A multi-center, open-label study to evaluate the effects of topically-applied DNA repair enzymes and substrates on photo-aged skin

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BACKGROUND

Skin quality and function deteriorates over one's lifetime due to both intrinsic factors (advancing age) as well as extrinsic factors (environmental stressors such as ultraviolet and smog exposure). Skin's mechanistic, protective and restorative abilities decline with advancing age. An age-related decline in the skin's DNA repair capacity has been observed.[1] Specifically, cellular energy metabolism is directly related to mitochondrial function, which deteriorates with advancing age as well as with exposure to free radicals; free radicals are generated after exposure to noxious exogenous stressors.[2] Chronic exposure to environmental factors including ultraviolet (UV) rays in sunlight as well as smog/pollution further accentuate the skin's underlying inherent deterioration associated with chronological aging. "Aged" skin (whether intrinsically and/or extrinsically induced) manifests as fine and coarse rhytides (e.g. periocular "crow's feet"), irregular texture and pigment/color, loss of elasticity and luster, as well as prominence of individual pores. Safe, effective, and well-tolerated topical treatments that reverse DNA damage manifesting as visible signs of "aged" skin is a novel and exciting frontier.

Cells generate energy within mitochondria in the form of ATP, which is subsequently stored in the form of phosphocreatine.[3] Declining mitochondrial function associated with intrinsic and extrinsic aging results in a decreased production of ATP, as well as an increased production of hydrogen peroxide and reactive oxygen species (ROS).[4] These ROS damage neighboring mitochondrial as well as nuclear DNA, resulting in the activation of genes that regulate matrix metalloproteinases (MMPs). MMPs degrade collagen within the dermis, further exacerbating visible signs of cutaneous aging.[5] Hydrogen peroxide and ROS cause oxidative damage to proteins such as collagen, elastin, keratin, and fibronectin, which allows these proteins to react with sugars within the skin, leading to glycation, or degradation of skin cells by sugar.[6,7]

Endogenous mechanisms that excise, extract, and repair damaged DNA as well as those that reverse oxidation and glycation exist; however, these functions decline with advancing age. Supplementation of these endogenous mechanisms is a novel approach to anti-aging and has been demonstrated with topically applied DNA repair enzymes (Roxisomes®, Ultrasomes®, and Photosomes®) and natural protein precursors (Unirepair Complex®) engineered within liposomal delivery systems to enhance lipid bilayer penetration, thus ensuring that the active enzymes/protein precursors reach their targeted areas of damaged DNA. For example, oxo-guanine glycosylase-1 (OGG-1) is an enzyme that repairs DNA damaged by oxidative stress. To penetrate the epidermis, OGG-1 is encapsulated within specially formulated pH-sensitive liposomes; these complexes are referred to as Roxisomes®. T4 endonuclease V (T4N5), known as Ultrasomes® upon liposomal encapsulation, acts to recognize distorted DNA molecules (i.e. UV-induced thymine dimers), break the DNA chain near the dimer, excise the small damaged region, and patch up the strand with the correct bases corresponding to bases on the intact strand. In addition, exposing skin to Ultrasomes® also reduces cytokines regularly released during stress [i.e. Interleukin-1 (IL-1), IL-6, IL-8, IL-10, and tumor necrosis factor-α (TNF-α)] and downregulates UV-induced elevated levels of MMP's (i.e. MMP-1, a.k.a. collagenase-1, which cleaves collagen I, the major subtype of collagen in the extracellular matrix of the dermis). Photolyase, referred to as Photosomes® when encapsulated within liposomes, absorbs visible light to cleave and reverse DNA damage in the form of CPD's caused by UV with shorter wavelengths; this process specifically cleaves the UV-induced cyclobutane ring and is known as photoreactivation. Natural DNA repair precursors such as acetyl tyrosine, proline, hydrolyzed vegetable protein, and ATP are also delivered to areas of damaged DNA via Unirepair® complexes.

A promising new modality for restoring quality and function of "aged" skin involves topical application of supplemental DNA repair enzymes and natural protein precursors. Specifically, application of products containing DNA repair enzymes such as T4 endonuclease 5 (T4N5) delivered within

Ultrasomes®, photolyase within Photosomes®, and OXO-guanine glycosylase-1 (OGG-1) within Roxisomes® as well as natural DNA repair precursor proteins such as acetyl tyrosine, proline, hydrolyzed vegetable protein and ATP within Unirepair Complex® has been shown to reverse DNA damage *in vitro* and purported to function similarly *in vivo*. Cyclobutane pyrimidine dimer (CPD) chains, formed after DNA is exposed to UV or other noxious stimuli, are manifestations of damaged DNA. Exposure to ROS, referred to as oxidative stress, also causes damage to both mitochondrial as well as nuclear DNA. The endonuclease within Ultrasomes® stimulates the body's own mechanisms for recognizing DNA mutations and subsequently extracting them. Photosomes® target and subsequently break these CPD chains. Roxisomes® target and subsequently excise DNA damaged by oxidation, the most common cause of nucleic damage.

As our understanding of the mechanisms of action behind DNA damage and skin "aging" increases, so does our ability to formulate topical supplements to reverse/repair this damage, manifesting clinically as healthier skin. Ultrasomes®, Roxisomes®, Photosomes®, and Unirepair Complexes® have been incorporated into three topical products (PRESCRIBED**solutions** DNA Repair line, Biopelle, Inc., Ferndale, MI) with anti-photoaging effects. These products include "Stop the Clock," which also contains a heat-activated antioxidant known as *Thermus thermophillis*, as well as physical and chemical SPF50 sun protection; "Urbane Renewal," which also contains urban antioxidants and creatine for mitochondrial repair; and "Cream – No Sugar," which also contains moisturizing ingredients as well as carnosine for its anti-glycation properties.

MATERIALS AND METHODS

Study population

Females and males aged 35-75 with Fitzpatrick skin types II & III demonstrating moderate to severe facial photodamage (measuring 3 or higher on the Glogau photodamage scale) as well as moderate to severe facial wrinkles (scoring 3 or higher on the Rao-Goldman's 5-point wrinkle evaluation scale) were eligible for enrollment. A total of twenty-seven subjects were enrolled at the three study sites (6 subjects at one site, 10 subjects at the second site, and 11 subjects at the third site).

Exclusion criteria included the following: unwillingness to avoid excessive sunlight or wear protective clothing and sunscreen, unwillingness to forego any other topical dermatological or drug therapy (including corticosteroids) on the face, as well as use of both alpha- as well as beta-hydroxy acids, retinoids, or Vitamins C or D containing topicals within 30 days prior to as well as throughout the course of the study.

Washout periods adhered to by subjects in this study included the following: six months free from dermabrasion, deep chemical peels, ablative laser treatments, neurotoxin or filler injections, and cosmetic surgery; three months free from non-ablative laser, light (including intense pulse light) or radiofrequency treatments; and one month free from microdermabrasion as well as light and medium depth chemical peels.

Treatment administration

A three-center, open-label, 12 week study to evaluate the safety and efficacy of a daily skin care regimen consisting of three PRESCRIBED**solutions** DNA Repair products, each containing Ultrasomes®, Roxisomes®, Photosomes®, and Unirepair Complex® ("Stop the Clock" day cream, "Urbane Renewal" twice daily serum, and "Cream – No Sugar" night cream, Biopelle, Inc., Ferndale, MI) in patients with moderate to severe photodamage was performed.

Each subject's treatment regimen included daily morning use of a bland cleanser followed by full-face application of "Urbane Renewal" serum followed by "Stop the Clock" cream. As "Stop the Clock" provides SPF50 coverage, no additional sunscreen was applied. The evening regimen included use of a bland cleanser followed by full-face application of "Urban Renewal" serum followed by "Cream – No Sugar" cream. This regimen was maintained for 12 weeks.

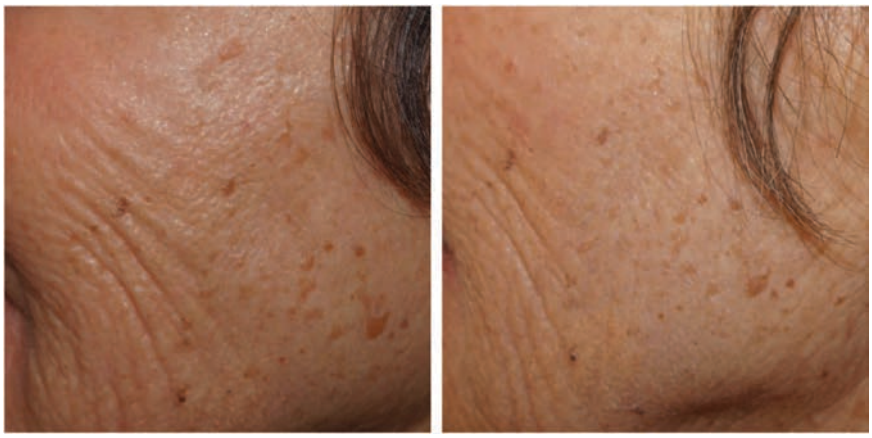
In addition to subjective patient evaluations of facial skin and adverse events, investigator assessments and digital photographs were performed at baseline (day 0) as well as at weeks 6 and 12.

Subjects and physician investigators rated the former on various common signs of photo-aging at baseline, week 6 and week 12. Additional investigator assessments were performed at the conclusion of the study to generate GAIS scores for each patient at both weeks 6 and 12.

Patient used a 12 week regimen of PRESCRIBED**solutions** DNA products that included AM use of Stop the Clock and Urbane Renewal and PM use of Urbane Renewal and Cream – No Sugar. Photos courtesy of Dr. Hema Sundaram

At each of the three visits, subjects used a 5-point scale to rate the appearance of their crow's feet, wrinkles elsewhere on the face, and fine lines elsewhere on the face (1 = none; 2 = minimal; 3 = mild; 4 = significant; 5 = severe). Subjects used another 5-point scale to rate their skin texture, luster/luminosity, color, suppleness, and overall satisfaction (1 = perfectly happy; 2 = mostly pleased; 3 = neither pleased nor displeased; 4 = displeased; 5 = very displeased).

At each of the three visits, physician investigators used the 5-point Rao-Goldman wrinkle evaluation scale to assess wrinkles on the cheeks, infraocular areas, lateral periocular areas (crow's feet), and forehead. Investigators used another 5-point scale to rate skin roughness, lack of brightness, lack of elasticity, mottled pigmentation, and overall photodamage (1 = none; 2 = minimal; 3 = mild; 4 = significant; 5 = severe). Following each patient's final, week 12 visit, the investigator generated two GAIS scores: one via comparison of the baseline photo with that of week 6 and the other via comparison of the baseline photo with that of week 12 (1 = exceptional improvement; 2 = markedly improved; 3 = improved. Further treatment may be recommended; 4 = no change; 5 = worsened compared to baseline pre-treatment evaluation).



Patient used a 12 week regimen of PRESCRIBED**solutions** DNA products that included AM use of Stop the Clock and Urbane Renewal and PM use of Urbane Renewal and Cream – No Sugar. Photos courtesy of Dr. Hema Sundaram

RESULTS & DISCUSSION

Twenty-seven subjects were enrolled in this study; both males as well as females were included. All 27 patients completed the study and had his/her data included in the final analysis. Between baseline and week 12, every parameter that was assessed by both the subject (Table 1) and the investigator (Table 2) showed improvement. The percentage of patients rating overall improvement on each parameter and the mean improvement is shown in the following table:

Table 1. Results of *Patient* Evaluations After 12 Weeks of Treatment

Parameter	Mean Improvement*	Percentage of Patients Showing Improvement (n = 27)
Crow's feet	0.59	59%
Wrinkles elsewhere on face	0.50	26%
Fine lines elsewhere on face	0.85	59%
Texture	0.85	56%
Luster (luminosity)	0.96	67%
Color	0.74	56%
Suppleness	0.85	56%
Overall facial skin satisfaction	0.81	56%

*As assessed on a 5-point scale.

Investigator assessments are shown in Table 2:

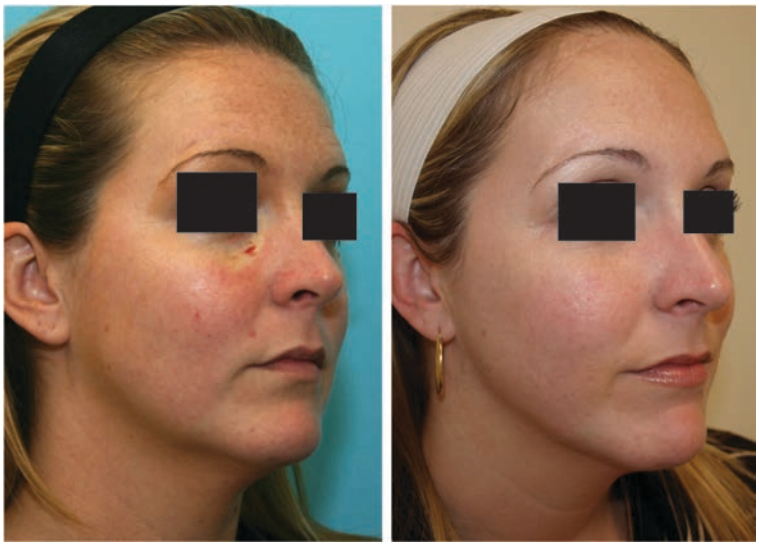
Table 2. Results of *Investigator* Evaluations After 12 Weeks of Treatment

Parameter	Mean Improvement*	Percentage of Patients Showing Improvement (n = 27)
Cheek wrinkles	0.74	59%
Under-eye wrinkles	1.28	85%
Crow's feet	1.17	85%
Forehead wrinkles	1.04	70%
Skin roughness	1.15	85%
Lack of skin brightness	1.33	93%
Lack of elasticity	1.11	81%
Mottled pigmentation	1.11	81%
Pore prominence	1.19	59%
Overall photodamage	1.11	81%

*As assessed on a 5-point scale.

The average overall GAIS at 12 weeks was 2.52, which translated to a score between "improved" and "markedly improved" on the spectrum of change. Specifically, one patient scored a 4, which signified "no change," thirteen patients scored a 3, which signified "improved. Further treatment recommended," twelve patients scored a 2, which signified "markedly improved," and one patient scored a 1, which signified "exceptional improvement."

This was a preliminary, open-label study. As such, there was no control group (nor was there a split-face design with each patient serving as his/her own control). Due to the lack of adverse events and the predominance of positive findings of improvement shown in each of the parameters representing the most common signs of photo-ageing as assessed by both the patients as well as the investigators, future studies that include a control group and a randomized, double-blind design are warranted to validate these favorable preliminary findings.



Patient used a 12 week regimen of PRESCRIBED**solutions** DNA products that included AM use of Stop the Clock and Urbane Renewal and PM use of Urbane Renewal and Cream – No Sugar. Photos courtesy of Dr. Hema Sundaram

CONCLUSION

Twice daily application of a skincare regimen containing DNA repair enzymes (to excise, extract, and repair damaged DNA) and supplemental protein substrates (to serve as repair process precursors) yielded improvement in all major parameters that signify cutaneous photo-aging after 12 weeks of use. The topical regimen used in this study included ingredients that targeted both oxidative as well as UV-induced DNA damage, ultimately yielding repaired DNA as well as preventing further DNA damage. Of note, this comprehensive regimen also contained creatine, which has been shown to protect against free radical damage and to prevent further mitochondrial breakdown, as well as carnosine, which has been shown to prevent glycation of oxidatively damaged proteins. In addition to being efficacious, this topical regimen containing DNA-repair enzymes proved to be well tolerated, with no adverse reactions reported throughout the study.

REFERENCES

1. Offredo H. Enzymes to repair damaged DNA. *Guide of Cosmetic Ingredients*. 2010; 120-123.
2. Harman D. Ageing: a theory based on free radical and radiation chemistry. *J Gerontol*. 1956; 11: 298-300.
3. Lenz H, Schmidt M, Welge V, et al. The creatine kinase system in human skin: protective effects of creatine against oxidative and UV damage in vitro and in vivo. *J Invest Dermatol*. 2005; 124(2): 443-452.
4. Sohal R. Hydrogen peroxide production by mitochondria may be a biomarker of ageing. *Mech Ageing Dev*. 1991; 60: 189-198.
5. Fisher G, Wang Z, Datta S, et al. Pathophysiology of premature skin ageing induced by ultraviolet light. *N Engl J Med*. 1997; 337: 1419-1428.
6. Pugliese P. Physiology of the skin: the impact of glycation on the skin, part 1. *Skin Inc*. 2008. Accessed in 2012 via www.skininc.com.
7. Pugliese P. Physiology of the skin: the impact of glycation on the skin, part 2. *Skin Inc*. 2008. Accessed in 2012 via www.skininc.com.