, GAGs and is commonly used as an indicator of changes in ground substance.

[0069] The quantification of stainable material was determined using a custom designed software program, integrated into the Axiovision image analysis system (Zeiss Corporation). The analysis is conducted in the following manner: (i) the reaction product (i.e., blue-black –

elastin; brown – pro-collagen; blue – GAGs) is detected from a histogram and only objects with that color are outlined on the micrograph. The total area occupied by the outlined areas is measured; (ii) the entire area of the dermis is outlined and measured; and (iii) area of reaction product divided by total area = the percentage of material deposited.

[0070] It should be noted that all photomicrographs were taken and analyses performed in a double blind manner, and only after the data was tabulated was the investigator informed about the identity of the R and L sites.

[0071] **Results**

[0072] Morphology

[0073] The epidermis did not appear to be morphologically altered in any of the subjects at the two treatment as well as the untreated site. In most instances, the undulating nature of the dermoepidermal interface was maintained. The granular layer was prominent in all specimens and there was little evidence of apoptosis (sunburn cells) within the epidermis. The "basketweave" architecture of the stratum cornea, characteristic of formalin-fixed human skin was maintained in all subjects in all sites.

[0074] For the most part, the fibrous components of the dermis (i.e., collagen, elastin) did not appear altered on the H&E sections from any of the treatment regimens or the untreated sites of the six subjects. In some cases, the dermis from the untreated site appeared more compact. In a few subjects, occasional areas of blue-gray staining material, usually associated with elastosis, were noted; however, frank signs of photodamage were not seen in any of the subjects. Importantly, there did not appear to be an unusual amount of inflammatory cells in biopsies from any of the treatment sites or in the untreated site from any of the subjects. Some increased cellularity was noted around portions of hair follicles present in some of the sections but this was

not deemed significant. Vascular profiles appeared normal and there was no evidence of increased vascularity, vasodilatation and/or extravasation of red blood cells.

[0075] Viable Epidermal Thickness (VET)

[0076] There was no consistent trend seen in the VET measurements (Tables 2 and 3). Subjects 1, 2, and 6 had similar VET values for the untreated, R and L sites. Subject 3 had a thinner VET measurement for the untreated site compared with the R and L sites. Subject 4 had a thinner VET for the R site compared with the untreated and L site, whereas Subject 5 had a thinner VET for the L site when compared with the R and untreated sites. Given the lack of inflammation, which usually is responsible for epidermal thickening, it is not surprising that VET was not affected by either R or L treatment.

[0077] Elastin

[0078] The overall area of the dermis occupied by elastin appeared to be greater in the untreated sites from all six subjects when compared with either the R or L treatment site (Tables 2 and 3). This finding in no way implies that either of the treatments had a negative impact on elastin fiber deposition, synthesis and/or destruction. It most likely represents inherent differences in elastin content between the volar forearm and the upper inner arm.

[0079] With respect to treatment sites, there was no obvious trend (Tables 2 and 3). Subjects 1 and 4 had significantly more elastin-stained material in the L site when compared with the R site. In contrast, subject 3 had significantly more elastin-stained material in the R site compared with the L site. There was no significant difference in elastin-stained material when the R and L sites were compared in Subjects 2, 5, and 6. When all six subjects were compared there was no change in elastin-stained material (Table 2). This is not surprising since elastin is one of the more stable components of the dermis with an extremely long turnover time. New

elastin deposition is most often seen during tissue regeneration following a wound. Thus the lack of evidence for skin perturbation due to either of the treatments could account for the failure to detect a change in elastin.

[0080] Procollagen

[0081] Five of the six subjects showed an increase in the immunostaining for procollagen when the R site was compared with the L site (Tables 2 and 3). Of these 5 subjects, one (#4) was significant at the $P<0.05$ level and two (#2 and #3) were highly significant ($P<0.01$ level). While an overall increase in procollagen immunostained material was detected for the R site versus the L site in Subjects 1 and 5, the difference was not statistically significant. The R and L treatment did not affect the procollagen-stainable material in Subject 6. When all six subjects were combined there was a greater amount of immunostaining for procollagen in the R site versus the L site; however due to subject to subject variability, this difference was not significant.

[0082] With respect to the untreated site, 4 subjects had less immunostained material corresponding to procollagen when compared with the R site; two subjects had more immunostained material. Due to potential differences in the dermis between the treated and untreated sites it is difficult to meaningfully interpret these changes.

[0083] GAGs

[0084] Four of the six subjects had significantly ($P<0.01$) increased Hale's-stainable material in the R site when compared with the L site (Tables 2 and 3). Subjects 4 and 6 had increases in Hale's-stainable material in the L site versus the R site; however, this difference was not statistically significant. When all six subjects were combined there was a greater amount of Hale's stainable material in the R site versus the L site; however due to subject to subject variability, this difference was not significant.

[0085] The untreated site showed the greatest subject to subject variability in Hale's-stainable material. Nevertheless 4 subjects had greater Hale's-stainable material in the R site when compared to the untreated site. As mentioned previously, due to regional differences it is difficult to draw meaningful comparisons between the treated and untreated sites.

[0086] Conclusions

[0087] There are several conclusions to be drawn from this small pilot study. The active and vehicle-only formulations did not have any deleterious effects on the skin that were discernable at the light microscopic level. This is important because any changes seen in the other parameters were not confounded by and/or secondary to an inflammatory response. The assumption is that any changes are the result of the cord serum complex. The R site, which received the cord serum complex daily for 26 days, had increased amounts of stained material corresponding to procollagen and GAGs compared to the vehicle-treated (L) site. Cord serum complex had no discernable effect on VET or elastin.

[0088] Since ground substance (GAGs) is the dermal component that is most rapidly turned over, it is not surprising that changes were seen. Furthermore, ground substance is well known for its water-holding capacity, and it is this increase in water binding that could be partially responsible for the disappearance of fine-line wrinkling after use of this product. The increase in procollagen-stained material was also significant. In contrast to the GAGs, collagen is slowly turned over. This suggests that some of the changes in skin quality that have been reported following use of the cord serum complex facial cream may be somewhat more long lasting than the GAG-induced changes because collagen fibers are a more stabile dermal component than GAGs. Taken together these findings suggest that facial cream with serum cord

complex stimulates the synthesis of GAGs and procollagen, which in part is responsible for the clinical changes seen after use of this product.

Table 2 - Histogeometric Analyses

VET (microns)	1	2	3	4	5	6	∑
NT	126 ± 7	99 ± 7	98 ± 8	115 ± 4	128 ± 16	114 ± 3	113 ± 12
R	132 ± 7	94 ± 4	120 ± 15	91 ± 2	118 ± 20	119 ± 3	96 ± 37
L	126 ± 5	91 ± 8	115 ± 10	108 ± 4	87 ± 9	113 ± 5	107 ± 14
Elastin (% dermis)							
NT	14.0 ± 1	10.4 ± 1.1	12.5 ± 0.7	12.2 ± 2.4	13.0 ± 2.0	7.6 ± 2.0	11.6 ± 2.0
R	8.0 ± 1.5	9.8 ± 0.3	8.3 ± 1.6**	5.8 ± 0.4	8.2 ± 1.1	5.0 ± 0.4	7.5 ± 1.6
L	11.8 ± 0.7**	8.0 ± 1.7	3.3 ± 0.3	8.6 ± 0.8**	9.5 ± 1.1	5.3 ± 1.1	7.8 ± 2.8
Procollagen (% upper dermis)							
NT	8.1 ± 0.6	9.9 ± 1.7	4.7 ± 1.8	16.2 ± 1.8	9.1 ± 1.5	16.4 ± 2.9	10.7 ± 4.3
R	9.2 ± 0.6	13.3 ± 1.6**	9.9 ± 1.7**	12.3 ± 2.3*	19.2 ± 5.4	10.0 ± 2.4	12.3 ± 3.4
L	9.1 ± 1.1	7.8 ± 1.3	5.2 ± 2.1	7.9 ± 1.8	15.8 ± 3.4	11.5 ± 1.4	9.6 ± 3.4
GAGs (% dermis)							
NT	1.8 ± 0.8	24.3 ± 2.8	22.6 ± 2.4	13 ± 3.8	14.7 ± 2.2	5.2 ± 0.9	13.6 ± 8
R	22 ± 5.0**	11.4 ± 0.9**	20.1 ± 2.2**	15 ± 2.7	23 ± 3.0**	10.8 ± 3.9	17.4 ± 4.9
L	4.4 ± 2.3	6.5 ± 1.5	8.3 ± 2.7	18 ± 5.5	14.3 ± 1.5	13.9 ± 3.9	10.9 ± 4.8

*P <0.05; **P< 0.01

Table 3 - Percentage Change

VET	1	2	3	4	5	6
R vs L	4.5 ↑	3 ↑	4.2 ↑	15.7 ↓	26.3 ↑	5.0 ↑
R vs NT	4.5 ↑	5.1 ↓	18.3 ↑	20.8 ↓	7.8 ↓	4.2 ↓
L vs NT	0	8.1 ↓	14.8 ↑	6.1 ↓	32.0 ↓	0.9 ↓
elastin						
R vs L	32.2 ↓	18.4 ↑	60.2 ↑	32.6 ↓	13.7 ↓	5.7 ↓
R vs NT	42.9 ↓	5.8 ↓	33.6 ↓	52.5 ↓	36.9 ↓	34.2 ↓
L vs NT	15.7 ↓	23.1 ↓	73.6 ↓	29.5 ↓	26.9 ↓	30.3 ↓
procollagen						
R vs L	1.1 ↑	41.4 ↑	47.5 ↑	35.8 ↑	17.7 ↑	13.0 ↓
R vs NT	12.0 ↑	25.6 ↑	52.5 ↑	24.0 ↓	52.6 ↑	39.0 ↓
L vs NT	11.0 ↑	21.2 ↓	9.6 ↑	51.2 ↓	42.4 ↑	6.1 ↓
GAGs						
R vs L	80.0 ↑	43.0 ↑	58.7 ↑	16.7 ↓	37.8 ↑	22.3 ↑

R vs NT	91.8 ↑	53.1 ↓	11.1 ↓	13.3 ↑	36.0 ↑	51.9 ↑
L vs NT	59.0 ↑	73.3 ↓	63.3 ↓	27.8 ↑	2.7 ↓	62.6 ↑

[0089] From the foregoing it will be seen that this invention is one well adapted to attain all ends and objectives herein-above set forth, together with the other advantages which are obvious and which are inherent to the invention. Since many possible embodiments may be made of the invention without departing from the scope thereof, it is to be understood that all matters herein set forth are to be interpreted as illustrative, and not in a limiting sense. While specific embodiments have been shown and discussed, various modifications may of course be made, and the invention is not limited to the specific forms or arrangement of parts and steps described herein, except insofar as such limitations are included in the following claims. Further, it will be understood that certain features and subcombinations are of utility and may be employed without reference to other features and subcombinations. This is contemplated by and is within the scope of the claims.

CLAIMS

What is claimed and desired to be secured by Letters Patent is as follows:

1. A skin care composition for topical application to the human skin for imparting an anti-aging benefit to the skin comprising an effective amount of umbilical cord blood serum.

2. The skin care composition of claim 1, wherein said umbilical cord blood serum is present in an amount from about 0.0001 wt% to about 90 wt%.

3. The skin care composition of claim 1, wherein said umbilical cord blood serum is present in an amount from about 0.01 wt% to about 25 wt%.

4. The skin care composition of claim 1, wherein said umbilical cord blood serum is present in an amount from about 0.01 wt % to about 10 wt%.

5. The skin care composition of claim 1 further comprising a peptide selected from the group consisting of tyr-arg, acetyl hexapeptide-3, palmitoyl oligopeptide, palmitoyl tetrapeptide-7, and mixtures thereof.

6. The skin care composition of claim 1 further comprising a biological additive selected from the group consisting of *Juglans regia*, *Centella asiatica*, *Pyrus germanica* extract, and mixtures thereof.

7. The skin care composition of claim 1 further comprising a biological additive selected from the group consisting of *Undaria pinnatifida* and *Stevia rebaudiana Bertoni*, and mixtures thereof.

8. The skin care composition of claim 1 further comprising one or more phosphospholipids.

9. The skin care composition of claim 8 wherein said phospholipid is selected from the group consisting of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, diphosphatidyl glycerol, and mixtures thereof.

10. The skin care composition of claim 1 further comprising one or more preservatives.

11. The skin care composition of claim 10 wherein said preservative is selected from the group consisting of one or more parabens.

12. The skin care composition of claim 1 further comprising one or more penetration enhancers.

13. The skin care composition of claim 12 wherein said penetration enhancer is selected from the group consisting of dimethyl isosorbide and diethyl-glycol-monoethylether.

14. The skin care composition of claim 1 further comprising one or more neutralizing agents selected from the group consisting of ammonium hydroxide, arginine, 2-amino-2-methyl-1-propanol, dimethanolamine, dibutanolamine, diisobutanolamine, tributanolamine, triisobutanolamine, tri-sec-butanolamine, tripropylamine, ethanolamine, diethanolamine, triethanolamine, PEG-15 cocamine, diisopropanolamine, methylethanolamine, diisopropylamine, dipropylenetriamine, tromethamine, isopropylamine ethylene diamine, triisopropanolamine, tetrahydroxypropyl ethylenediamine, trimethamine, 2-aminobutanol, aminoethyl propanediol, aminomethyl propanediol, aminomethyl propanol, sodium hydroxide, potassium hydroxide and mixtures thereof.

15. The skin care composition of claim 1 further comprising one or more hyaluronans.

16. The skin care composition of claim 1 further comprising one or more skin-conditioning emollients selected from the group jojoba oil, almond oil, capric/caprylic triglyceride and mixtures thereof.

17. The skin care composition of claim 1 comprising about 0.1 to about 0.35 wt% umbilical cord blood serum, about 3 to 5 wt% palmitoyl oligopeptide, about 3 to about 5 wt% palmitoyl tetrapeptide-7.

18. The skin care composition of claim 17 further comprising about 2 to about 3 wt% of the dipeptide tyr-arg and about 0.5 to 1.5 wt% acetyl hexapeptide-3.

19. The skin care composition of claim 18 further comprising about 0.5 to about 1.2 wt% phosphatidyl choline, about 0.5 to about 1.5 wt % hyaluronans, and 0.01 to 0.085 of a preservative selected from the group consisting of methyl paraben and propyl paraben, or mixtures thereof.

20. The skin care composition of claim 19 further comprising *juglans regia*, *centella asiatica*, *pyrus germanica*, *Undaria pinnatifida*, and *Stevia rebaudiana Bertoni*.

21. A method for imparting an anti-aging benefit to human skin comprising: topically applying to the skin of an individual in need thereof a composition of claim 1 to impart the anti-aging benefit to the skin.

22. The method according to claim 21, wherein said umbilical cord blood serum is present in an amount sufficient to increase procollagen or glycosaminoglycans.

23. The method according to claim 21, wherein said umbilical cord blood serum is present in an amount from about 0.0001 wt% to about 90 wt%.

24. The method according to claim 21, wherein said umbilical cord blood serum is present in an amount from about 0.01 wt% to about 25 wt%.

25. The method according to claim 21, wherein said umbilical cord blood serum is present in an amount from about 0.01 wt % to about 10 wt% .

26. The method according to claim 21, wherein said anti-aging benefit is selected from the group consisting of: (a) treatment, reduction, and/or prevention of fine lines or wrinkles, (b) reduction of skin pore size, (c) improvement in skin thickness, plumpness, and/or tautness; (d) improvement in skin suppleness and/or softness; (e) improvement in skin tone, radiance, and/or clarity; (f) improvement in procollagen and/or collagen production; (g) improvement in maintenance and remodeling of elastin; (h) improvement in skin texture and/or promotion of retexturization; (i) improvement in skin barrier repair and/or function; (j) improvement in appearance of skin contours; (k) restoration of skin luster and/or brightness; (l) replenishment of essential nutrients and/or constituents in the skin; (m) improvement of skin appearance decreased by menopause; (n) improvement in skin moisturization; (o) increase in and/or preventing loss of skin elasticity; (p) treatment, reduction, and/or prevention of skin sagging; or (q) treatment, reduction, and/or prevention of discoloration of skin.

27. The method according to claim 26, wherein said anti-aging benefit is treatment, reduction, and/or prevention of fine lines or wrinkles.

28. The method according to claim 26, wherein said anti-aging benefit is treatment, reduction, and/or prevention of skin sagging.

29. The method according to claim 26, wherein said anti-aging benefit is treatment, reduction, and/or prevention of discoloration of skin.

30. The method according to claim 26, wherein said anti-aging benefit is increase in and/or preventing loss of skin elasticity.

TOPICAL COMPOSITION COMPRISING UMBILICAL CORD BLOOD SERUM

ABSTRACT OF THE DISCLOSURE

Cosmetic compositions comprising umbilical cord blood serum and methods of using such compositions to impart anti-aging benefits to the skin are disclosed.

“UnderEye recovery cream with Cord Serum Complex”

Part I: removing dark circles

Under-eye circles are created when red blood cells leak from capillaries in the eye area, releasing hemoglobin when they explode in nearby skin tissue. The iron-rich hemoglobin degrades into the yellow pigment—bilirubin—as well as other various colored pigments, resulting in a bruised appearance to the skin under the eyes and sometimes on the eyelids. It still is not clear why the capillaries leak, although inflammation—due to stress, genetics, allergies and lack of sleep—is believed to be a key trigger.

Under eye recovery cream with cord serum complex is a combination of proprietary components/systems that binds to the iron released by the red blood cells, trapping the molecules before bilirubin can be formed and making the iron soluble enough to be eliminated from the skin. (Haloxyl) and stimulates a natural enzyme that clears bilirubin that already has been formed. Working with these molecules are components providing support to the fragile connective tissue around the eyes. When it begins to lose its tone and elasticity, the irritation that leads to dark circles can increase.

Studies show a reduction in the production of the key inflammatory enzyme prostaglandin E2 (PGE2) in epidermal keratinocytes by 93% and in dermal fibroblasts by 86% when these cells are exposed to ultraviolet B (UVB) irradiation—long believed by researchers to be a significant contributor to aging around the eye area. In a two-month clinical study of 22 females whose average age was 32.7 years, application of the cream, two times a day showed an average 63% decrease in under-eye darkening.

Part II: relieving puffiness

Helps reduce puffiness and bags under the eyes. Puffy eyes are due to fluid build-up caused by poor drainage, fragile capillaries, and loss of elasticity. Fluid leaks into surrounding tissues and produces "bags". The Recovery Cream targets all three conditions by improving drainage, reducing capillary fragility, and reducing irritation and skin slackening while increasing skin firmness and elasticity (Eyeliss)

Part III: Instant and gradual skin brightening

Following the application of the Under Eye Regeneration serum with CSC we notice an even complexion with less visible spoting. Luminosity and skin appearance: lines, pores and blemishes are improved and the yellow or red skin color is brighter. Daily use of the UnderEye serum results in significant progressive and durable reduction of the quantity of melanin in the skin leading to a clearer skin by 16%. Pores are less apparent and lines are optically erased.

Part IV: Light Manipulation

The appearance of fine lines and wrinkles on the skin's surface is a result of uneven textures. The UnderEye serum through the addition of microscopic clear lenses (Chronosphere opticals) to the formula is able to manipulate light striking the skin surface in the eye area creating an illusion on the adjacent skin areas reducing the darkness and shadows caused by fine lines and wrinkles on the skin. The clarity of the microlenses allows the product to be used on all skin tones.

Part V: Reduction of fine lines and wrinkles

Charged coupled biopeptides, neuropeptides, micro algae's and botanicals reduce the depth of fine lines and wrinkles, additional-synergistic support is provided by the Cord Serum Complex (Human Umbilical Extract) see attachment "Histogeometric Analysis of the effects of Product A versus product B on human skin.

Note: Clinical data and trial information available