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(54) Title: COMBINED ENERGY AND TOPICAL COMPOSITION APPLICATION FOR REGULATING THE CONDITION OF MAMMALIAN SKIN

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COMBINED ENERGY AND TOPICAL COMPOSITION APPLICATION FOR REGULATING THE CONDITION OF MAMMALIAN SKIN

FIELD OF THE INVENTION

The present invention relates to combined applications of energy and topical compositions to mammalian skin for regulating the condition of the skin. Regimens for the most efficient use of energy delivery devices and methods for determining efficacy of an energy delivery device, the composition and the regimen are also disclosed.

BACKGROUND OF THE INVENTION

Treatment of skin to avoid or reduce effects of intrinsic chronological and extrinsic environmental aging of skin is a multi-billion dollar commercial industry underpinned by even greater dollar investment in the development and validation of new technologies. Administration of electromagnetic (EM) energy to skin via application to the surface of skin has been known for decades and implemented in a wide range of forms and through a variety of delivery devices. Generally EM-based skin treatment methods may be divided into ablative and nonablative procedures although both exploit the thermolytic effect of EM energy application.

A variety of products are available to consumers to improve the condition of skin and to delay and/or prevent typical signs of aging. Such signs include, for example, fine lines, wrinkles, hyper-pigmentation, shallowness, sagging, dark under-eye circles, puffy eyes, uneven skin tone, enlarged pores, diminished rate of epidermal cell turnover, and abnormal desquamation or exfoliation. For some consumers, however, the wide variety of available products and the advancements in skin care technology still fail to produce the desired results, and some feel the need to turn to more invasive medical procedures.

Ablative procedures such as ablative laser have proven to be effective methods for gross morphological resurfacing or removal of skin, such as in scar and tattoo removal procedures, and have also proven effective for treating and improving appearance of aged and photo-damaged skin. Although ablative procedures are effective for improving the appearance of fine lines and wrinkles in the cosmetically vulnerable perioral and periorbital areas of facial skin, major disadvantages include prolonged periods of healing and recuperation which impose a seriously compromised cosmetic appearance to the consumer for undesirably long time periods. Further,

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the potential side effects of infection, scarring and pigmentation irregularities which may result are often considered cosmetically unacceptable to consumers in particular where facial skin is implicated.

Recent research and development efforts have therefore focused on providing consumers with cheaper, more convenient and safer nonablative skin anti-aging and rejuvenation treatments. Cosmetic regimens involving administration of thermal energy to the skin for the purpose of promoting improved appearance of the skin are well known in the art. Electromagnetic energy delivery technology in the form of handheld devices targeted for consumer home use have been available on the market for nearly a decade. Improvements and advances in the technology center around maximizing a thermally induced benefit to deeper target skin tissues while minimizing undesirable damage both to the target tissue to peripheral and surface tissues. It is believed that selective thermal treatment induces new collagen formation and selective thermal damage induces, inter alia, dermal matrix remodeling. Currently available technologies, however, are known to result in undesirable sustained negative side effects of problematic damage including overheating, burning, erythema and pigment irregularities.

In the past, efficacy of these devices and compositions was determined by exterior methods only. That is, if there was a visible improvement in the exterior layer of the skin the energy delivery device must be working. But there are few methods for determining the optimal time, temperature and composition for these energy delivery devices. Moreover, it is generally accepted that many of the visible changes that occur on the surface of the skin are the results of changes that occur below the surface in the dermis and epidermis layers of the skin. It is difficult to know exactly how to control and maximize the performance of a device without knowing how it affects the underlying layers of skin.

Selective photothermolysis of skin tissue is a widely practiced cosmetic treatment form, in particular in treatments comprising administration of monochromatic laser energy and broad spectrum intense pulsed light (IPL) energy. In these technologies, optical energy is applied directly to the surface of the skin and penetration relies on transmission through the epidermis and absorption in the dermis. Dark skin and hyper-pigmented spots on the epidermis may impede transmission and hinder efficacy of the treatment by absorbing energy, and may also result in overheating of the pigmented areas resulting in blistering, burning, and other cosmetically undesirable effects.

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As an alternative to EM-based thermolysis, electrically conducted radio frequency (RF) current has also been investigated as a cosmetic skin treatment modality. The use of RF current and pulsed radio frequencies (pRF) in the medicinal arts is known, although the use of RF current as a nonablative skin rejuvenation technology for self-delivery by consumers is still relatively innovative. In the application of RF current to skin, a transfer of biopolar RF current takes place through two electrodes that are applied directly to the skin. The current, therefore, runs directly through the dermal layer conducted from electrode to electrode, distinguishing it from application of EM energy which is focused through the epidermis and limited by factors which affect wavelength penetration depth.

RF current administration theoretically appears to offer significant skin treatment advantages over EM energy application. Unlike electromagnetic energy, for example, electrically conducted RF energy is chromophore-independent, which avoids complications and efficacy problems relating to the existence of an absorption differential between pigmented and non-pigmented skin and the resulting problems in treating darker skin, which has more energy-absorbing melanin and lighter skin, which may reflect optical wavelengths. In both cases, consistency of results is compromised and thermal control in chromophore-containing skin remains problematic.

RF current administration to skin at energy levels which may provide thermal treatment efficacy, however, is plagued by an inability by investigators to optimize parameters to achieve a desired benefit in the absence of undesirable skin damage. RF current is delivered through the dermal tissue below the skin surface, whereas effect-monitoring by temperature or moisture sensors is limited to the accessible surface of skin. RF current impedance is a function of tissue composition and various skin tissue attributes including collagen density and integrity, hydration level, and the like. Although the distance between electrodes and control of parameters such as pulse length and frequency may theoretically be adjusted to optimize effect and avoid safety concerns, such adjustments are nearly impossible without benefit of an apparatus or other means to monitor changes in tissue condition. In the case of EM energy-based delivery, the surface skin typically reaches the highest treatment temperatures so that temperature monitoring at the surface can prevent undesired damage to sub-epidermal tissue. However, in the case of RF current the sub-epidermal tissues actually reach a higher treatment temperature than the surface skin so that damage may occur to deeper tissues without being measurably manifest at the surface. Outside of clinical settings under the supervision of highly trained medical personnel and using sophisticated

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instrumentation, devices and regimens targeted for personal use by consumers based on delivery of RF current alone have therefore been generally avoided since safety considerations continue to exist at effective treatment levels in the absence of an appropriate sub-epidermal monitoring mechanism. Handheld energy delivery devices which provide RF-current as a sole treatment modality and targeted for home consumer use are virtually unknown. One such purported device (STOP™, Ultragen Ltd) is marketed to consumers for personal use in Europe, but the treatment tolerances of the device are set so low in order to avoid undesirable damage, that objective evidence of clinical efficacy under controlled conditions is not available.

Hence, the role of RF in skin treatment is substantially limited to an adjunctive or preparative function in combination with other thermolytic procedures. For example, in 2002, Bitter and Mulholland ("Report of a new technique for enhanced non-invasive skin rejuvenation using a dual mode pulsed light and radio frequency energy sources: selective radiothermolysis," *J. Cosmet Dermatol* 2002; 1: 142-145) proposed a treatment protocol based on a combination of RF current and IPL and reported results based on facial treatment of 100 test subjects, although the authors failed to disclose specific treatment design parameters. In that study, RF was reported to augment the effects of IPL treatment. Side effects included reports of cosmetically undesirable pigmentation effects, and consumer perception of pain was controlled by superficial cooling.

RF has also been suggested and investigated as useful for cosmetic skin treatment in conjunction with targeted optical energy application. Generally, according to this treatment protocol design, the RF is used adjunctively to the optical energy and is applied in accordance with some parameter of the optical energy. For example, in Hammes et al. ("Electro-optical synergy (ELOS™) for nonablative skin rejuvenation: a preliminary prospective study," *Journal of European Academy of Dermatology. and Venereology* 2006, 20, 1070-1075), the authors focus on a coordinating pulse frequency between the RF current and the optical energy and suggest that synergy exists between these energy forms which may permit use of lower, less invasive levels of optical energy and further suggest that side effects associated with RF application alone are reduced or avoided by the combined protocol. Further, the regimens which employ these devices include means to mechanically cool the skin in response to overheating, or to prevent overheating.

In another example, U.S. Published Application No. 2008/0033516 A1 to Altshuler discloses "temperature controlled photobiostimulation" of skin tissue which involves a combination of heating skin to a target depth and irradiation of a target area with electromagnetic

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radiation. Altshuler notes the existing technologies of low-level light, low-level laser, monochromatic and quasi-monochromatic photostimulation based skin treatment methods, which are generally thought to increase ATP production, cellular proliferation and protein production, as well as trigger a growth response by induction of a low-grade inflammatory response, but notes reports of inconsistent results and lack of clinical confirmation of efficacy. Altshuler posits that application thermal energy may enhance the photostimulatory response. Altshuler teaches that hyperthermia of a volume of skin may be achieved by any known source capable of raising the temperature of the volume to preferable between 37° and 45°C, and specifically exemplifies heating by hot air, AC or DC electrical current, use of a conductive heat source, ultrasound or microwave radiation or any suitable wavelength or wavelengths of EM radiation in the range of 380-2700 nm. In all Altshuler embodiments, however, EM energy is relied upon to achieve the desired treatment effect. Altshuler teaches that heat provides synergistic enhancement of the desired effects of photostimulation, but also suggests that heat in the absence of EM may result in undesirable biostimulation such as slowing repair of radiation-induced DNA damage, production of heat shock proteins, which build tolerance to subsequent heat applications, and modification of enzymatic processes including those involved in skin tissue regeneration and repair and generally teaches away from heat in the absence of light as a skin treatment modality. Altshuler does not suggest how to overcome deficiencies relating to an inability to assess or monitor sub-epidermal skin conditions.

Moreover, consumer compliance is always an issue for currently available devices. Consumers have a limited amount of time each day for their beauty regimen. While device manufacturers would like to recommend that the consumers use there devices for extended periods to insure they get the maximum benefit, the consumers are unlikely to comply. Hence there is a trade off between recommending extended use of the device and recognizing that consumers have a limited amount of free time in their day to use the device.

The consumer experience is also important when designing a device, a composition and a regimen. For example, sonograms are commonly performed procedures and provide an enormous medical benefit. But the gel used in sonogram procedures is thick and difficult to remove causing consumer discomfort. Moreover, many energy delivery devices heat the exterior skin too quickly or too hot causing an unpleasant consumer experience.

Therefore, there is a continuing need for methods of improving the condition of skin sufficiently to avoid the need for more invasive procedures and the risks associated therewith.

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And there exists a need for better methods of determining the efficacy of energy delivery devices, which methods can then be used to develop more consumer acceptable experiences, while achieving the desired results of improved skin appearance.

There remains a need in the art for safe and effective nonablative skin treatment and rejuvenation devices, therapies and regimens suitable for personal use by consumers. In particular, there remains a need for a means to treat sub-epidermal skin tissue by enhancing collagen synthesis and dermal remodeling without causing undesirable damage to the treated target tissue or to surrounding tissue. There is a specific need in the art for methods of assessing and monitoring the effects of RF-current based consumer-conducted treatments in order to optimize RF-current administration for provision of desired benefits, and there remains a need for optimized RF-current based therapies which avoid the problems associated with EM-based therapies and which do not rely on mechanical cooling in conjunction with treatment.

SUMMARY OF THE INVENTION

The present invention relates to a method for regulating the condition of mammalian skin. The skin has at least three layers: a stratum corneum exterior layer; an epidermis; and a dermis. And the method comprises the steps of, applying a first personal care composition to an area of skin where regulation is desired, wherein the first personal care composition comprises a gel composition. Another step comprises delivering energy to the dermis to heat collagen in the dermis such that the heated collagen in the dermis heats the epidermis and stratum corneum until the stratum corneum reaches an external temperature of from about 37°C to about 48°C. The energy delivery to the dermis is then controlled to maintain the temperature of the stratum corneum in the range of from about 37°C to about 48°C. The energy delivered can be from an RF energy device and the RF energy is delivered via two or more electrodes that contact the stratum corneum via the gel composition. It is sometimes preferred that the energy delivery device does not emit light and it does not produce electromagnetic energy. And in one embodiment the gel composition has an electrical conductivity of from about 1,000 to about 2,000 $\mu\text{S}/\text{cm}$.

In another embodiment of the present invention, when the RF energy device is turned on it delivers the RF energy in the range of 35% to about 65% of full power for the for about 20 to about 50 seconds, then the power is increased to from about 65% to about 100% for about 20 to

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about 50 seconds, then energy delivery is controlled such that the temperature of the stratum corneum is maintained in the range of from about 37°C to about 48°C.

In yet another embodiment of the present invention the energy delivery device is handheld and is applied under an eye of a consumer and moved underneath the eye to just above the crows feet area, and then the direction is reversed, and the energy delivery device is moved back and forth across this path for from about 3 to about 6 minutes. This method can be repeated underneath the other eye of the consumer. Moreover, the energy delivery device can be applied above an eye of a consumer and moved over the eye to just below the crows feet area, and then the direction is reversed, and the energy delivery device is moved back and forth across this path for from about 3 to about 6 minutes. This method can be repeated above the other eye of the consumer.

A multi-step regimen is disclosed comprising the steps of applying the energy delivery device under one eye of a consumer and moving it to just above the crows feet area in a continuous back and forth motion for from about 3 to about 6 minutes, then applying the energy delivery device above an eye of the consumer and moving it to just below the crows feet area in a continuous back and forth motion for from about 3 to about 6 minutes, repeating these two steps on the other eye of the consumer such that the crows feet area adjacent both eyes of the consumer are each treated for from about 6 to about 12 minutes. This multi-step regimen can be completed at least once per day, for a regimen period of 3 to 5 days per week, for from about 3 weeks to about 6 weeks. The consumer can then wait for about 2 to about 8 months and then repeats the multi step regimen for the regimen period. Preferably, the fine line and wrinkles around the eyes of the consumer are visibly reduced after each regimen period.

BRIEF DESCRIPTION OF THE DRAWINGS

While the specification concludes with claims particularly pointing out and distinctly claiming the present invention, it is believed the same will be better understood from the following description taken in conjunction with the accompanying drawings in which:

Figure 1 is a schematic representation of naturally occurring damage and repair cycle in human skin;

Figure 2A, 2B illustrates the properties of Human Collagen;

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Figure 3 is a schematic of Biological Model of RF current administration efficacy;

Figure 4A, 4B is the 24 hour average fold-change for compilation genes;

Figure 5A, 5B is the 24 hour average fold-change for exemplary genes;

Figure 6A, 6B is the 1 month average fold-change for compilation genes;

Figure 7A, 7B is the 1 month average fold-change for exemplary genes;

Figure 8A, 8B are two graphs of RF simple heat transfer;

Figure 9 is a schematic of RF simple heat transfer;

Figure 10 is a schematic of the Crow's Feet area around a consumer's eyes;

Figure 11 is zone A of a consumer's skin;

Figure 12 is zone B of a consumer's skin;

Figure 13 is zone C of a consumer's skin;

Figure 14 is zone D of a consumer's skin;

Figure 15 is zone E of a consumer's skin; and

Figure 16 is zone F of a consumer's skin.

DETAILED DESCRIPTION OF THE INVENTION

In all embodiments of the present invention, all percentages are by weight of the total composition, unless specifically stated otherwise. All ratios are weight ratios, unless specifically stated otherwise. All ranges are inclusive and combinable. The number of significant digits

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conveys neither limitations on the indicated amounts nor on the accuracy of the measurements. All numerical amounts are understood to be modified by the word “about” unless otherwise specifically indicated. All measurements are understood to be made at 25°C and at ambient conditions, where “ambient conditions” means conditions under one atmosphere of pressure and at 50% relative humidity.

It is to be understood that the steps recited in any method claims appended hereto can be performed in any order unless specified otherwise. For example, in a method claim reciting steps (a), (b) and (c), step (c) could be performed prior to or between steps (a) and (b). Furthermore, the individual steps, although recited as distinct steps, can be performed during time periods with some or complete overlap.

Herein, “regulating the condition of skin” means improving the condition of skin and/or prophylactically regulating the condition of skin, and includes, for example, protecting the tissue from ultraviolet radiation, and regulating the signs of skin aging. Herein, “improving the condition of mammalian skin” means effecting a visually and/or tactilely perceptible positive change in the appearance and feel of the tissue. Conditions that may be regulated and/or improved include, but are not limited to, one or more of the following: Reducing the appearance of wrinkles and coarse deep lines, fine lines, crevices, bumps, and large pores; thickening of skin (e.g., building the epidermis and/or dermis and/or sub-dermal layers of the skin, and where applicable the keratinous layers of the nail and hair shaft, to reduce skin, hair, or nail atrophy); increasing the convolution of the dermal-epidermal border (also known as the rete ridges); preventing loss of skin or hair elasticity, for example, due to loss, damage and/or inactivation of functional skin elastin, resulting in such conditions as elastosis, sagging, loss of skin or hair recoil from deformation; reduction in cellulite; change in coloration to the skin, hair, or nails, for example, under-eye circles, blotchiness (e.g., uneven red coloration due to, for example, rosacea), sallowness, discoloration caused by telangiectasia or spider vessels, dryness, brittleness, and graying hair.

As used herein, “signs of skin aging,” include, but are not limited to, outward visibly and tactilely perceptible manifestations, as well as any macro- or micro effects, due to skin aging. These signs may result from processes which include, but are not limited to, the development of textural discontinuities such as wrinkles and coarse deep wrinkles, fine lines, skin lines, crevices, bumps, large pores, unevenness or roughness; flaking; dryness; loss of skin elasticity; discoloration (including under eye circles); blotchiness; shallowness; hyperpigmented skin

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regions such as age spots and freckles; keratoses; abnormal differentiation; hyperkeratinization; elastosis; collagen breakdown, and other histological changes in the stratum corneum, dermis, epidermis, vascular system (e.g., telangiectasia or spider vessels), and underlying tissues (e.g., fat and/or muscle), especially those proximate to the skin.

“Hyperpigmentation,” as used herein, refers to an area of skin wherein the pigmentation is greater than that of an adjacent area of skin (e.g., a pigment spot, an age spot, and the like).

Herein, “personal care composition” means compositions suitable for topical application on mammalian skin. The personal care compositions described herein may contain one or more skin care actives. “Skin care actives,” or “actives,” as used herein, means compounds that aid in regulating the condition of skin and of other mammalian skin, for example, by providing a benefit or improvement to the skin.

“Energy delivery device,” as used herein, means any device used to deliver energy to mammalian skin and/or hair. Herein, “delivery of energy,” means that the surface and/or layers of the skin are exposed to the energy emanating from the energy delivery device, where it may penetrate to desired layers of the skin, including the hair shaft and/or hair follicle.

“Continuous level,” as used herein, means that the energy delivered by the device, or energy output, remains at an essentially constant level between the time of device activation and the time of device deactivation.

“Pulsed,” as used herein, means that between the time of device activation and the time of device deactivation, the energy output varies in a predictable manner, characterized by periods of higher output (pulses) alternating with periods of lower output. The onset of pulses may be sudden or gradual. “Predictable” means that the pulse peak intensities, pulse shapes, pulse durations, and the temporal spacing between the pulses are substantially identical. The duration of the pulses and the time between pulses may vary.

“Hand-held,” as used herein, means that the device is of a weight and dimension suitable for an average adult human to comfortably hold.

The human skin may be divided into two major structural layers: the epidermis and the sub-epidermal or underlying dermis. The epidermis with the stratum corneum serves as a biological barrier to the environment. In the basilar layer of the epidermis, pigment-forming cells called melanocytes are present, which are the main determinants of skin color.

The underlying dermis provides the main structural support of the skin. It is composed mainly of an extra-cellular protein called collagen. Collagen is produced by fibroblasts and

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synthesized as a triple helix with three polypeptide chains that are connected with heat labile and heat stable chemical bonds. When collagen-containing tissue is heated, alterations in the physical properties of this protein matrix occur at a characteristic temperature. Structural transition of collagen contraction and remodeling of the collagen matrix occurs with heat.

Within the skin, some amount of repair activity occurs to promote continual collagen production. During aging the rate at which damage occurs may increase faster than repair activity, or damage may continue to occur at substantially constant rates, but repair activity slows. In either case the result is reduced collagen and compromised appearance manifest as signs of aging, including appearance of surface defects such as fine lines, wrinkles and hyper-pigmented spots. The normal repair activity cycle and resultant impact on appearance over time is illustrated in Figures 1 and 3.

In Fig. 1 the boxes represent normal causes of collagen production. For many reasons, the increase of collagen in the skin, and the speed with which it is repaired and replaced contributes to fuller, healthier, and more attractive looking skin. Ongoing proliferation and dermal remodeling occur naturally, but unfortunately, these processes slow as we age. Collagen is also produced when skin is damaged, for example, after inflammation or insult from, for example, radiation from the sun. The circles in Fig. 1 represent some of the mechanisms by which collagen is formed and how its formation can be tracked. MMP and cytokine activity are just two measurable quantities that help track the collagen repair and replenishment cycle.

Fig. 3 is a schematic of the dual action biological model for elure efficacy 20. Skin 21 is divided into three layers, the stratum corneum 30, the epidermis 32 and the dermis 34. The collagen remodeling and production of new collagen occurs in the dermis layer. Normal insult 22 to skin 21 occurs constantly and includes normal aging, UV insult, changes in pH, chemical insults and others. Normal insults 22 result in damaged collagen 24. Likewise, normal low level inflammation 26 occurs that results in cytokine, HSP and HSF activity. Ultimately, repeated low level thermal energy yields increased MMP activity 36 results in the production of collagen fragments. The increase in heat cause an up regulation 38 of cellular activity that causes the formation and repair of collagen. Both mechanisms 36 and 38 result in the formation and remodeling of healthy collagen 28.

Although collagen is measured to have a melting temperature of up to 50°C, the repair cycle may be altered and enhanced by the addition of heat at lower levels. Thermal cleavage of intramolecular hydrogen bonded crosslinking is created by the balance between cleavage events

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and relaxation events (reforming of hydrogen bonds). No external force is required for this process to occur. As a result, intermolecular stress is created by the thermal cleavage of intramolecular hydrogen bonds. Contraction of the tertiary structure of the cross-linked molecule creates the initial intermolecular vector of contraction. RF model heating curves are illustrated in Figures 8A, 8B and 9.

The dermal structure is predominantly comprised of collagen 1, 50, Figures 2A and 2B. Collagen is expressed as procollagen 52, a single stranded protein, by fibroblasts. Procollagen 52 is clipped upon expression to collagen 1 50 and folded into a triple helix conformation called "tropocollagen" 54. This process is illustrated in Figures 2A and 2B.

Collagen crosslinking may be intramolecular (covalent or hydrogen bond) or intermolecular (covalent or ionic bonds). Causes of collagen denaturation as a function of age include thermal energy insult, mechanical insult, effects of pH on collagenase and MMP rate, hydration status, and general disruption in the natural equilibrium of collagen microfibrils which may "zip" or "unzip," making them vulnerable to MMP digestion. Although these represent multiple insult types, all are rate controlled by temperature. Further, the normal collagen turnover cycle may be regulated within a temperature range of $<37^{\circ}\text{C}$ - 43°C .

Cleavage of collagen bonds also occurs at lower temperatures but at a lower rate. Low-level thermal cleavage is frequently associated with relaxation phenomena in which bonds are reformed without a net change in molecular length.

Dermal remodeling is a biophysical phenomenon that occurs at cellular and molecular levels. Molecular contraction or partial denaturization of collagen involves the application of an energy source, which destabilizes the longitudinal axis of the molecule by cleaving the heat labile bonds of the triple helix. As a result, stress is created to break the intermolecular bonds of the matrix. This is essentially an extra-cellular process, whereas cellular contraction requires a lag period for the migration and multiplication of fibroblasts into a damaged area. A healing response generally involves an initial inflammatory process, which consists of infiltration by white blood cells or leukocytes that dispose of cellular debris. This is followed by proliferation of fibroblasts at the injured site and/or an increase in turnover with an ultimate increase in collagen available for deposition. Fibroblast cells differentiate into contractile myofibroblasts, which are the source of cellular soft tissue contraction. Following cellular contraction, collagen is laid down as a static supporting matrix in the tightened soft tissue structure. The deposition and

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subsequent remodeling of this nascent scar matrix provides the means to alter the consistency and geometry of soft tissue for aesthetic purposes.

Application of thermal energy to initiate the damage and repair cascade in order to ultimately achieve an improvement in surface appearance of skin is known in the art, however currently available technologies are associated with known deficiencies. For example, laser delivery devices use specific wavelengths of light that penetrate the skin, bind to specific chromophores and, through a process called selective photothermolysis, remove various colors and pigments from the skin. The lasers are large, expensive pieces of capital equipment, only attack specific problems or colors in the skin, are prone to laser burns, scars, can cause hyper and/or hypopigmentation and may result in user and patient ocular injuries. Intense broad band light systems emit multiple wavelengths of light, and through selective photothermolysis, also improve skin discoloration and, through skin heating, non-specific skin texture improvement. The systems are also larger and expensive, the skin textures and wrinkle improvements are minimal and there is also the risk of skin burns, hypo or hyperpigmentation and scars. Generally, application of electromagnetic energy is achieved through the epidermis with penetration limited by pigmentation factors at the surface and composition of the dermal layer.

Radio frequency technologies are also known in the art of skin treatment. RF technology uses electrical current to heat the dermis and stimulate production of collagen and elastin fibers that firm and tighten the skin. Substantial drawbacks exist, however, in the current state of the art due to an inability to optimize treatment parameters. RF current application creates a thermal gradient in the skin that is reverse to other thermal energy delivery technologies. Administration of RF current is conducted between electrodes placed some distance apart on the skin. The current is conducted between the electrodes, through the dermis so that the temperature of the dermis rises more rapidly than the temperature at the skin surface. Since most skin parameter measuring devices are designed for skin surface measurement, excessive heating of the dermis may occur before realization.

Hence, treatment by RF current in the absence of thermal quenching, mechanical cooling or other technologies designed to control internal heating has been avoided in the art.

However, utilizing recent advances in the biotechnologies of genomics and proteonomics, the present inventors developed methods for assessing the effects of dermal administration of RF current. In particular, the present inventors screened a group of potential genes identified as involved in the dermal collagen matrix, dermal inflammation and remodeling, and in epidermal

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differentiation. Genetic signatures and gene chip constituents based on resultant genetic expression profiles were determined by analysis and inspection of differential regulation of the potential genes when subject to specific RF current treatment parameters, conditions and regimens.

Energy delivered to and/or into layers of the skin may be in the form of RF energy, including, for example, radiofrequency waves and microwaves. Exemplary RF energy devices are disclosed in the following U.S. Patent Nos.: 6,889,090; 6,702,808; 6,662,054; 5,569,242; 5,755,753; 6,241,753; 6,430,446; 6,350,276; 5,919,219; 5,660,836; 6,413,255; 6,228,078; 5,366,443; and 6,766,202.

The method of the present invention comprises the step of applying a first personal care composition and optionally a second personal care composition to an area of mammalian skin. The first and second personal care compositions may be in a variety of forms, including but not limited to lotions, creams, serums, foams, gels, sprays, ointments, masks, sticks, moisturizers, patches, powders, and/or wipes. In one embodiment, the first personal care composition is applied prior to and/or during delivery of energy. In an alternative embodiment, the second personal care composition is applied after the application of the first composition and the delivery of energy. Optionally, the method of the present invention may comprise the step of applying a third personal care composition to the skin, wherein the third composition comprises a conditioning agent. In one embodiment, the third personal care composition is applied prior to application of the first personal care composition. Preferably, the third personal care composition is applied at least 24 hours prior to the delivery of energy. In an alternative embodiment, the first personal care composition is applied twice daily and energy is delivered once daily, alternatively once weekly, and alternatively once monthly. In one embodiment, the first personal care composition is applied to the skin twice daily and energy is delivered to the skin once weekly. The first, second and third personal care compositions may contain a variety of ingredients, non-limiting examples of which may be found in The CTFA International Cosmetic Ingredient Dictionary and Handbook, Tenth Edition (2004).

The compositions of the present invention may comprise from 50% to 99.9% of a dermatologically acceptable carrier. The carrier of the present invention is in the form of an emulsion. Herein, "emulsions" generally contain an aqueous phase and an oil phase. The oils may be derived from animals, plants, or petroleum, may be natural or synthetic, and may include

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silicone oils. Emulsion carriers include, but are not limited to, oil-in-water, water-in-oil, water-in-oil-in-water, and oil-in-water-in-silicone emulsions. In one embodiment, the dermatologically acceptable carrier comprises an oil-in-water emulsion, and alternatively, a silicone-in-water emulsion. The emulsion further may comprise a humectant, for example, glycerin and a non-ionic, cationic and/or anionic emulsifier. Suitable emulsifiers are disclosed in, for example, U.S. Patent No. 3,755,560 issued to Dickert et al., U.S. Patent No. 4,421,769, issued to Dixon et al., and *McCutcheon's Detergents and Emulsifiers*, North American Edition, pages 317-324 (1986).

A wide range of quantities of the compositions of the present invention can be employed to improve the condition of the skin. The quantity of the personal care composition that is applied to the skin can vary depending on the bodily location and desired benefit. Exemplary quantities include from 0.1 mg/cm² to 40 mg/cm². One useful application amount is 0.5 mg/cm² to 10 mg/cm².

A temperature change may be simultaneously induced in the skin or alternatively, in a composition applied to the surface of the skin. This temperature change is in addition to any temperature change induced by the delivered energy itself. For example, the skin may be heated prior to delivery of energy, or alternatively, the skin may be cooled before, during, and/or after delivery of energy.

Using the gene panels and genetic signatures after treatment, as well as extensive consumer research, regimen zones were developed that optimize size of the treatment area and the optimal treatment time were developed. It was discovered, contrary to the teachings in the art, using the device over large areas is not the best treatment method. When too large an area is treated, the beginning portion can cool down and recover before the consumer sweeps over it again. If too small an area is treated the consumer risks overtreatment and unnecessary damage. Moreover, the treatment itself is work intensive for the consumer and to breaking the treatment into discreet chunks makes it easier for the consumer to fully comply with the regimen. Likewise, the personal care composition used with the device is much more appealing to the consumer if they can apply it to a small area, treat that area and then remove any remaining personal care composition. Putting the personal care composition on the entire face, or even half of the face, can render the treatment experience unpleasant. This, in turn, has a negative impact on consumer compliance.

Turning now to Figure 10 wherein crow's feet area 60 of consumer 62 is identified with a dashed circle and occur above and below eyes 64 and adjacent the outside corner of each eye. It

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is well known that crow's feet areas 60 are prone to wrinkle and fine line formation as human age and are subject to environmental insults. Figure 11 shows Zone A 70 wherein arrows within Zone A are showed to demonstrate a exemplary treatment path for the device (not shown). The times of treatment are given above. Figure 10 illustrates Zone B 72 which is over the same eye of consumer 62 and overlaps crow's feet area 60. Exemplary arrows are shown to illustrate a possible path of treatment. By defining Zone A 70 and Zone B 73 in this manner crow's feet area 60 is treated twice as long as non-crow's feet portion of Zones A and B. This is an important discovery because most of the fine lines and wrinkles on a consumer's face that need treating are in the crow's feet area.

Figures 13 and 14 illustrate Zones C 74 and D 76 on the other eye 64 of the consumer 62. As one would expect, these treatment areas are substantially similar and treated in the same manner as Zones A and B.

An alternate treatment protocol is shown in Figures 15 and 16 wherein Zones E 80 and F 82 are shown on consumer 62. These "C" shaped treatment areas treat the crow's feet area 60, Figure 10, only once on each pass, but the longer "C" shaped treatment area allows for each area of skin that is treated to cool a bit before the device returns for another pass. Thus, consumer's comfort is increased, but the crow's feet area 60, Figure 10, is treated only during the one treatment cycle.

A second personal care composition may optionally be used in conjunction with the above-described method. The second personal care composition may be used between successive treatment periods that employ the first personal care composition and thermal heat device. The second personal care composition preferably comprises at least one skin care active not present in the first personal care composition.

Application of RF and treatment regimens comprising administration of RF current through the dermis may be designed for the first time in order to optimize the desired effects of RF treatment. Upon analysis and inspection of the genetic expression data, it was surprisingly discovered that the potential gene dataset could be reduced into three subsets with particular utility in optimizing treatment regimens to provide increase in dermal collagen and desired hormetic stress-induced dermal remodeling, in the absence of a more problematic inflammatory cytokine damage response. To the best of the present inventors knowledge, this represents the first time that genomics has been applied to cosmetic treatment employing RF current technology. The expression profiles reveal that controlled and optimized administration results

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in the provision of positive hormetic stress which initiates and sustains desirable dermal remodeling, while avoiding the traditional damage associated with undesirable biological effects. The present invention provides novel gene chips, genetic signatures, methods of screening and optimizable regimens based on these discoveries.

Genes investigated as potential genes include genes associated with integrity of the dermal matrix (FBN1, FBLN1, TNXB, FN1, LOXL2, COL3A1, COL1A1, ELN and LOXL1), genes associated with dermal inflammation initiated remodeling (TIMP2, IL1A, TIMP1, TNF, MMP1, MMP9, MMP3, SOD2 and IL1B) and genes associated with epidermal barrier function (KRT2, KRT6A, CLDN1, LOR, FLG, IVL, DRT10, AQP3, and KRT14). Subsets derived from analysis of the expression data for these genes are set forth in Table 1 (dermal markers), Table 2 (matrix remodeling markers, i.e. positive hormetic stress initiators) and Table 3 (markers of an inflammatory cytokine response, i.e. damage outside the repair response).

A genetic expression profile provides information about cellular response to a set of conditions. Genes contain the instructions for making messenger RNA (mRNA). At any given point in time, however, each cell makes mRNA from only a fraction of the genes it carries. A gene is referred to as being turned "on" if it is being used to produce mRNA and is otherwise referred to as being turned "off." The term "regulation" refers to triggering a transcriptional status that is different from a gene's control status. For example, "up-regulation" may include merely turning on, or may refer to increasing a transcriptional rate over a base line rate derived from a control or reference condition.

In expression profiling, the relative amount of mRNA expressed in two or more experimental conditions is measured. Altered levels of mRNA suggest a changed need for the protein coded for by the mRNA. For example, increased transcription of enzyme catalysts or cofactors is observed in response to increased levels of the enzyme's substrate in the cellular environment.

In general, a gene expression profile includes those genes that demonstrate significant differences under changed experimental conditions. This is typically a subset of some dataset, which may include the entire genome. For a type of cell, a group of genes whose combined expression pattern is uniquely characteristic to a given condition constitutes a gene signature of the condition. Gene signatures may be used, for example, to select patients which may benefit from a particular treatment, or to design treatment protocols to maximize a desired signature.

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Genetic expression profiles for the potential gene dataset may be determined using a microarray. Exemplary cDNA microarrays are commercially available and may be purchased from such companies as Agilent Technologies, Affymetrix Inc. (Santa Clara, Calif.), Nanogen (San Diego, Calif.) and Protogene Laboratories (Palo Alto, Calif.). Specific hybridization technology which also may be practiced to generate the expression profiles employed in the subject methods includes the technology described in U.S. Pat. Nos. 5,143,854; 5,288,644; 5,324,633; 5,432,049; 5,470,710; 5,492,806; 5,503,980; 5,510,270; 5,525,464; 5,547,839; 5,580,732; 5,661,028; 5,800,992; the disclosures of which are herein incorporated by reference; as well as WO 95/21265; WO 96/31622; WO 97/10365; WO 97/27317; EP 373 203; and EP 785 280. Generally in these methods, an array of "probe" nucleic acids that includes a probe for each of the phenotype determinative genes whose expression is being assayed is contacted with target nucleic acids as set forth above. Contact is carried out under hybridization conditions, e.g., stringent hybridization conditions and unbound nucleic acid is then removed. The resultant pattern of hybridized nucleic acid provides information regarding expression for each of the genes that have been probed, where the expression information is in terms of whether or not the gene is expressed and, typically, at what level, where the expression data, i.e., expression profile, may be both qualitative and quantitative. Alternatively, the expression profile is determined by quantitative PCR or other quantitative methods for measuring mRNA.

One embodiment of the invention provides a gene panel comprising genes regulated in mammalian skin in response to generation of a radio frequency current in a tissue volume of the mammalian skin sufficient to heat the tissue volume to a treatment temperature. At least one gene is selected from Table 1 or Table 2 and at least one gene is selected from Table 3. In specific embodiments at least one gene is selected from each of the three tables. All gene panels according to the invention are contemplated to include at least one gene from Table 3, since lack of expression shift in these genes indicates lack of a "bad" damage response. Probes may be designed to target each gene constituting a gene chip of the invention in order to construct very specific microarrays with utility in designing, screening, adapting or monitoring treatment regimens for the desired effect, or for validation of the treatment regimen in treated subjects. Microarrays comprising a set of immobilized nucleic acid probes capable of hybridizing to and detecting genes constituting a gene panel according to the invention are contemplated.

As used herein, a "probe" refers to an oligonucleotide, polynucleotide or DNA molecule, whether occurring naturally or produced synthetically, which is capable of specifically

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hybridizing to a nucleic acid with sequences complementary to the probe. The probes of the present invention refer specifically to the oligonucleotides attached to a solid support in the DNA microarray substrate. A probe may be either single-stranded or double-stranded. The probe typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides. The probes herein are selected to be complementary to different strands of a particular target nucleic acid sequence and therefore must be sufficiently complementary so as to be able to specifically hybridize with their respective target strands under a set of pre-determined conditions. Therefore, the probe sequence need not reflect the exact complementary sequence of the target. For example, a non-complementary nucleotide fragment may be attached to the 5' or 3' end of the probe, with the remainder of the probe sequence being complementary to the target strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with the sequence of the target nucleic acid to anneal therewith specifically.

Another embodiment of the invention includes methods for providing a benefit to mammalian skin. The benefit comprises inducing collagen formation and/or dermal remodeling in a dermal layer of the mammalian skin in the absence of a skin-damaging inflammatory cytokine response and the method comprises generating a radio frequency current in a tissue volume of the mammalian skin for a treatment cycle sufficient to heat the tissue volume to a treatment temperature while avoiding an upregulation in expression of genes listed in Table 3. The RF current may be generated a plurality of times in one treatment cycle.

As used herein the terms "treat" and "treatment" and the like generally refer to obtaining a desired cosmetic or aesthetic effect, underpinned by a targeted biological response. "Treatment" as used herein covers treatment in a mammal, particularly a human, and includes: (a) preventing or avoiding development of a cosmetically undesirable skin condition, for example fine lines, wrinkles, hyper-pigmented spots, and other skin irregularities that result from either chronological or environmental aging or impact on the skin, (b) inhibiting, ameliorating or delaying appearance of a cosmetically undesirable skin condition; (c) reversing or causing regression of the cosmetically undesirable skin condition.

One or more treatment cycles according to the invention may be conducted across a treatment period. Treatment cycles may be as short in duration as necessary to effectuate a desired response. In specific embodiments the treatment cycle is about one minute or less, while in other specific embodiments the treatment cycle is greater than about one minute. In other

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specific embodiments a treatment cycle lasts between about 1 and about 6 minutes and in other embodiments lasts between about 2 and about 6 minutes. A treatment period according to the invention comprises one or more treatment cycles and in specific embodiments is at least one week and comprises at least one treatment cycle. In other specific embodiments the treatment period is between one week and 12 weeks. In more specific embodiments the treatment period is between 3 and 8 weeks. In certain embodiments each week of a treatment period comprises between one and six treatment cycles, although it is understood in the art that this may vary with unique features of individual being treated.

Generally, although RF current administration effectuates a higher temperature in the dermis than at the skin surface, skin temperature is most conveniently and noninvasively measured at the surface. Hence, in certain embodiments a treatment temperature, defined herein as the temperature of the tissue volume through which the RF current is conducted, effectuates a skin surface temperature over the tissue volume of less than about 45°C. In more specific embodiments the treatment temperature effectuates a skin surface temperature over the tissue volume of between about 37°C and about 43°C.

Desired benefits according to the invention may be assessed by extracting mRNA from a sample obtained from the tissue volume through which the RF current is conducted; and determining an expression profile of a gene panel consisting of at least one gene selected from Table 1 and/or Table 2 and at least one gene selected from Table 3. A benefit is indicated where an expression profile reflects upregulation of genes selected from Tables 1 and/or 2 combined with substantially no change in expression of genes selected from Table 3 is indicative of a benefit being provided.

Generally, regulation of genes in accordance with the invention is reflected in expression fold change data. It is known in the art that many methods exist for analysis of microarray based experiments to identify genes that are differentially expressed between conditions, and that choice of methods may affect the set of genes that are identified. Fold-change appears to provide the most reproducible results. Fold-change may be defined as the ratio of the mean control and mean treatment observations (or as the difference of the mean log control and mean log treatment data). A fold-change of 1, therefore, represents no change over the control observation. A positive fold-change indicates an increase in expression across a time period referred to herein as upregulation, and a negative fold change indicates a decrease in expression across a time period referred to

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herein as downregulation. The significance of a fold-change may be determined by ordinary statistical methods.

According to certain aspects of the invention, the benefit may be optimized by adjusting the treatment temperature and/or other treatment parameters in response to the expression profile wherein upregulation of the genes selected from Table 1 and/or Table 2 is maximized while expression of genes selected from Table 3 is maintained at substantially no change. The treatment regimens of the invention may comprise maintenance treatments wherein a benefit is maintained beyond the treatment period by one or more maintenance treatments, each maintenance treatment comprising at least one maintenance cycle.

The inventive skin treatment regimens contemplate application of thermal energy by electrically conducted RF current via a handheld RF current delivery device. Preferred RF delivery devices according to the invention deliver RF as a current electrically conducted between two electrodes placed at some distance from one another on the skin. The delivery device that effectuates the benefits of the invention conducts the radio frequency current through the tissue volume in the absence of electromagnetic radiation in the visible light or infrared frequencies of the electromagnetic spectrum and in the absence of supplemental monochromatic or polychromatic light sources directed toward the tissue volume, as well as in the absence of mechanical cooling.

Certain embodiments of the invention provide the capability for assessing efficacy of any energy delivery device or combination of energy delivery technologies in providing benefits according to the invention. Hence, specific methods for assessing treatment efficacy of an energy delivery device designed to provide a benefit to skin by heating the skin comprise: treating the skin by application of the energy delivery device; extracting mRNA from a sample of the treated skin; and generating an expression profile for a gene panel according to the invention. As illustrated in **Example 3**, below, RF current delivery devices exist comprising parameter specification tolerances capable of being dialed in to yield the expression profile according to the invention. A suitable device is manufactured by Syneron. An example of a device that is not designed to achieve the genomic response goals of the instant invention is a handheld personal use RF delivery device manufactured by Ultragen Ltd for distribution in Europe under the brand STOP™. This device, also illustrated in Example 3 for comparative purposes, fails to elicit the upregulation of genes in Tables 1 and 2. The device specifications are designed to accommodate a regimen that is based on provision of substantially less thermal energy applied over a larger

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skin area and may not reach the levels required to achieve the positive homeotic effect achieved by devices and regimens in accordance with the present invention.

According to another embodiment of the invention, methods of screening a facial skin treatment regimen are provided. The methods are screened for efficacy in providing a collagen and/or dermal remodeling benefit to mammalian skin without stimulating a skin-damaging inflammatory cytokine response. The methods generally comprise treating facial skin according to a treatment regimen; extracting mRNA from a sample of the treated facial skin; generating a gene expression profile for a gene panel according to the invention; comparing the gene expression profile to a reference profile; and determining that the facial treatment regimen is efficacious where the expression profile reflects upregulation of genes selected from Table 1 and/or Table 2 and a substantial lack of regulation of genes selected from Table 3. In specific embodiments the facial skin treatment regimen comprises generating a pulsed radio frequency current through a first tissue volume of the facial skin over a treatment cycle with a radio frequency current generating device. The treatment regimen may comprise moving the radio frequency current generating device and generating a pulsed radio frequency current through a second tissue volume of the facial skin during the treatment cycle.

In screening methods, areas of skin are selected for concordance with characteristics of target treatment areas, in particular of the face. Cosmetically vulnerable areas of facial skin include the periorbital and perioral areas. Hence, facial skin around the ear, known as periauricular skin, is close in structure to the treatment target areas but not readily visible in the case of blemishes which may result from minor biopsy procedures. In aspects of the invention requiring biopsy therefore, the required sample is obtained by biopsy of treated periauricular skin and the reference is obtained by biopsy of pre-treated or non-treated periauricular skin. Generally, the pre-treated or non-treated periauricular reference skin comprises skin substantially adjacent to the treated periauricular skin. In very specific embodiments the expression profile of genes selected from Table 1 and/or Table 2 reveals a fold-change over the reference that is statistically greater than one.

Notably, the instant invention may provide benefits to skin other than facial skin, however facial skin is highlighted in illustrative examples because it poses the greatest cosmetic treatment challenges in that it manifests most readily the effects of both chronological and environmental aging and damage, and is initially thinner and more vulnerable than skin over other areas of the

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body. Further, facial skin is an area of paramount aesthetic significance to consumers of cosmetic treatment technologies.

In certain embodiments the benefit provided by application of RF-current may be augmented or enhanced by application of a cosmetic active or composition to the facial skin in conjunction with generating the pulsed radio frequency current. The active or composition may have a potentiating or synergistic effect on the gene expression profiles according to the invention. Non-limiting examples of suitable cosmetic actives include Retinol Propionate and derivatives thereof, caffeine, Hyaluronic Acid, and generally plant extracts.

Screening methods according to the invention comprise substantially the same parameters as treatment methods according to the invention with respect to treatment cycles, treatment periods, treatment temperatures and delivery devices, adjusted for desired screen tolerances.

In accordance with further embodiments of the invention, a gene signature of differentially expressed genes suitable for identifying a cosmetic skin benefit is provided. The benefit comprises induction of collagen formation and/or dermal remodeling in a dermal layer of mammalian skin in the absence of a skin-damaging inflammatory cytokine response, the genetic signature comprising at least one gene selected from each of Table 1, Table 2, and Table 3. Generally a gene signature is a subset of genes obtained from a dataset of genes related to a particular characteristic, trait or biological function. Gene signatures may be obtained from all or a part of a gene dataset and signatures according to the invention may comprise information from at least about two genes, or any number of genes up to the number constituting the total dataset. Where a subset of the dataset is used, the subset may comprise upregulated genes, downregulated genes, substantially unregulated genes or combinations thereof.

The following Examples are provided to illustrate certain features and advantages of various embodiments of the invention and should not be construed as limiting the scope thereof.

EXAMPLES

Example 1: Treatment protocol

This example illustrates treatment of the periauricular region of facial skin for purposes of conducting genomic and histological assessments. Therefore, all treatments are technician-

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administered in a clinical setting for control purposes. Treatments are administered to four zones within the treatment area for a total of 16 minutes with 4 minutes per zone, 3 times per week for six weeks. The protocol is according to a split face/neck study in a within-subject control design with the treatment applied only to the periauricular region of one side and a sham treatment consisting of gel plus an unpowered device applied to the corresponding periauricular region of the other side. Treatments are monitored with thermal imaging cameras to ensure that target skin surface temperatures of 40-43°C are reached within the first minute and maintained but not exceeded during each 4 minute treatment.

The treatment site is located and centered just below the ear. The site is approximately 1.5 inches wide and about 4 inches long. If an area of treated skin is too dry or gets too hot, extra gel may be applied. The face and neck of the subject are clean and free of lotions, perfumes and the like. Hair is clipped back from the treatment area.

Device treatment:

1. One pump (approximately 1.4 g gel) is applied to treatment site and spread evenly without rubbing. An additional pump of gel to the cheek above the treatment area may be applied as needed during treatment to cool skin if it gets too hot, as expressed by the subject.
2. The device is turned on and a 4 minute timer is set.
3. The device applicator head is applied to the skin and moved along the treatment site using a back and forth motion and a light touch. Connectivity is maintained while maneuvering the device head across the treatment area. Proper contact with the skin is indicated by a lens on the applicator which should continue to flash red to indicate contact.
4. The thermal camera image is monitored during treatment. Within 30-60 seconds, the skin in the treatment area reaches the optimal temperature of 40-43°C. Color patterns on the thermal image indicate temperature. Generally, as the device warms up the applicator is moved rapidly across the skin to avoid discomfort from the rising temperature. As the treatment continues, movement is slowed to maintain ideal temperature. Subjects are warned that the device will feel warm, but will not burn them.
5. The temperature is in excess of 43°C if the thermal image shows solid red areas. Temperature is controlled by increasing treatment speed in the back and forth motion, by slightly increasing the size of the treatment area, by alternating the back and forth motion with a figure

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eight pattern, or by adding more gel by touching the applicator head to extra gel deposited on the cheek for this contingency.

6. If the treatment area fails to reach optimal temperature, or if it cools off, the back and forth motion should be slowed until the bulk of the treatment area is at the target temperature.

Example 2: Biopsy protocol

This example illustrates the relatively gentle biopsy protocol used to collect samples for assessment and monitoring purposes in accordance with certain aspects of the invention and support data disclosed herein.

A clinical biopsy study was conducted using an RF-current delivery device in 30 adult female subjects. Treatments were provided in the periauricular (around the ear) region of the facial skin. Periauricular skin is known in the cosmetic arts as a suitable periorbital substitute. Treatments were clinically administered using a Syneron V8 device (see Example 3), and a four minute heating profile as the target profile. Subjects received treatment three times per week for a total of six weeks. Biopsies were conducted to inspect skin histology as a function of treatment, and to generate a genomics profile.

Biopsy procedure:

One 4mm biopsy was taken from the periauricular area from both the right and left sides of the neck (this area is located just below the ear). Using a 30 gauge sterile needle, an anesthetic containing 2% xylocaine with epinephrine is injected just under the skin to be biopsied. Once the subject indicates that the area is numb, an appropriate size punch biopsy is collected using standard aseptic techniques, followed by suture closure. A 20% aqueous solution of aluminum chloride is used for hemostasis, as needed. Following biopsy the punch site is monitored for normal healing and sutures are removed after 7 days.

Post biopsy sample handling:

The biopsied tissue is split into two separate samples, one for gene expression testing and the other for histological evaluation. The sample is divided into equal halves in a line parallel to a line drawn from the stratum corneum to the dermis. Sample handling is in accordance with industry standards.

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Example 3: mRNA extraction and analysis

This example illustrates RNA biomarker identification and analysis that underpins certain embodiments of the invention including gene chips, genetic signatures and discovery of the biological model that guides RF delivery regimen design.

4mm biopsies in accordance with Example 2 were taken from both sides of the face/neck just below the ear as described above 2 times, once at 24 hours post final treatment, and once at 4 weeks post treatment.

The biopsied samples were transferred to 2 ml centrifuge tubes containing 1.5 ml tissue storage reagent (RNAlater® solution, invitrogen, Life Technologies, Carlsbad, CA). Tubes were refrigerated overnight at 2-8°C. Storage reagent was removed and the samples were placed in a freezer at -80°C until processing. Just prior to processing, samples were removed from the freezer and 1.5 ml monophasic solution of phenol and guanidine isothiocyanate (TRIzol® reagent, Invitrogen, Life Technologies, Carlsbad, CA) and one 3 mM Tungsten carbide bead (3 mM Tungsten Carbide beads, Qiagen, Catalog #69997) were added to each tube. The samples were immediately homogenized in a mixer mill (Quiagen Inc.) with four 3 minutes shades at 30/second, flipping the adapter after each. Samples were centrifuged for 10 minutes at 12,000 rpm to remove debris. The supernatant was then transferred to pre-spun Phase Lock gel heavy tubes (Phase Lock gel heavy tubes, Eppendorf, New York, NY, catalog #0032-005-152), 300 µL chloroform (Sigma) was added, and the tubes shaken vigorously without over-vortexing. The samples were centrifuged for 10 minutes at 12,000 rpm and the supernatant was thereafter transferred into new 2 ml centrifuge tubes.

Binding conditions were adjusted by adding 800 µl 70% ethanol, and the tubes were vortexed to mix and then spun briefly. 830 ml of the sample was transferred to an RNeasy mini spin column in a vacuum manifold, and a vacuum was applied. The remaining sample (approx. 830 ml) was transferred onto the same RNeasy column and a vacuum was applied. Contaminants were removed by sequentially pipetting 700µl and 500µl of buffer RW1 onto the RNeasy column with vacuum applied after each rinse.

The RNeasy spin column was transferred to a new 2ml collection tube and the tube was centrifuged for 2 minutes at 14,000 rpm, and the column was transferred into anew 1.5 ml collection tube. Residual ethanol was aspirated from the inside ridge of each column. Thirty µl

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of pre-heated Rnase-free water was pipetted directly onto the RNeasy membrane and the membrane was incubated for 5 minutes and then centrifuged for 2 minutes at 14,000 rpm. The elute was then collected, providing ready-to-use RNA in water.

After isolation, RNA yield was determined using the RNA 6000 Nano LabChip® Kit #5065-4476, available from Agilent Technologies, Inc. of Santa Clara, CA. RNA was evaluated by one-step RT-PCR. For the RT-PCR biomarker analysis, RNA was diluted to a final concentration of 5 ng/well.

Purified RNA was converted to cDNA with an RT-PCR kit (QScript™ One-Step Fast MGB qRT-PCR kit, available from Quanta BioScience, Inc., Gaithersburg, MD). Five hundred nanograms of RNA was then mixed with the QScript enzyme/buffer mix and run on a thermal cycler according to kit instructions. One µl of the resulting cDNA was then mixed with the Quanta Perfecta Master Mix, and aliquoted across a custom array plate (Custom RT Profiler™ PCR Array available from SABiosciences, Corp., Frederick, MD) containing pre-validated primers for the following genes: KRT2, KRT6A, CLDN1, LOR, FLG, IVL, KRT10, AQP3, KRT14, FBN1, FBNL1, TNXB, FN1, LOXL2, COL3A1, COL1A1, ELN, LOXL1, TIMP2, IL1A, TIMP1, TNF, MMP1, MMP9, MMP3, SOD2, and IL1B. The array plates were then sealed and run on a thermal cycler (StepOnePlus™ Real-Time PCR System Upgrade, from Applied Biosystems, Inc. Foster City, CA).

Data analysis was performed using the data analysis software provided by SABiosciences of Frederick, MD.

Expression fold change data for the 27 compilation genes listed above at the 24 hour and 4 week target times are set forth in Figures 4A, 4B and Figures 6A, 6B, respectively, above. Genes associated with the dermal matrix are grouped as 90 and 91, the genes associated with dermal inflammation remodeling are grouped as 92 and 93, and the genes associated with epidermal differentiation are grouped together as 94 and 95. Fold change data for exemplary genes are set forth in Figures 5A collagen 1A1, 5B elastin, and Figure 7A collagen 1A1 and 7B elastin. Note the co-regulation between many of the collagen1A1 genes and the elastin genes.

The fold change data was subject to inspection and analysis and groupings it was surprisingly discovered that when genes demonstrating very little regulation in response to the treatment were segregated (IL1A, IL1B and TNF), they comprised the inflammatory cytokine response genes, while genes relating to collagen synthesis and turnover and dermal remodeling

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were upregulated under treatment conditions. The genes selected on the basis of fold change data are tabled accordingly in Tables 1, 2 and 3, below.

Further, it was surprisingly found that the positive impact of the treatment regimen on gene expression that existed at 24 months were maintained nearly unabated at the 1 month out target. Maintenance treatments may be desirable to maintain the genomic effects for periods beyond this.

It is contemplated that the gene expression profile reflects a downstream upregulation in the translation products. Although the observed fold change may be relatively low, the sustained upregulation in synthesis of certain proteins and enzymes over an extended period of time is considered to confer substantially enduring cosmetic benefit to the skin.

Example 4 Personal Care Composition

The following is an exemplary personal care composition for use with the devices of the present invention.

TRADE OR COMMON NAME	INCI NAME	Function	PERCENT COMPOSITION		By Wt %
			As Added	Chem. Content	Possible Range
Purified Water	Water (Aqua)	Vehicle	87.75	87.75	50.0-96.0
Glycerin	Glycerin	Humectant	6.00	6.00	1.0-50.0
Simulgel INS 100	Hydroxyethyl acrylate Sodium Acryloyldimethyl taurate Copolymer	Thickener	3.00	1.08	0.0-3.0
	Isohexadecane	Skin Conditioner		0.72	0.0-2.5
	Polysorbate 60	Emulsifier Surfactant		0.12	0.0-0.30
	Water	Vehicle		1.08	0.0-3.0
Dow Corning 1503	Dimethicone	Skin Conditioner	1.00	0.88	0.0-3.0
	Dimethiconol	Skin Conditioner		0.12	0.0-0.03
Hexylene Glycol	Hexylene Glycol	Emulsifier Surfactant	1.00	1.00	0.0-1.00
Phenoxyethanol	Phenoxyethanol	Emulsifier Surfactant	0.50	0.50	0.0-2.0
Nipagin M	Methylparaben	Preservative	0.20	0.20	0.0-2.0
Dissolvine NA2 S	Disodium EDTA	Chelating Agent	0.05	0.05	0.0-1.0
Microthene FN-510	Polyethylene	Acute Powder	0.50	0.50	0.0-1.0

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100.0	100.0	100.0
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The dimensions and values disclosed herein are not to be understood as being strictly limited to the exact numerical values recited. Instead, unless otherwise specified, each such dimension is intended to mean both the recited value and a functionally equivalent range surrounding that value. For example, a dimension disclosed as “40 mm” is intended to mean “about 40 mm”.

All documents cited in the Detailed Description of the Invention are, in relevant part, incorporated herein by reference; the citation of any document is not to be construed as an admission that it is prior art with respect to the present invention. To the extent that any meaning or definition of a term in this written document conflicts with any meaning or definition of the term in a document incorporated by reference, the meaning or definition assigned to the term in this written document shall govern.

While particular embodiments of the present invention have been illustrated and described, it would be obvious to those skilled in the art that various other changes and modifications can be made without departing from the spirit and scope of the invention. It is therefore intended to cover in the appended claims all such changes and modifications that are within the scope of this invention.

Table 1
Dermal Markers

Gene	Expression Product	Function
FBN1 (SEQ ID NO: 1)	A member of the fibrillin family; a large, extracellular matrix glycoprotein that serve as a structural component of 10-12 nm calcium-binding microfibrils which provide force bearing structural support in elastic and nonelastic connective tissue throughout the body.	Fibrillins are structural components of 10-12 nm extracellular calcium-binding microfibrils, which occur either in association with elastin or in elastin-free bundles. Fibrillin-1-containing microfibrils provide long-term force bearing structural support
FBLN1 (SEQ ID NO: 2)	A secreted glycoprotein that becomes incorporated into a fibrillar extracellular matrix. Calcium-binding is apparently required to mediate its binding to laminin and nidogen. It mediates platelet adhesion via binding fibrinogen.	Incorporated into fibronectin-containing matrix fibers. May play a role in cell adhesion and migration along protein fibers within the extracellular matrix (ECM). Could be important for certain developmental processes and contribute to the supramolecular organization of ECM architecture, in particular to those of basement membranes. Implicated in a role in cellular transformation and tumor invasion; appears to be a tumor suppressor. May play a role in haemostasis and thrombosis owing to its ability to bind fibrinogen and incorporate into clots. Could play a significant role in modulating

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		the neurotrophic activities of APP, particularly soluble APP
TNXB (SEQ ID NO: 3)	A member of the tenascin family of extracellular matrix glycoproteins. The tenascins have anti-adhesive effects, as opposed to fibronectin which is adhesive. This protein is thought to function in matrix maturation during wound healing.	Appears to mediate interactions between cells and the extracellular matrix. Substrate-adhesion molecule that appears to inhibit cell migration. Accelerates collagen fibril formation. May play a role in supporting the growth of epithelial tumors
FN1 (SEQ ID NO: 4)	fibronectin, a glycoprotein present in a soluble dimeric form in plasma, and in a dimeric or multimeric form at the cell surface and in extracellular matrix. Fibronectin is involved in cell adhesion and migration processes including embryogenesis, wound healing, blood coagulation, host defense, and metastasis.	Fibronectins bind cell surfaces and various compounds including collagen, fibrin, heparin, DNA, and actin. Fibronectins are involved in cell adhesion, cell motility, opsonization, wound healing, and maintenance of cell shape. Interaction with TNR mediates inhibition of cell adhesion and neurite outgrowth (By similarity)
LOXL2 (SEQ ID NO: 5)	A member of the lysyl oxidase gene family.	The prototypic member of the family is essential to the biogenesis of connective tissue, encoding an extracellular copper-dependent amine oxidase that catalyses the first step in the formation of crosslinks in collagens and elastin. A highly conserved amino acid sequence at the C-terminus end appears to be sufficient for amine oxidase activity, suggesting that each family member may retain this function. The N-terminus is poorly conserved and may impart additional roles in developmental regulation, senescence, tumor suppression, cell growth control, and chemotaxis to each member of the family.
COL 3A1 (SEQ ID NO: 6)	The pro-alpha1 chains of type III collagen, a fibrillar collagen that is found in extensible connective tissues such as skin, lung, uterus, intestine and the vascular system, frequently in association with type I collagen.	Collagen type III occurs in most soft connective tissues along with type I collagen
COL 1A1 (SEQ ID NO: 7)	The pro-alpha1 chains of type I collagen whose triple helix comprises two alpha1 chains and one alpha2 chain.	Type I is a fibril-forming collagen found in most connective tissues and is abundant in bone, cornea, dermis and tendon.
ELN (SEQ ID NO: 8)	A protein that is one of the two components of elastic fibers. The encoded protein is rich in hydrophobic amino acids such as glycine and proline, which form mobile hydrophobic regions bounded by crosslinks between lysine residues.	Major structural protein of tissues such as aorta and nuchal ligament, which must expand rapidly and recover completely. Molecular determinant of the late arterial morphogenesis, stabilizing arterial structure by regulating proliferation and organization of vascular smooth muscle.
LOXL1 (SEQ ID NO: 9)	A member of the lysyl oxidase gene family.	The prototypic member of the family is essential to the biogenesis of connective tissue, encoding an extracellular copper-dependent amine oxidase that catalyses the first step in the formation of crosslinks in collagens and elastin.

Table 2
Matrix Remodeling Markers

Gene	Expression Product	Function
TIMP2 (SEQ ID NO: 10)	Inhibitors of the matrix metalloproteinases: Complexes with metalloproteinases (such as	Has a unique role among TIMP family members in its ability to directly suppress the

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ID NO: 10)	collagenases) and irreversibly inactivates them. Known to act on MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-13, MMP-14, MMP-15, MMP-16 and MMP-19	proliferation of endothelial cells. As a result, the encoded protein may be critical to the maintenance of tissue homeostasis by suppressing the proliferation of quiescent tissues in response to angiogenic factors, and by inhibiting protease activity in tissues undergoing remodelling of the extracellular matrix
TIMP1 (SEQ ID NO: 11)	Complexes with metalloproteinases (such as collagenases) and irreversibly inactivates them. Also mediates erythropoiesis in vitro; but, unlike IL-3, it is species-specific, stimulating the growth and differentiation of only human and murine erythroid progenitors. Known to act on MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-11, MMP-12, MMP-13 and MMP-16. Does not act on MMP-14	Promotes cell proliferation in a wide range of cell types, and may also have an anti-apoptotic function. Transcription of this gene is highly inducible in response to many cytokines and hormones.
MMP1 (SEQ ID NO: 12)	Matrix metalloproteinase (MMP) family of proteins: Cleaves collagens of types I, II, and III at one site in the helical domain. Also cleaves collagens of types VII and X. In case of HIV infection, interacts and cleaves the secreted viral Tat protein, leading to a decrease in neuronal Tat's mediated neurotoxicity	Proteins of the Matrix metalloproteinase (MMP) family of proteins are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. Activated when cleaved by extracellular proteinases. A secreted enzyme which breaks down the interstitial collagens, types I, II, and III.
MMP9 (SEQ ID NO: 13)	Matrix metalloproteinase (MMP) family of proteins Cleaves KiSS1 at a Gly- -Leu bond. Cleaves type IV and type V collagen into large C-terminal three quarter fragments and shorter N-terminal one quarter fragments. Degrades fibronectin but not laminin or Pz-peptide	Degrades type IV and V collagens; The enzyme may be involved in IL-8-induced mobilization of hematopoietic progenitor cells from bone marrow, and may have a role in tumor-associated tissue remodeling. May play an essential role in local proteolysis of the extracellular matrix and in leukocyte migration. Could play a role in bone osteoclastic resorption.
MMP3 (SEQ ID NO: 14)	Matrix metalloproteinase (MMP) family of proteins	Degrades fibronectin, laminin, collagens III, IV, IX, and X, and cartilage proteoglycans. The enzyme is thought to be involved in wound repair, progression of atherosclerosis, and tumor initiation.

Table 3
Markers of Inflammatory Cytokine Response

Gene	Expression Product	Function
IL1B (SEQ ID NO: 15)	Member of the interleukin 1 cytokine family. This cytokine is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1 (CASP1/ICE)	This cytokine is an Important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. The induction of cyclooxygenase-2 (PTGS2/COX2) by this cytokine in the central nervous system (CNS) is found to contribute to inflammatory pain hypersensitivity. Produced by activated macrophages, IL-1 stimulates thymocyte proliferation by inducing IL-2 release, B-cell maturation and proliferation, and fibroblast growth factor activity. IL-1 proteins are involved in the inflammatory response, being identified as endogenous pyrogens, and are

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		reported to stimulate the release of prostaglandin and collagenase from synovial cells
TNF (SEQ ID NO: 16)	A multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily and mainly secreted by macrophages; binds to and thus function through its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFR1 .	Involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation; Implicated in a variety of diseases, including autoimmune diseases, insulin resistance, and cancer. May have a neuroprotective function. It is mainly secreted by macrophages and can induce cell death of certain tumor cell lines. It is potent pyrogen causing fever by direct action or by stimulation of interleukin-1 secretion and is implicated in the induction of cachexia, Under certain conditions it can stimulate cell proliferation and induce cell differentiation
IL1A (SEQ ID NO: 17)	A member of the interleukin 1 cytokine family; produced by monocytes and macrophages as a proprotein, which is proteolytically processed and released in response to cell injury, and thus induces apoptosis.	Stimulates thymocyte proliferation by inducing IL-2 release, B-cell maturation and proliferation, and fibroblast growth factor activity. IL-1 proteins are involved in the inflammatory response, being identified as endogenous pyrogens, and are reported to stimulate the release of prostaglandin and collagenase from synovial cells

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CLAIMS

What is claimed is:

1. A method for regulating the condition of mammalian skin, wherein the skin has at least three layers: a stratum corneum exterior layer; an epidermis; and a dermis, comprising the steps of:
 - (a) applying a first personal care composition to an area of skin where regulation is desired, wherein the first personal care composition comprises a gel composition; and
 - (b) delivering energy to the dermis to heat collagen in the dermis such that the heated collagen in the dermis heats the epidermis and stratum corneum until the stratum corneum reaches an external temperature of from about 37°C to about 48°C, wherein the energy delivery to the dermis is then controlled to maintain the temperature of the stratum corneum in the range of from about 37°C to about 48°C.
2. The method of claim 1, wherein the energy is delivered by an RF energy device and the RF energy is delivered via two or more electrodes that contact the stratum corneum via the gel composition.
3. The method of claim 2, wherein when the RF energy device is turned on it delivers the RF energy in the range of 35% to about 65% of full power for the for about 20 to about 50 seconds, then the power is increased to from about 65% to about 100% for about 20 to about 50 seconds, then energy delivery is controlled such that the temperature of the stratum corneum is maintained in the range of from about 37°C to about 48°C.
4. The method of claim 1, wherein the energy delivery device is handheld and is applied under an eye of a consumer and moved underneath the eye to just above the crows feet area, and then the direction is reversed, and the energy delivery device is moved back and forth across this path for from about 3 to about 6 minutes.
5. The method of claim 1, wherein the energy delivery device is handheld and is applied above an eye of a consumer and moved over the eye to just below the crows feet area, and then the direction is reversed, and the energy delivery device is moved back and forth across this path for from about 3 to about 6 minutes.

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6. The method of claim 4, wherein the method is repeated underneath the other eye of the consumer.
7. The method of claim 5, wherein the method is repeated above the other eye of the consumer.
8. The method of claim 1 wherein the gel composition has an electrical conductivity of from about 1,000 to about 2,000 $\mu\text{S}/\text{cm}$.
9. The method of claim 1 wherein the device is handheld and is used in a multi-step regimen comprising the steps of applying the energy delivery device under one eye of a consumer and moving it to just above the crows feet area in a continuous back and forth motion for from about 3 to about 6 minutes, then applying the energy delivery device above an eye of the consumer and moving it to just below the crows feet area in a continuous back and forth motion for from about 3 to about 6 minutes, repeating these two steps on the other eye of the consumer such that the crows feet area adjacent both eyes of the consumer are each treated for from about 6 to about 12 minutes.
10. The method of claim 9 wherein the multistep regimen is completed at least once per day, for a regimen period of 3 to 5 days per week, for from about 3 weeks to about 6 weeks.
11. The method of claim 10, wherein the consumer waits for about 2 to about 8 months and then repeats the multi step regimen for the regimen period.
12. The method of claim 10, wherein fine line and wrinkles around the eyes of the consumer are visibly reduced after each regimen period.
13. The method of claim 2 wherein the RF energy delivery device does not emit light and it does not produce electromagnetic energy.

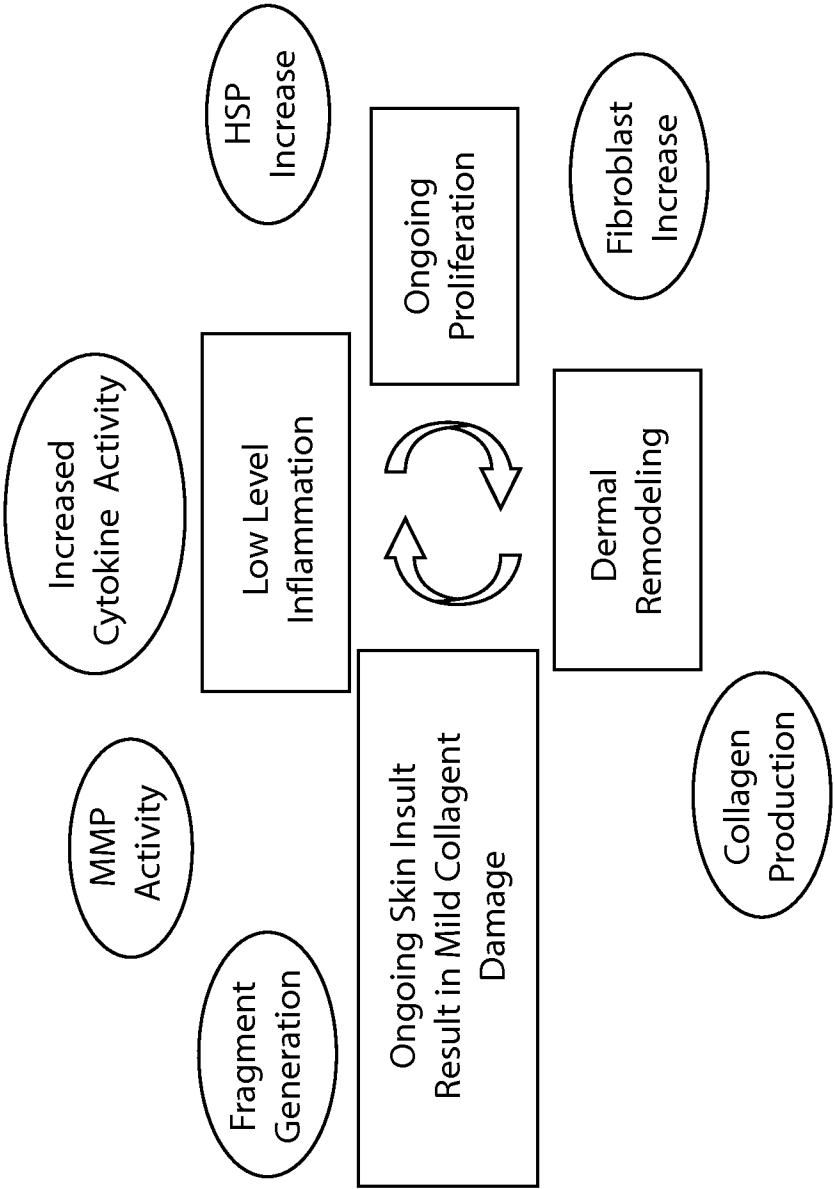


Fig. 1

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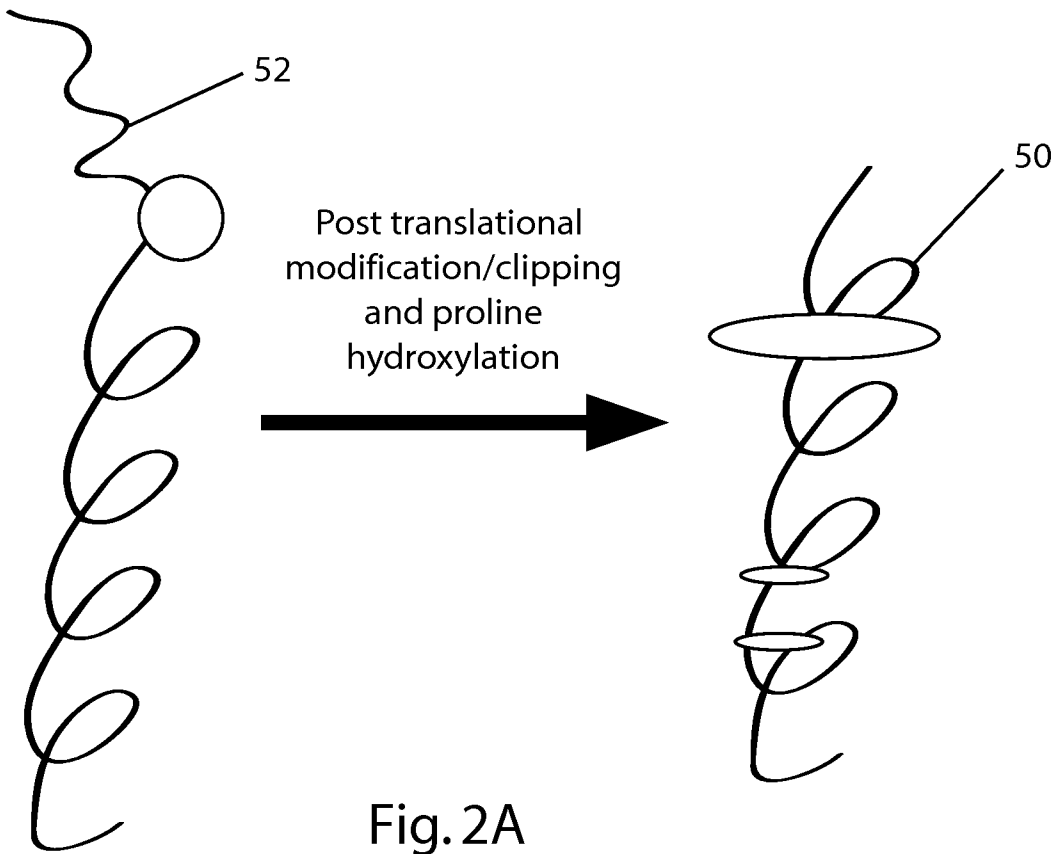


Fig. 2A

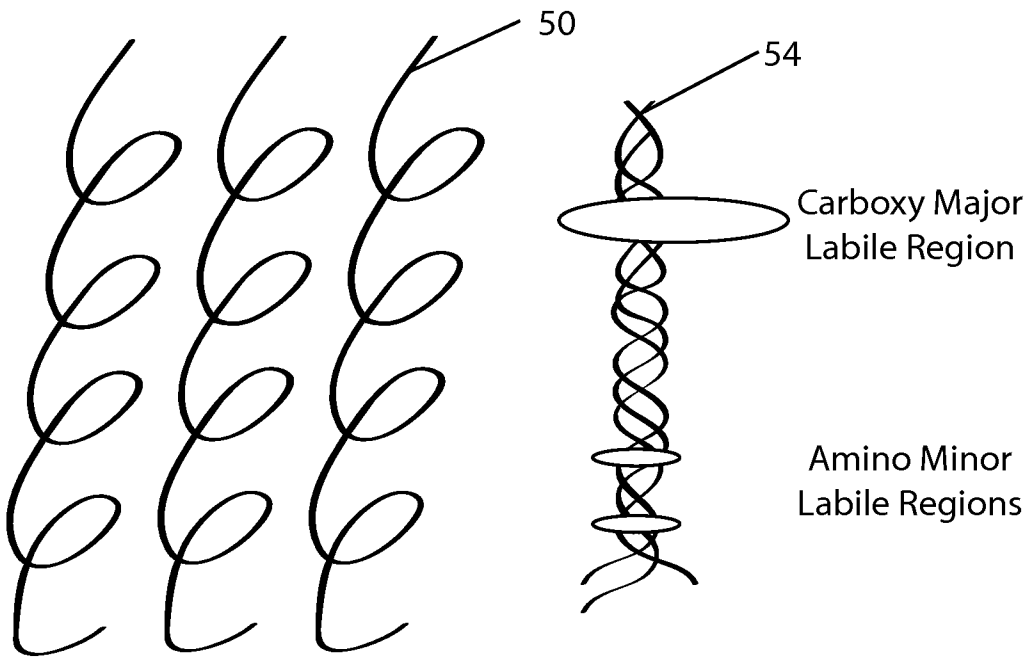


Fig. 2B

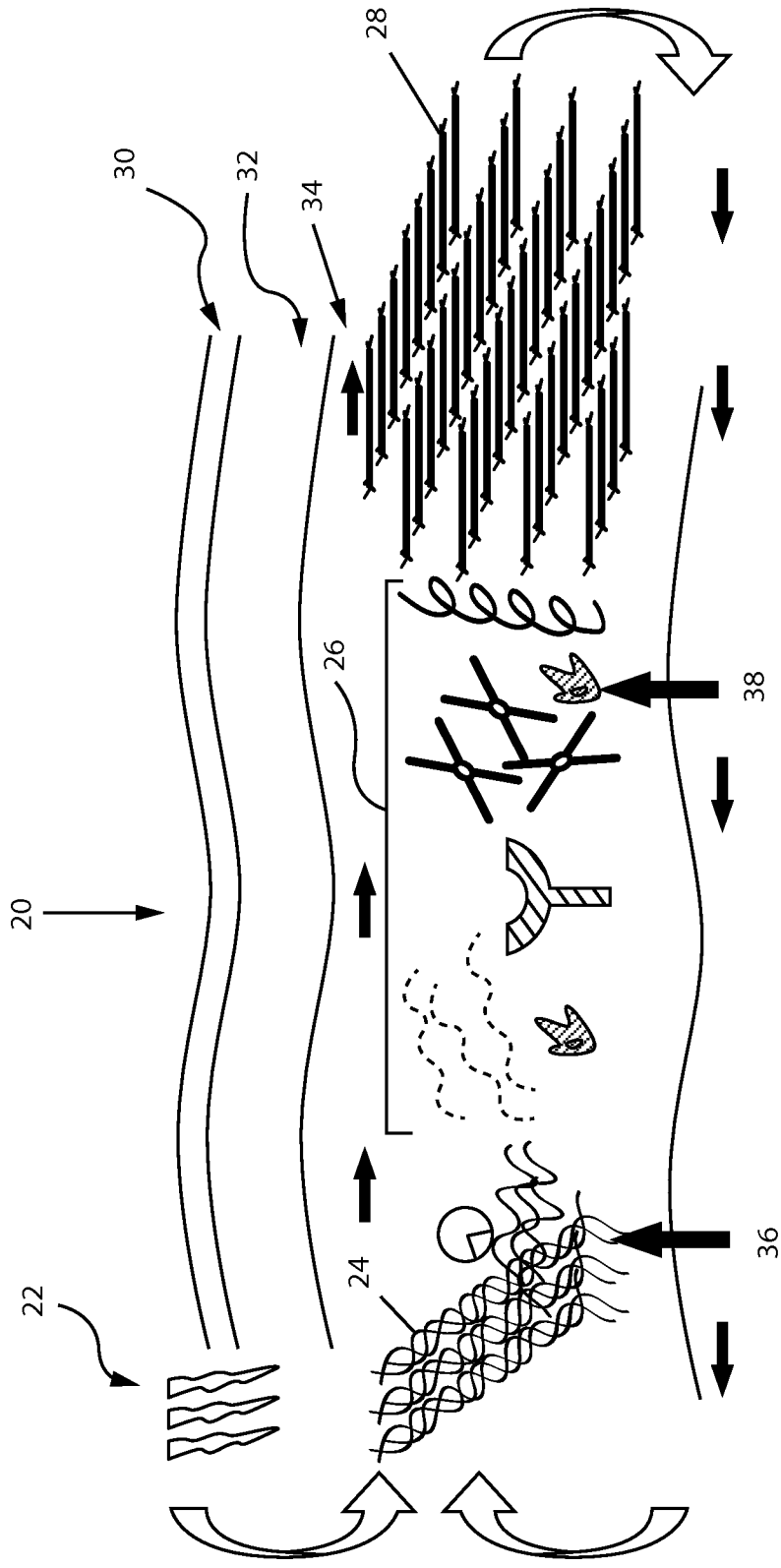


Fig. 3

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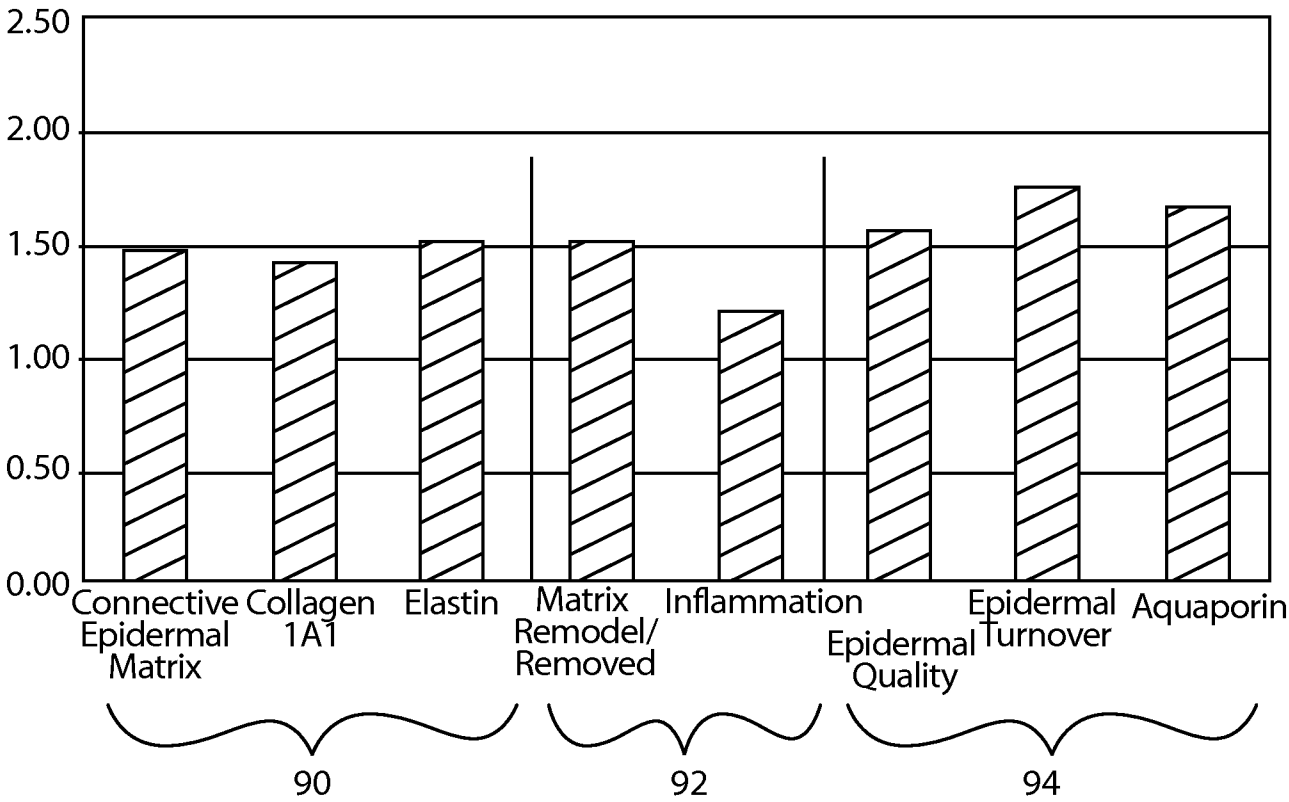


Fig. 4A

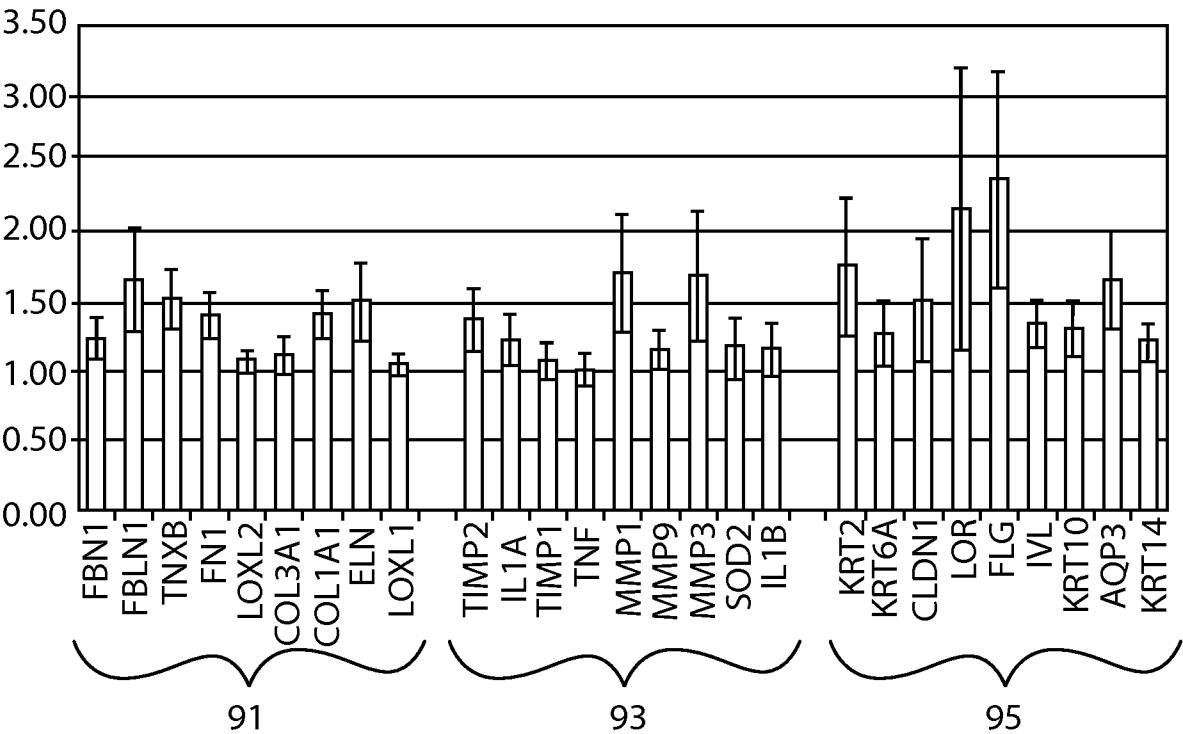


Fig. 4B

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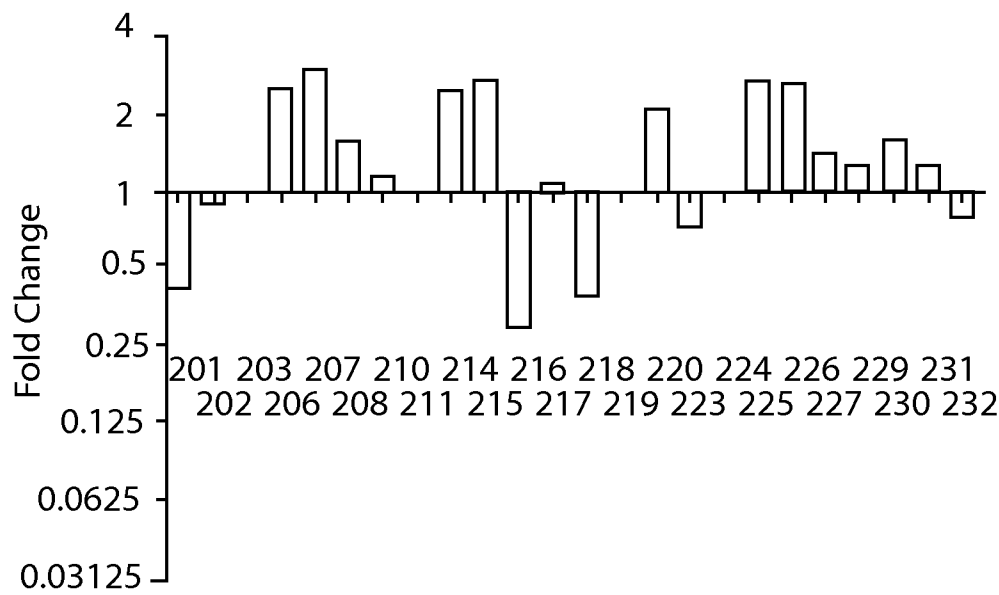


Fig. 5A

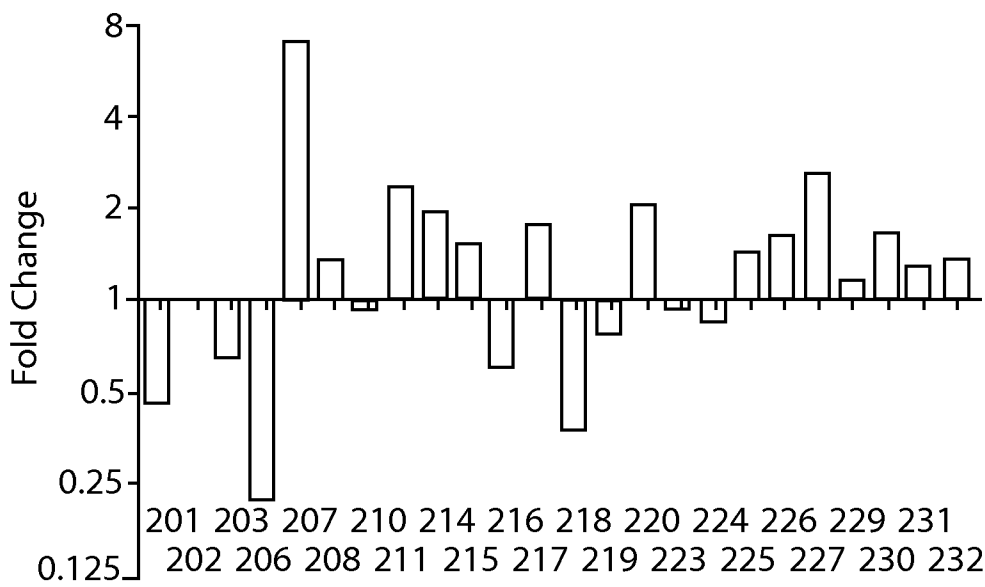


Fig. 5B

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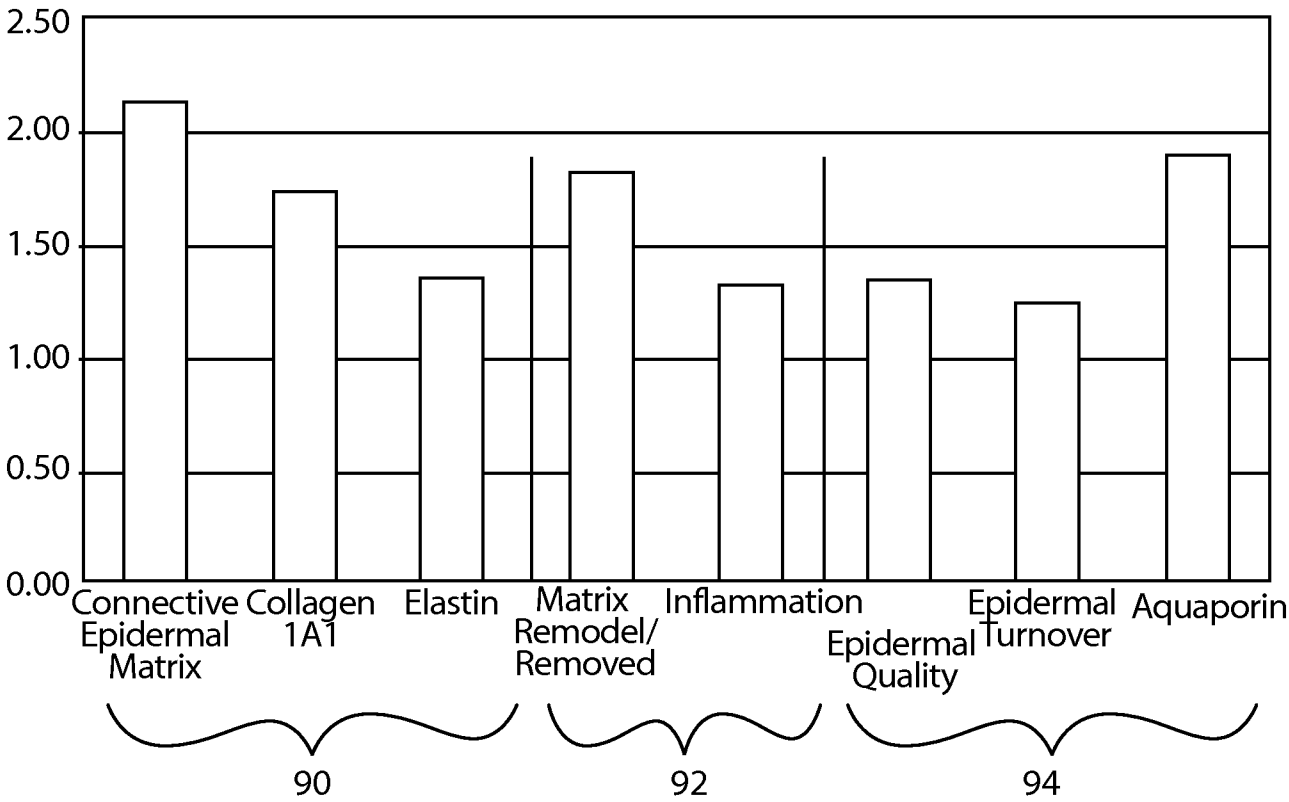


Fig.6A

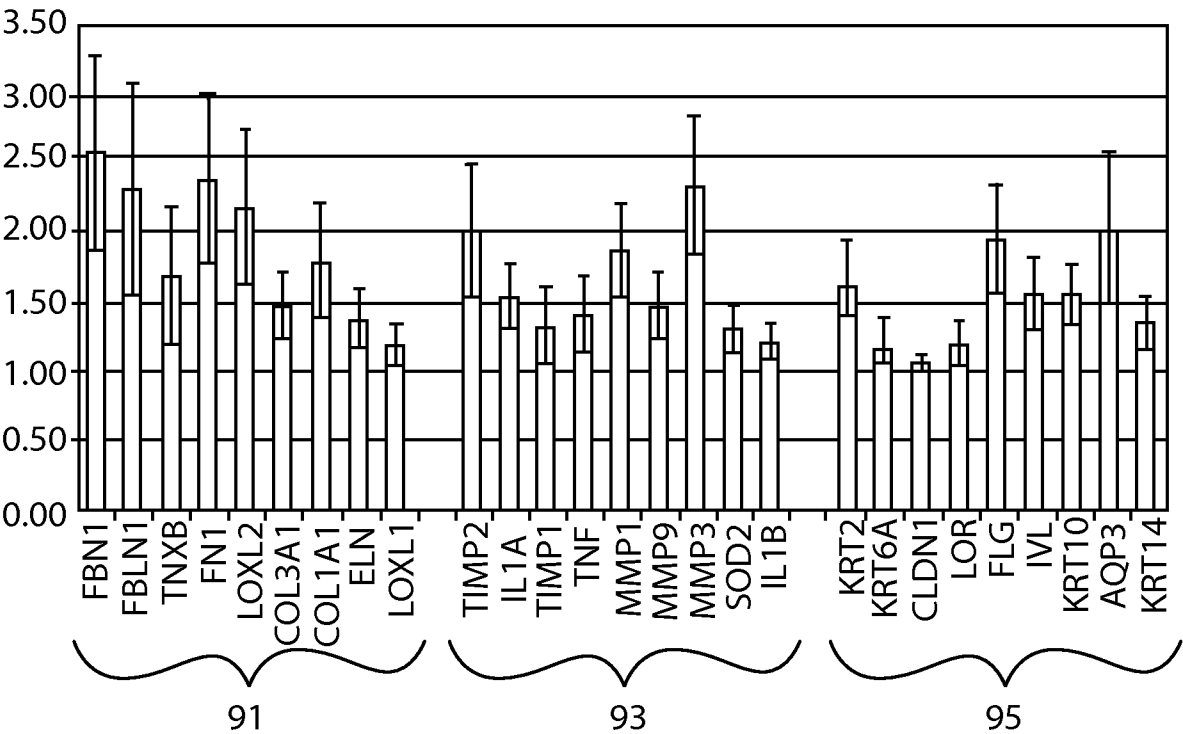


Fig.6B

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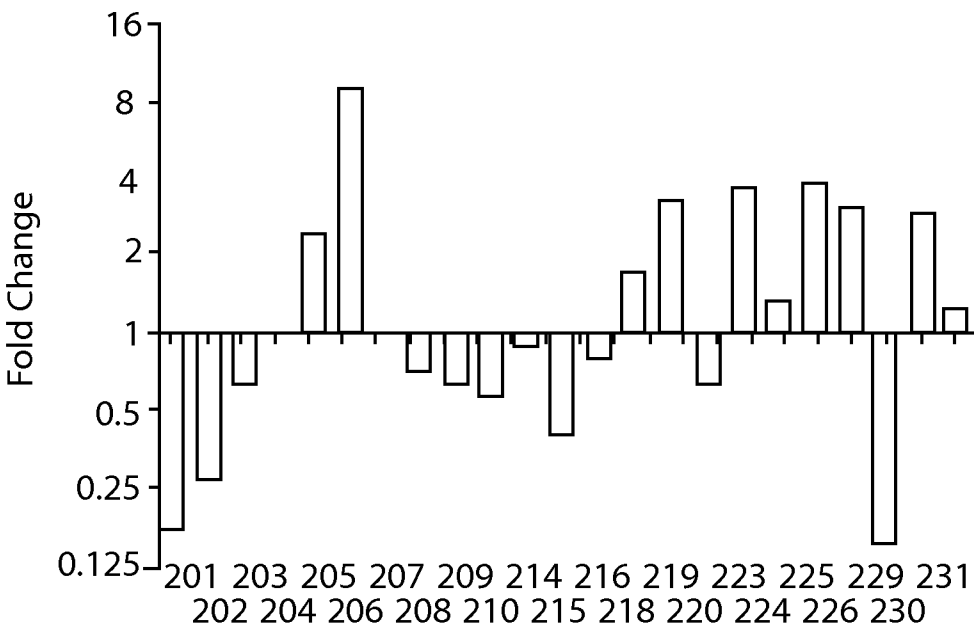


Fig. 7A

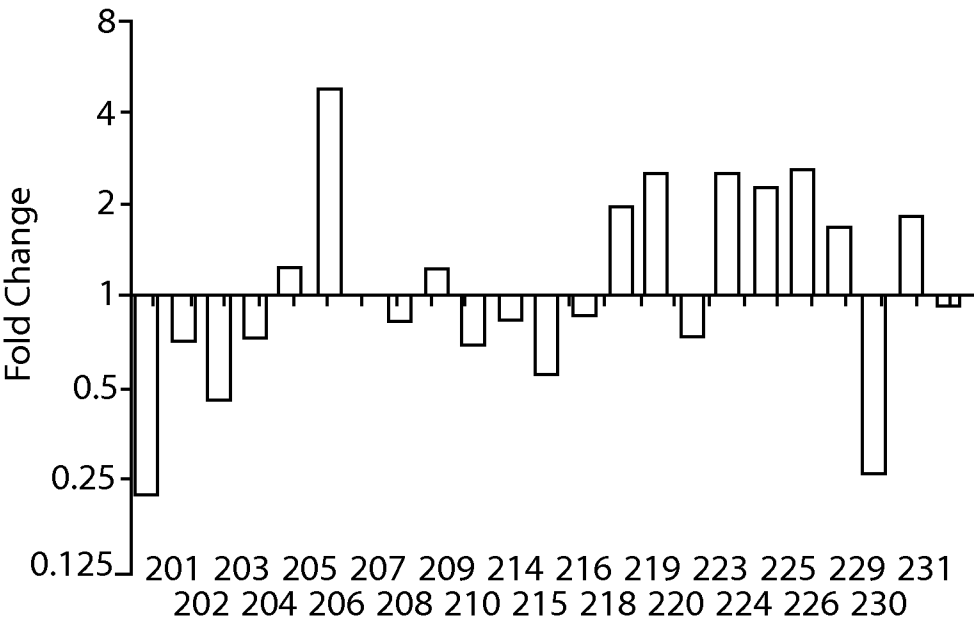


Fig. 7B

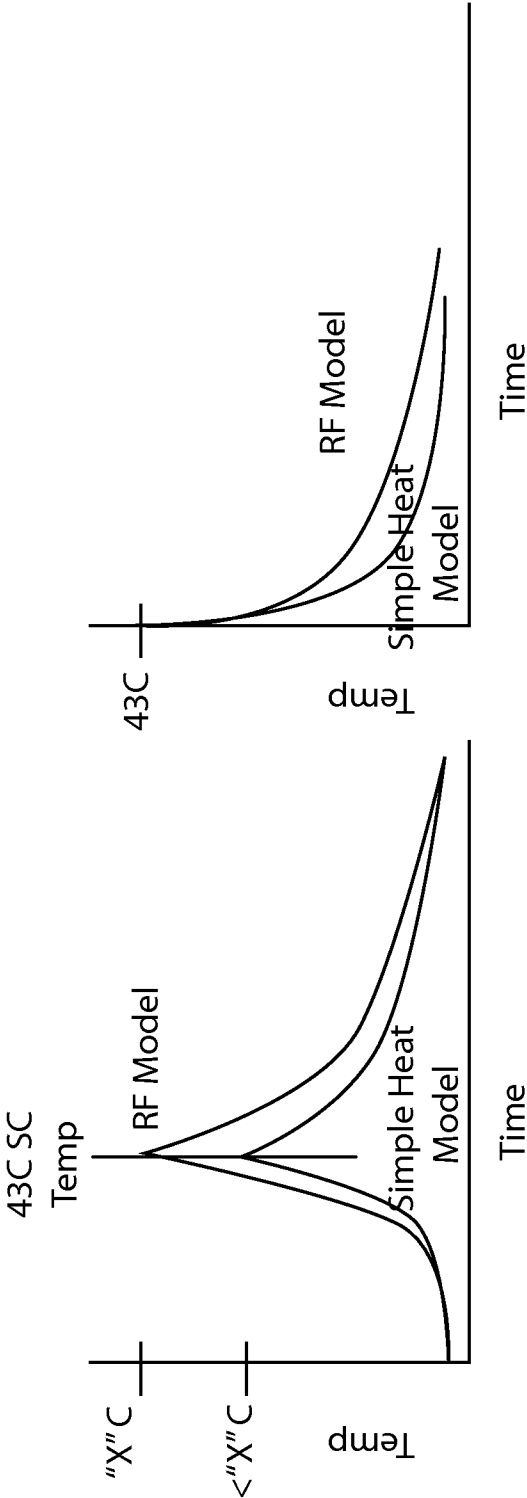


Fig. 8A

Fig. 8B

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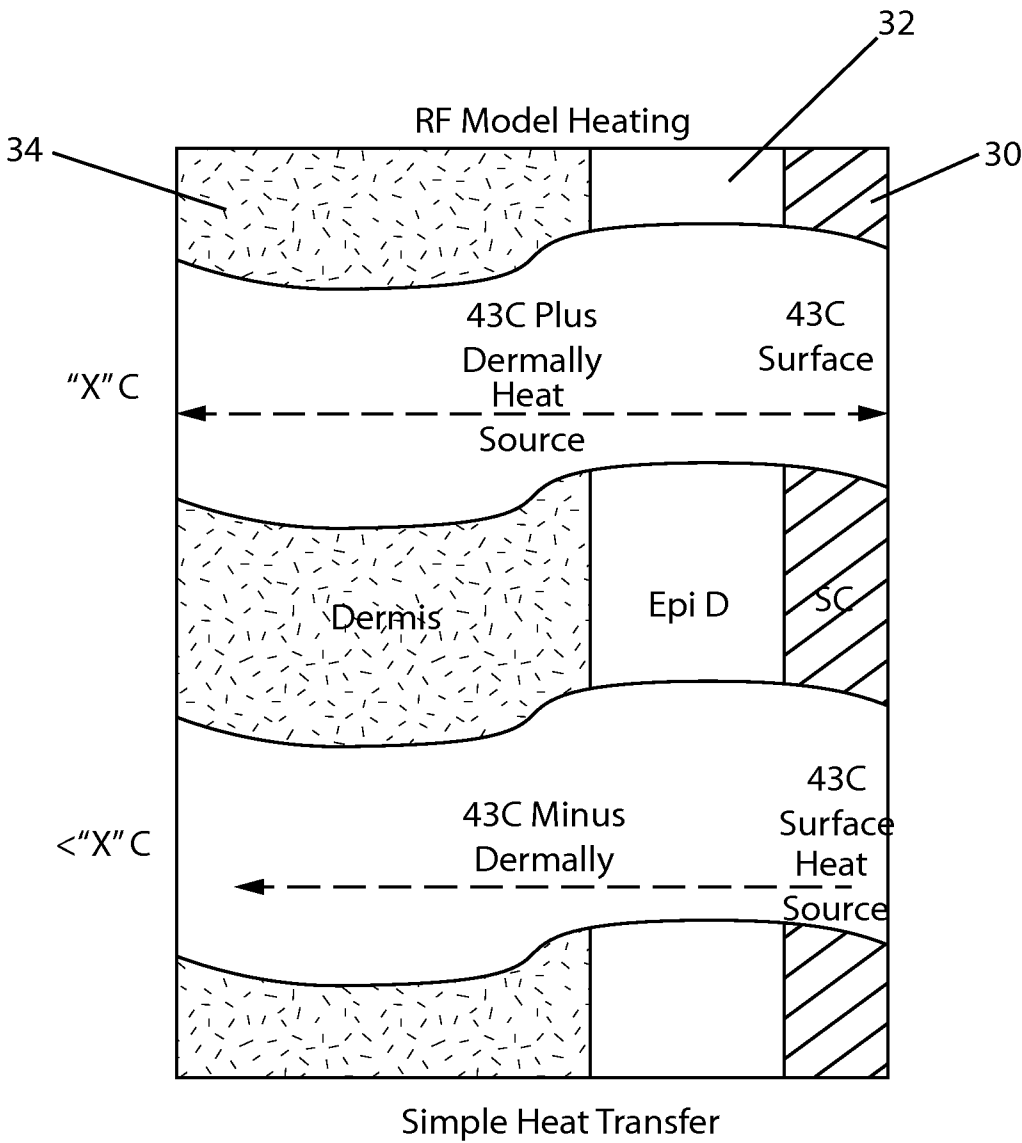


Fig. 9

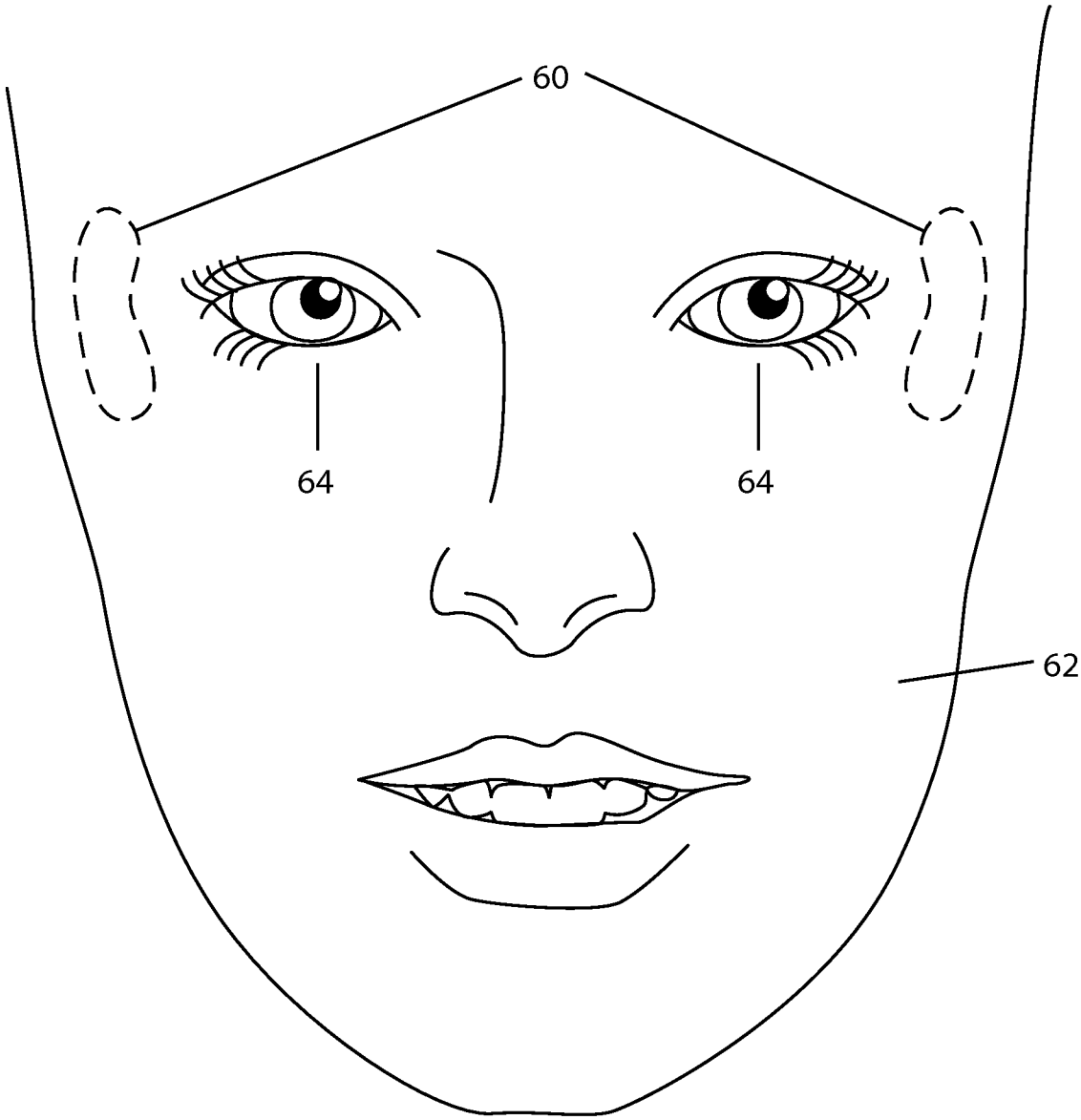


Fig. 10

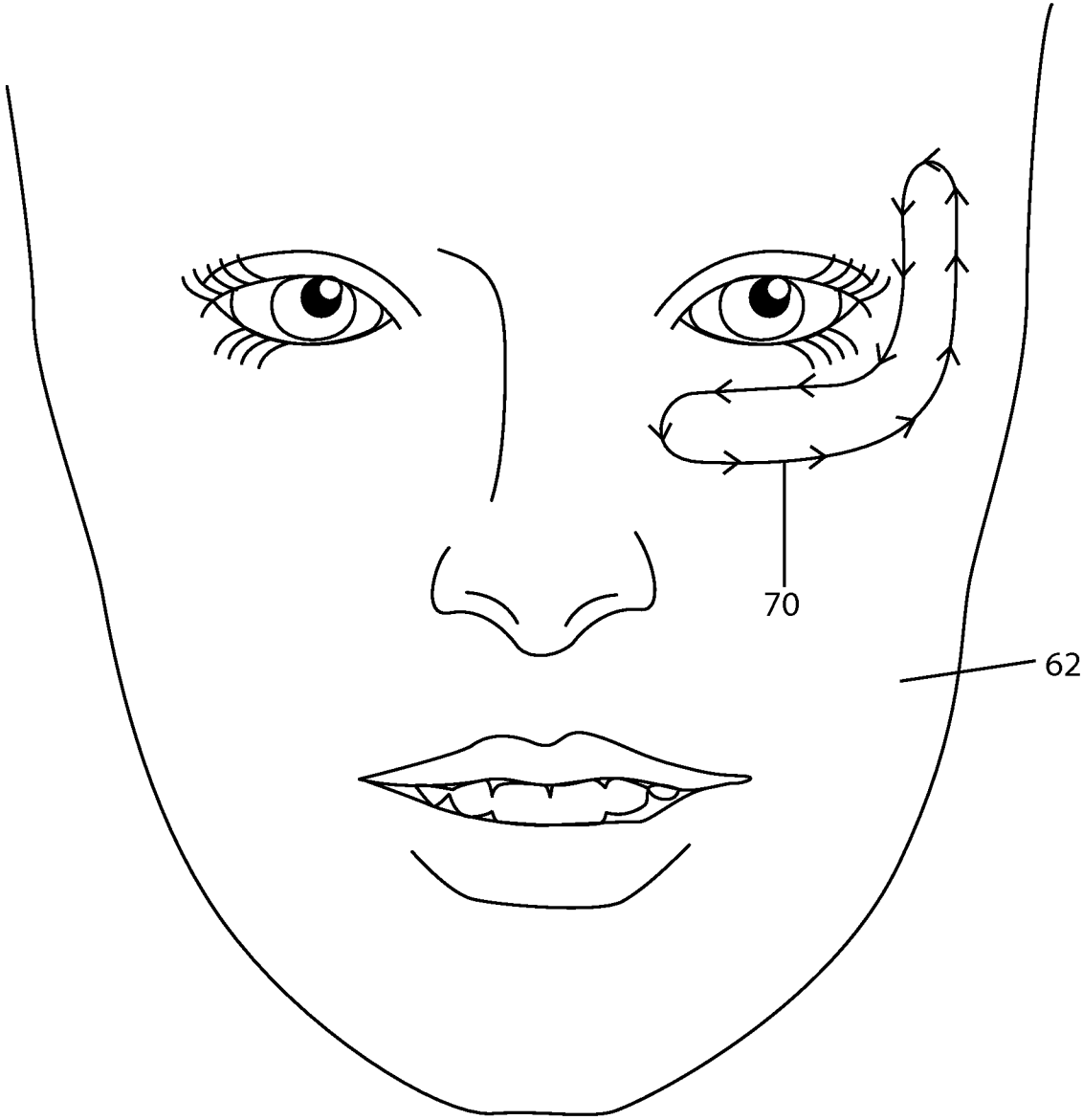


Fig. 11

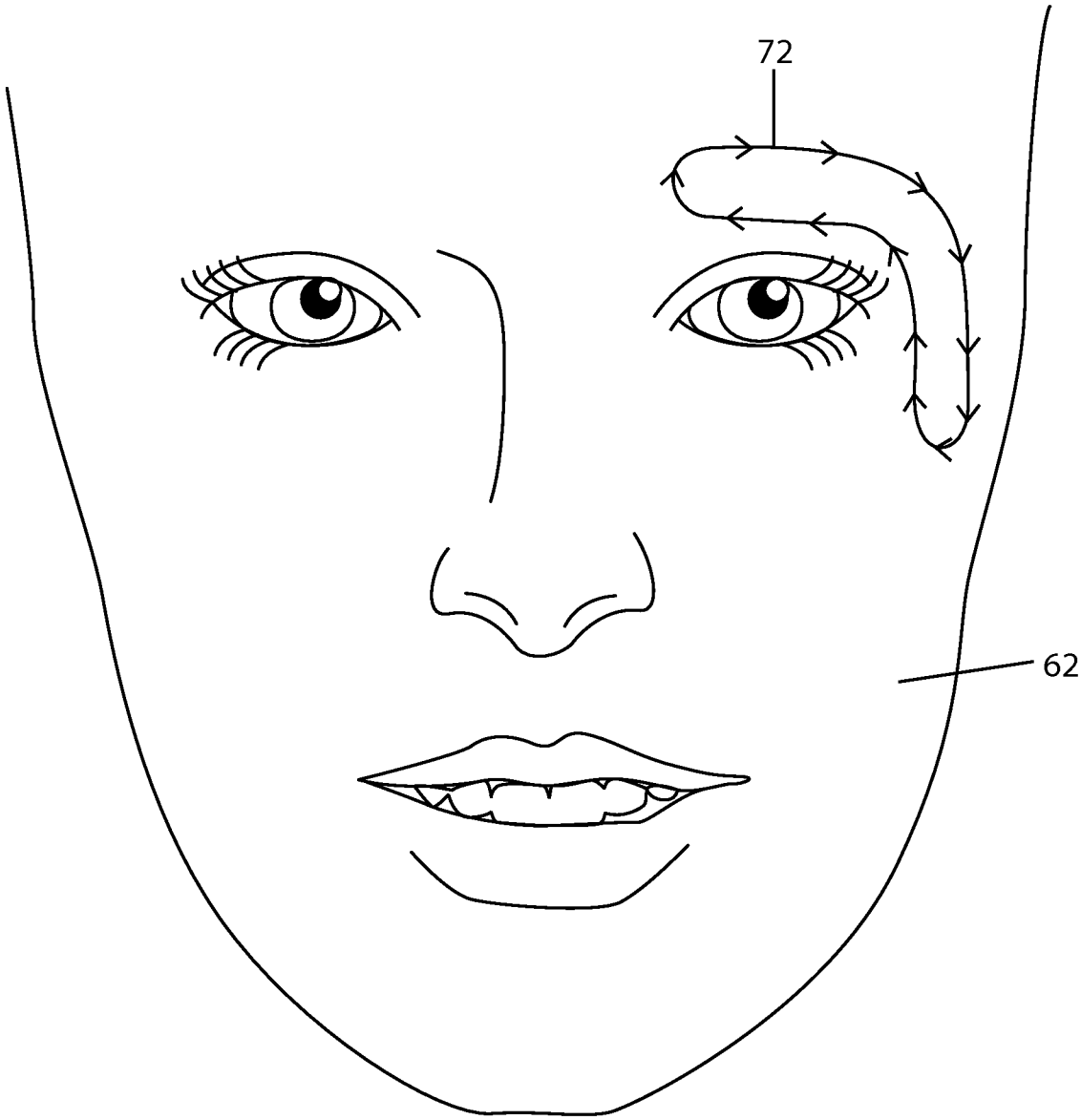


Fig. 12

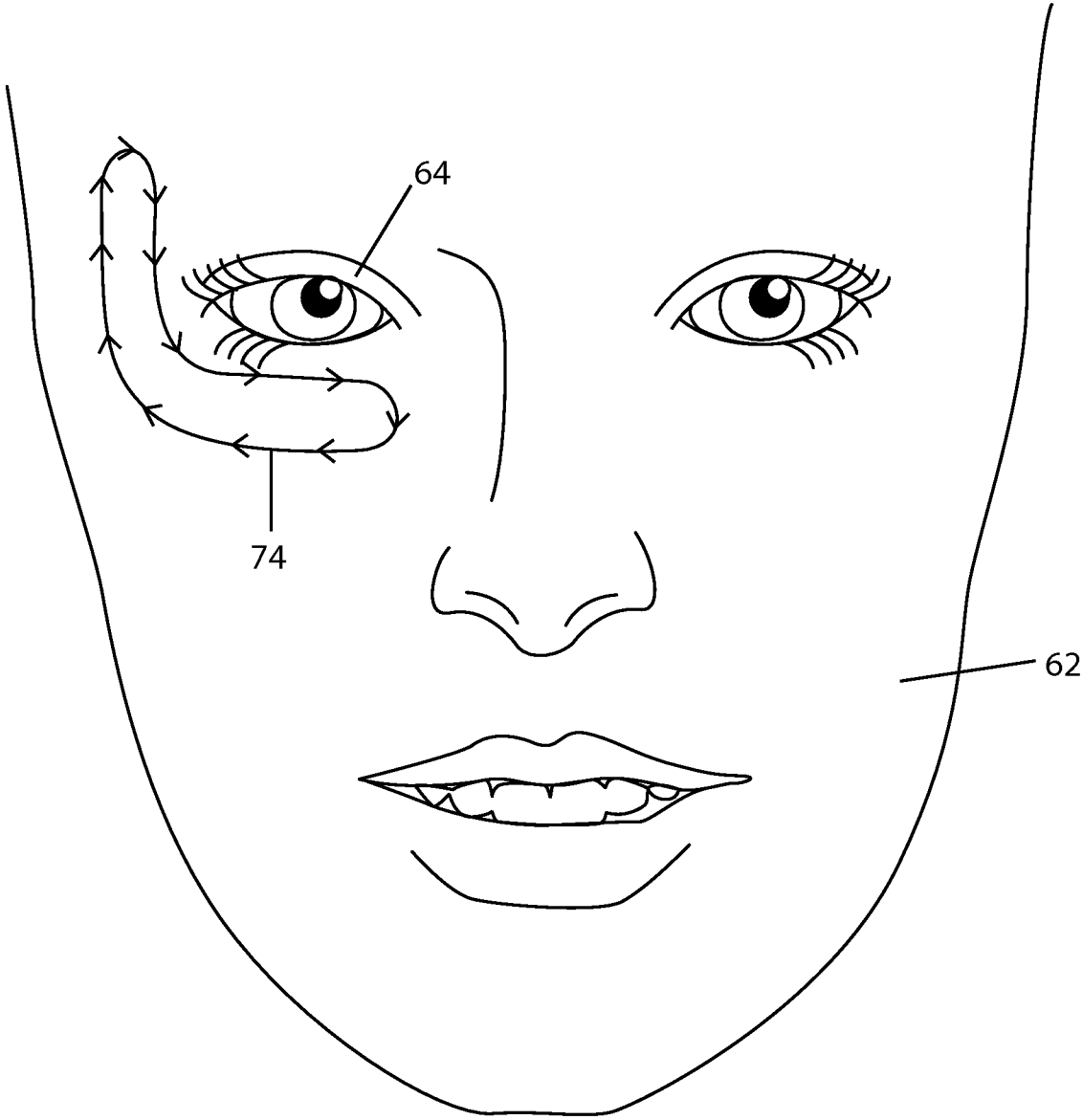


Fig. 13

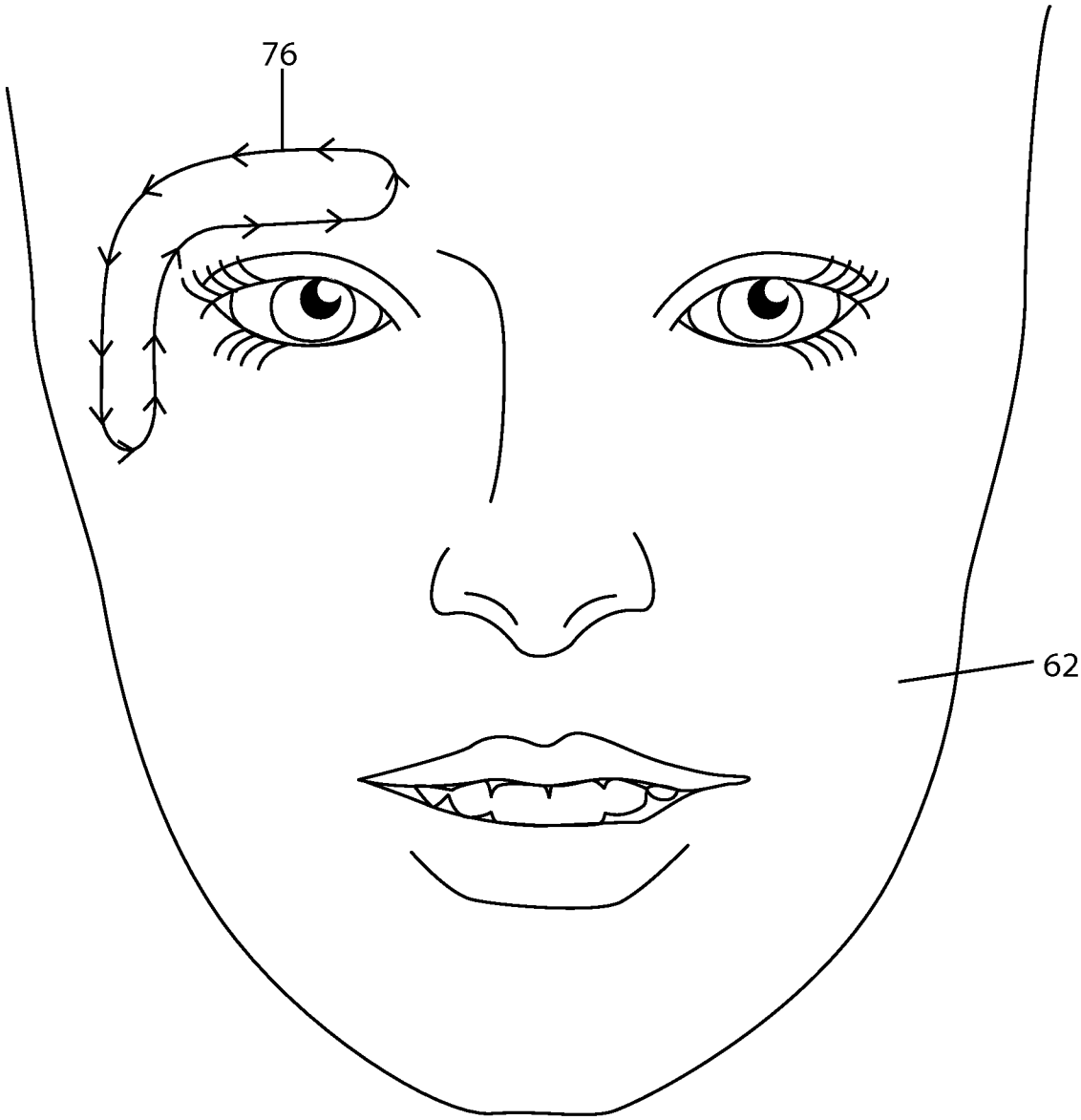


Fig. 14

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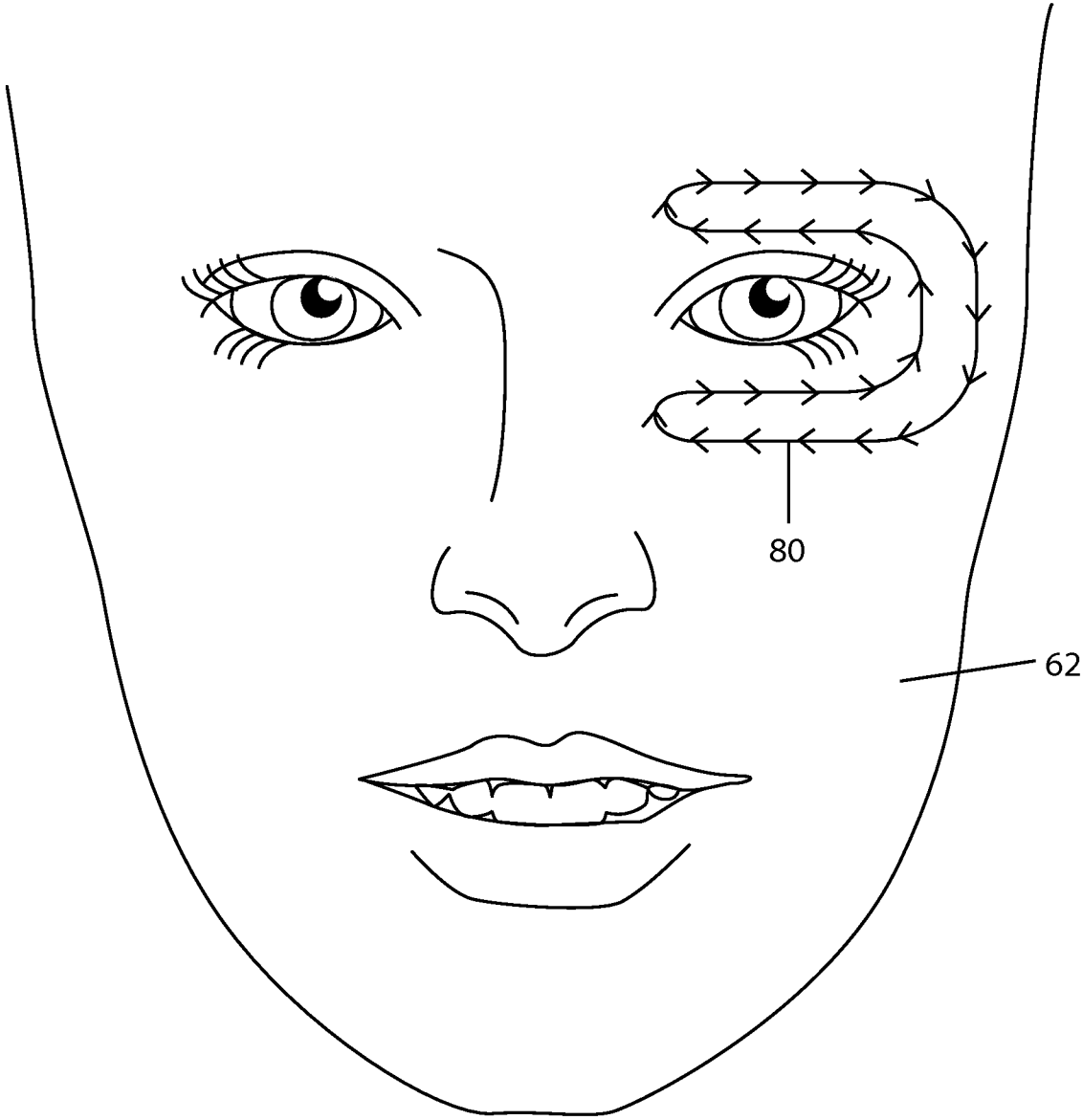


Fig. 15

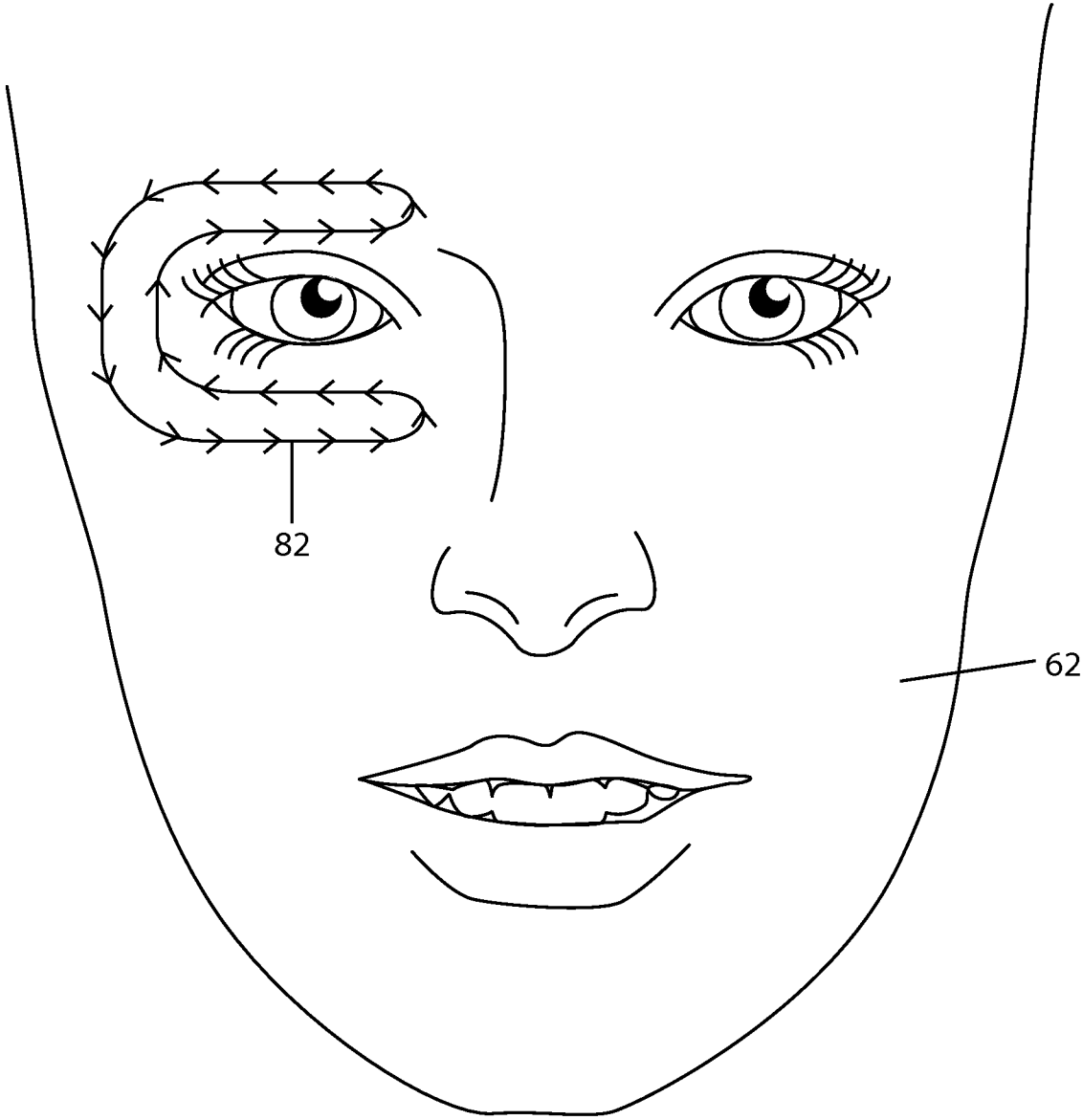


Fig. 16

PATENT COOPERATION TREATY

PCT

DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a), Rules 13ter.1(c) and Rule 39)

Applicant's or agent's file reference 11689M-JC	IMPORTANT DECLARATION	Date of mailing(<i>day/month/year</i>) 19 September 2011 (19-09-2011)
International application No. PCT/US2011/033034	International filing date(<i>day/month/year</i>) 19 April 2011 (19-04-2011)	(Earliest) Priority date(<i>day/month/year</i>) 19 April 2010 (19-04-2010)
International Patent Classification (IPC) or both national classification and IPC A61B18/12, A61B18/14, A61M35/00, A61N1/40		
Applicant THE PROCTER & GAMBLE COMPANY		

This International Searching Authority hereby declares, according to Article 17(2)(a), that **no international search report will be established** on the international application for the reasons indicated below

1. ☒ The subject matter of the international application relates to:
 - a. ☐ scientific theories
 - b. ☐ mathematical theories
 - c. ☐ plant varieties
 - d. ☐ animal varieties
 - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes
 - f. ☐ schemes, rules or methods of doing business
 - g. ☐ schemes, rules or methods of performing purely mental acts
 - h. ☐ schemes, rules or methods of playing games
 - i. ☒ methods for treatment of the human body by surgery or therapy
 - j. ☒ methods for treatment of the animal body by surgery or therapy
 - k. ☐ diagnostic methods practised on the human or animal body
 - l. ☐ mere presentations of information
 - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art
2. ☒ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:

☐ the description
 ☒ the claims
 ☐ the drawings
3. ☐ A meaningful search could not be carried out without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).
4. Further comments:

Name and mailing address of the International Searching Authority  European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040 Fax: (+31-70) 340-3016	Authorized officer SAN MIGUEL, Eva Tel: +49 (0)89 2399-7414
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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 203

Claims 1-13 relate to subject-matter considered by this Authority to be covered by the provisions of Article 17.2(a)(i) and Rule 39.1(iv) PCT as the said claims define therapy methods (see for example p 9 of the description: such procedures like protection from UV, treatment of skin atrophy, reduction of cellulite, treatment of rosacea or spider vessels are mentioned). Consequently, no search has been carried out for those claims.

Furthermore, for the same reason, no examination of said claims is required, see Article 34.4(a)(i) and Rule 67.1(iv) PCT.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.2), should the problems which led to the Article 17(2) declaration be overcome.