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Reduction of Facial Redness With Resveratrol Added to Topical Product Containing Green Tea Polyphenols and Caffeine

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ABSTRACT

Background/Objective: Many topical formulations include antioxidants to improve the antioxidant capability of the skin. This study evaluated the ability of a unique combination of antioxidants including resveratrol, green tea polyphenols, and caffeine to reduce facial redness.
Methods: Subjects (n=16) presenting with facial redness applied the resveratrol-enriched product twice daily to the entire face. Reduction in redness was evaluated by trained staff members and dermatology house staff officers. Evaluators compared clinical photographs and spectrally enhanced images taken before treatment and at 2-week intervals for up to 12 weeks.
Results: 16 of 16 clinical images showed improvement and 13 of 16 spectrally enhanced images were improved. Reduction in facial redness continued to evolve over the duration of the study period but was generally detectable by 6 weeks of treatment. Adverse effects were not observed in any subject.
Conclusion: The skin product combination of resveratrol, green tea polyphenols, and caffeine safely reduces facial redness in most patients by 6 weeks of continuous treatment and may provide further improvement with additional treatment.

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INTRODUCTION

Facial redness can occur in association with a large number of medical problems. The most common causes of facial redness include inflammatory dermatoses, such as rosacea, perioral oral/ocular dermatitis, contact, seborrheic and atopic dermatoses and chronic sun damage. While redness is the final clinical manifestation, the biologic pathway leading to the redness may be quite varied. We refer to the common denominator in all of these as inflammation, and we now understand many molecules are involved in the inflammatory process. Many of the pathways of inflammation involve reactive oxygen species (ROS). Therefore one may conclude that molecules quenching ROS should be considered anti-inflammatory agents.

There are a number of topical formulations that include antioxidants to improve the antioxidant capability of the skin.[1,2,3,4] Two antioxidants, green tea polyphenols and caffeine, have been shown in the laboratory[5,6,7] to be very effective and have been used in a commercially available product that has been well tolerated. A third compound that has received considerable attention is resveratrol (3,5,4'-trihydroxystilbene), a polyphenolic phytoalexin found in red wines, colored berries, and peanuts.[8] Resveratrol has also been shown in the laboratory[9] to be a potent antioxidant. The myriad of clinical benefits of resveratrol led to the hypothesis that the addition of this agent to a topical preparation containing green tea polyphenols and caffeine (both of which protect skin from UV injury[10,11]) might create an even more effective skin care product. The present study demonstrates that this combination of GTP, caffeine, and resveratrol reduces facial redness.

METHODS

Stage 1

In a preliminary split-face study, volunteers applied topical antioxidant product containing green tea polyphenols and caffeine to one side of the face and the same product with resveratrol added to the other side of the face. Product was applied twice daily for 8-12 weeks. Both products were well tolerated. Facial redness was reduced on the side treated with resveratrol-enriched product (data not shown). These results led to the present study in which subjects presenting with facial redness applied resveratrol-enriched product to the entire face to evaluate the consistency of the clinically apparent reduction in redness.

Stage 2

Subjects (n = 16) presenting with facial redness applied the resveratrol-enriched product twice daily to the entire face. Reduction in redness was evaluated and photographed at 2-week intervals for up to 12 weeks. Photography was obtained by Canfield Visia Software Version 5.2.0 2010-0503a. This unit has a mode that spectrally separates the red portion of the image allowing enhanced ability to see changes in skin redness. Improvement was evaluated by nine trained staff members and 21 dermatology residents on a scale of 1 to 9. The baseline score was assigned a value of 5 for each subject. Post treatment scores lower than 5 denoted redness reduction while scores above 5 indicated an increase in redness. Evaluators compared photographs taken before treatment and at 2-week intervals for up to12 weeks. All subjects provided signed informed consent to treatment and photography.

FIGURE 1. Clinicians (red) and trained observers (blue) independently rated clinical images of the same 16 patients. There was 100% agreement between the two groups of observers. All 16 patients exhibited signs of reduced redness after 6 weeks of treatment, based on clinical images. Note: All lines above baseline (level 5) denote improvement.

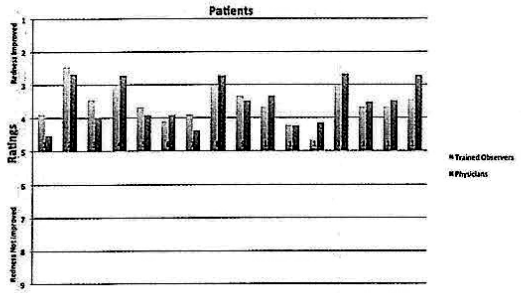
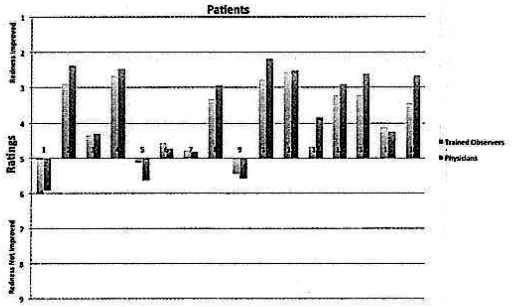


FIGURE 2. Clinicians (red) and trained observers (blue) independently rated computer-generated images of the same 16 patients. There was 100% agreement between the two groups of observers. Thirteen out of 16 patients showed reduced redness following 6 weeks of treatment, based on computer-generated images. Patients 1, 5, and 9 did not show signs of reduced redness based on computer-generated images. Note: All lines above baseline (level 5) denote improvement.



RESULTS

All subjects completed the study. Adverse effects were not observed in any subject. Two sets of images were evaluated. One set were clinical photos, the other spectrally enhanced red images that were computer generated by the Canfield software. The sets of observers evaluated the before and after images independently. During their evaluations there was no inter-observer discussion. There was 100% agreement between the groups as to which patients improved and which did not. Improvement once attained was sustained throughout the course of the study. The data suggest that 3 to 6 weeks may be sufficient time for most subjects to achieve a reduction score of 2 to 4. (Note added in proof: most of the patients have now been informally followed for more than 1 year and have maintained their improvement.)

DISCUSSION

As has been said, now that we know it works in fact, how does it work in theory? The product that reduced facial redness is a combination of a number of products produced by mother nature that each has individual histories of providing benefits by association with epidemiologic data. Uniquely these products are associated with a plethora of benefits but a noticeable absence of deleterious effects. Scientists, including our own group, have studied these molecules in test tube and animal models and drawn lots of conclusions about the nature of their activities. Discussed here are some of our favorites, but do they answer the question of how this product produces its admirable effects? Historically the first product that we evaluated clinically contained just green tea polyphenols. Note that this is was a concentrated assortment of all the molecules available from gentle extraction and concentration of the green tea leaf, intentionally not focusing on any single component as the epidemiology of benefits is for green tea and not individual components. This product was cosmetically accepted and conceptually was a potent topical antioxidant. Clinical observation suggested it had a calming effect on the users' skin, including those with inflammatory dermatoses, suggesting its anti-inflammatory nature. Our laboratory found that the addition of caffeine increased the antioxidant capacity of green tea polyphenols in a test tube model using human fibroblasts. Caffeine had already been administered topically and was being redeemed as systemically beneficial and therefore might add to the antioxidant qualities of our existing product. Back in the lab, resveratrol had proven to have antioxidant capacities and some other unique activities.[12] Guided by this laboratory data and an excellent epidemiology story, we added resveratrol producing a unique product in which all of these components were present in high concentrations in a stabilizing base. Each translation from bench top to commercial product has a period in which the older product is compared to the new for cosmetic acceptability. Everyone liked the qualities of the new product and we noticed that in those individuals with facial redness, the side treated with the resveratrol addition had reduced redness. Translational work is thought of as from the bench to the bedside but clinical observation yields facts and what remains is why.

A suggestion for the mechanism of action of this combination product centers on the concept that individual cells in an environment, in this case the skin, produce molecules that influence all the surrounding tissues and on some level the host.[13] This is now a more accepted doctrine in the cancer literature and recently in the aging literature.[14] The data presented here on redness in the skin may be explained by having the mixture of active principles in this product change how individual cells

FIGURE 3. A 35-year-old male (skin type 2) before treatment (left) and 9 weeks after treatment with resveratrol-enriched product (right). Clinical image. Redness reduction was scored at 3.

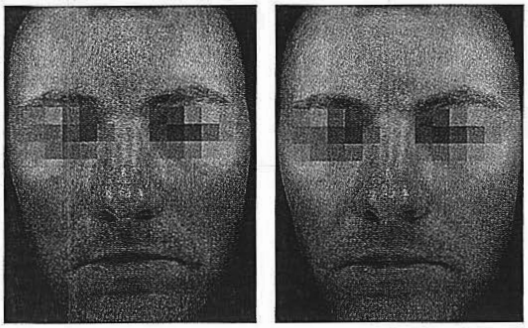
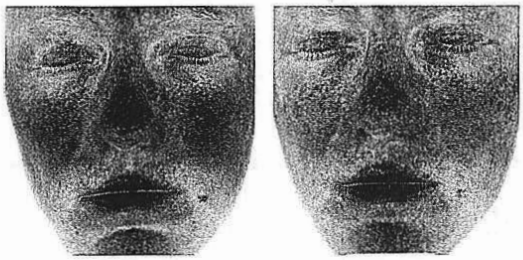


FIGURE 4. A 35-year-old female (skin type 2))before treatment (left) and 9 weeks after treatment with resveratrol-enriched product (right). Spectrally enhanced image. Redness reduction was scored at 3.



view injury and therefore the array of molecules they produce or induce their neighbors to produce. The injured cell has at least four alternatives: complete repair, autophagy, apoptosis or necrosis. Each of these pathways has a consequence to the surrounding tissue. An example of complete repair may be the excision-repair proteins for damaged DNA that may be going on continuously without upsetting the cellular environment. Additional stress to the cell is a reactive oxygen species (ROS) assault, which could be quenched by the cell's store of antioxidants or by the exogenous antioxidants applied to the skin or consumed. This simplistic explanation needs to be expanded. Cell necrosis is the most inflammatory path for the injured cell with release of all the contents initiating myriad inflammatory pathways and immune activation. This kind of pathway probably accounts for the exacerbation of lupus after acute UV damage. Apoptosis or programmed cell death is the least inflammatory method of removing non-repairable cells as represented by the sunburn cell. The combination of resveratrol, green tea pholyphenols, and caffeine in our product proved effective in reducing redness. Each of these three compounds yields an acclamatory effect on individual cells in the surrounding environment, which may account for the observed benefits.

Green tea polyphenols (GTPs) are antioxidants shown in mice to protect against skin inflammation, associated tumorigenesis[15,16] and phototoxicity induced by psoralen plus UV-A radiation.[17] The polyphenol portion of green tea (the catechins) includes epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate derivatives. When only the catechin portion of green tea is administered topically in mice, epigallocatechin-3-gallate (EGCG) protects best in a photocarcinogenesis model.[18] Because of this and similar models, EGCG is regarded as the most effective catechin.[19] It is important to remember that the epidemiology is for green tea and not any individual molecules. Surrogates are valid for their models. They are used for their expediency. Intentionally, the studied product uses all of the molecules in green tea leaves that would be present in the beverage on which the epidemiology is based.

Another approach to discerning mechanisms by which the combination product of the present study reduces facial redness involves pathways of inflammation. Facial redness may occur in a variety of inflammatory dermatologic disorders.[20] Since the molecular targets of each component are not identical, the components may act independently or synergistically to reduce cutaneous inflammation. All three of the components in our product have been shown to improve or protect against UV-induced skin damage. Exposure of the skin to UV radiation induces formation of ROS, which leads to inflammatory responses associated with a variety of skin disorders, including cancer. Inflammatory responses are characterized by erythema, edema, hyperplastic responses, and increases in blood vessel permeability. Both topical GTP-application and GTPs in drinking water reduce inflammation.[21] One study[22] on the anti-inflammatory component of GTPs showed that, after pretreating human skin with green tea extract and then exposing the treated area to solar-simulated light, the green tea extract inhibited UV-induced erythema in a dose-dependent manner, reduced the number of sunburn cells, and protected the epidermal Langerhans cells. Resveratrol, as a natural polyphenol, is also a pigment. This property allows it to absorb UV radiation, and when applied topically, it can reduce the penetration of UV radiation into the skin.[23] In this way, topical resveratrol acts as a natural sunscreen and reduces the inflammation and oxidative damage associated with UV exposure. Furthermore, pre-treatment of keratinocytes with resveratrol increases cell survival after these cells have been exposed to UV radiation.[24] This is also associated with a reduction in the production of ROS, and subsequent anti-inflammatory effects. Green tea phenols add to this anti-inflammatory effect. GTPs can inhibit the UV-induced infiltration of neutrophils and macrophages.[25] In our product, this effectiveness is further supplemented by caffeine. Topical caffeine has been shown to protect against UV damage in mice by eliminating UV-damaged keratinocytes,[26] and subsequently inhibiting skin

cancer development. The topical application of caffeine to human skin provides protection from UV light via DNA repair mechanisms.[27] Caffeine has been shown to prevent or reverse UV damage by inhibiting the ataxia-telangiectasia and Rad3-related protein (ATR)-checkpoint kinase 1 (Chk1) pathway[28] involved in cell cycle control.[29] By inhibiting the ATR-Chk1 pathway, caffeine prevents tumor growth and promotes apoptosis. Lastly, should UV-damage occur, topical caffeine can eliminate UV-damaged keratinocytes[30] and subsequently inhibit skin cancer development. While this discussion frequently uses UV light as the inducer of ROS, it has been shown that both visible light and infrared also induce ROS.[31]

Our product provides a "second line" of defense in that its components also directly inhibit various inflammatory pathways. The cyclooxygenase (COX)-2 and lipoxygenase (LOX) pathways catalyze the production of pro-inflammatory substances, including prostaglandins and leukotrienes.[32,33] Various biochemical pathways are also associated with the induction of inflammatory cytokines (tumor necrosis factor-α, IL-6, and IL-1β) that stimulate the growth of tumor cells.[34] Resveratrol works to diminish inflammation by stopping COX-2 activity,[35] likely by inhibition of the protein kinase C (PKC) signal transduction suppressing COX-2 expression.[36] This finding is important because PKC is up regulated in some types of cancer.[37,38,39] Green tea phenols (GTPs) have also been shown to have an effect on the COX pathway. GTPs in drinking water reduced inflammation markers COX-2, prostaglandin E2, proliferating cell nuclear antigen, and cyclin D1 in mice with skin damage that developed after exposure to UV radiation.[40] Other studies showed that GTPs: (1) inhibit ornithine decarboxylase, COX, and LOX; and (2) inhibit release of interleukins 1, 8, 10, and 12,[41] which are all pro-inflammatory molecules. The third compound in our product, caffeine, takes yet another approach in countering inflammation. Topical caffeine inhibits cyclic AMP phosphodiesterase, which results in increased levels of cAMP in skin, which, in turn, reduces inflammatory reactions.[42,43]

Lastly, it is important to consider that an increase in cutaneous blood supply would carve a convenient pathway for inflammatory markers to reach the skin. Angiogenesis is defined as the production of new blood vessels and/or altered permeability of existing blood vessels. A key element that stimulates angiogenesis is vascular endothelial growth factor (VEGF). Resveratrol, GTPs, and caffeine down regulate angiogenesis. A study by Pietrasik and colleagues[44] demonstrated that resveratrol modulates normal somatic cells, leading to a decrease of the angiogenic activity of endothelial cells. Mesothelial cells treated with resveratrol created an angiogenesis-suppressive milieu, reflected by the inhibited proliferation and migration of endothelial cells. This suppressive effect continued even after the cells were removed from resveratrol exposure. Endothelial cells treated directly with resveratrol also showed anti-angiogenic activity. The anti-angiogenic effect of resveratrol may be associated with its activation of glycogen-synthase kinase 3b (GSK3b), which results in decreased production of VEGF via down-regulation of b-catenin.[45] GTPs play an anti-angiogenic role by inhibiting phosphorylation of VEGF receptors[46] required for VEGF binding. Meanwhile, pretreatment of cells with caffeine significantly reduces adenosine-induced VEGF promoter activity and VEGF and IL-8 expression.[47] The anti-angiogenic effects of all three compounds in our product may directly reduce redness.

CONCLUSION

The skin product's unique combination of resveratrol, green tea polyphenols, and caffeine reduces facial redness in most patients after 3 to 6 weeks of continuous treatment and may provide further improvement with additional treatment.

DISCLOSURE

The study was initiated and funded by one of the authors (N.I.B.). That author contributed to the conceptualization and design of the product but holds no patents and does not benefit from its sale. This product is commercialized as Replenix Power of Three by Topix Pharmaceuticals.

REFERENCES

1. Brody, N. Effective antioxidants from bench to the clinic. *Lecture Hawaii Derm.* 2008
2. Berson, D. Natural antioxidants. *J Drugs Dermatol* 2008;7(7 Suppl):S7-S12.
3. Farris, P. Idebenone, green tea, and coffeeberry extract: new and innovative antioxidants. *Dermatol Ther.* 2007 Sep-Oct;20(5):322-9.
4. Palmer DM, Kitchin JS. Oxidative damage, skin aging, antioxidants and a novel antioxidant rating system. *J Drugs Dermatol.* 2010 Jan;9(1):11-5.
5. Bradu SM, Jagdeo J, Bajor E, Shwereb C, Hannan R, Brody N. Inhibition of hydrogen peroxide-generated lipid peroxidation product 4-hydroxy-2-nonenal by green tea polyphenols in human fibroblast WS-1 cells. Society for Investigative Dermatology 65th Annual Meeting, Providence, RI. 2004 Abstract. Poster.
6. Jagdeo J, Bradu SM, Sorace M, Bajor E, Ellis L, Sieminska J, Brody NI. Inhibition of hydrogen peroxide- generated intracellular free radicals by caffeine and green tea polyphenols alone and in combination in human fibroblast WS-1 cells quantified by dihydrorhodamine. Anti-Aging World Conference, 2005. Abstract.
7. Silverberg JI, Jagdeo J, Patel M, Siemenska J, Michl J, Brody N. Green Tea Extract Protects Human Skin Fibroblasts From reactive Oxygen Species-Associated Cell Death. *Accepted Journal of Drugs in Dermatology* 2011.
8. Baxter RA. Anti-aging properties of resveratrol: review and report of a potent new antioxidant skin care formulation. *J Cosmet Dermatol.* 2008 Mar;7(1):2-7.
9. Jagdeo J, Adams L, Lev-Tov H, Sieminska J, Michl J, Brody N. Anti-Oxidant Function of Resveratrol Demonstrated via Modulation of Reactive Oxygen Species in Normal Human Skin Fibroblasts in Vitro. *Journal of Drugs and Dermatology.* December 2010.
10. Elmets CA, Singh D, Tubesing K, Matsui M, Katiyar S, Mukhtar H. Cutaneous photoprotection from ultraviolet injury by green tea polyphenols. *J Am Acad Dermatol.* 2001 Mar;44(3):425-32.
11. Heffernan TP, Kawasumi M, Blasina A, Anderes K, Conney AH, Nghiem P. ATR-Chk1 pathway inhibition promotes apoptosis after UV treatment in primary human keratinocytes: potential basis for the UV protective effects of caffeine. *J Invest Dermatol.* 2009 Jul;129(7):1805-15. Epub 2009 Feb 26.
12. Pinnell SR. Cutaneous photodamage, oxidative stress, and topical antioxidant protection. *J Am Acad Dermatol.* 2003 Jan;48(1):1-19; quiz 20-2.
13. Kalish R, Brody N. The effects of tumor facilitating factor of B16 melanoma on the macrophage. *J Invest Dermatol.* 1983 Mar;80(3):162-7.
14. Villeda S, Luo J, Mosher K, Bende Z, Britschgi M, Gregor B, Stan T, Fainberg N, Ding Z, Eggel A, Lucin K, Czirr E, Park J, Couillard-Despres S, Aigner L, Li G, Peskind E, Kaye J, Quinn F, Galasko D, Xie X, Rando T, Wyss-Coray T. The ageing systemic mileu negatively regulates neurogenesis and cognitive function. *Nature.* 2011 December;90-94.

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Enhanced Post-Procedure Healing And Reduced Discomfort With Use Of A Sterile Treatment-Serum Infused Biocellulose Mask.

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ABSTRACT

Background. Dermatologic procedures may be accompanied by slow healing and post-treatment discomfort. A novel biocellulose mask is designed to relieve post-procedure discomfort, improve rates of healing, and reduce the appearance of redness for 1 week or longer.

Objectives. To evaluate the efficacy and safety of a biocellulose mask to accelerate healing, enhance improvement, and reduce discomfort following a RF/microneedling procedure of the face.

Methods. Ten healthy females aged 35 to 60 years, Fitzpatrick skin type II (n=7) and III (n=3), and mild to moderate wrinkles (Glogau grade II[n=8] or III [n=2]) enrolled in the open-label, single-site pilot study. Subjects were treated once with 2 passes of a microneedle radiofrequency (RF) device (EndyMed PRO™, Intensif Handpiece, EndyMed Medical, Cesarea, Israel) Treatment was immediately followed by application of the biocellulose mask to the entire face for 15 to 20 minutes. Subjects were given an additional six masks for daily home use and asked to return to the office 3 and 7 days later for evaluation of efficacy and safety. Skin responses were tracked by photography of subjects' faces immediately post procedure (pre-and post-mask application), and on days 3 and 7. Clinical grading was performed on days 1, 3 and 7.

Results. Subjects achieved statistically significant improvement in skin radiance, smoothness, texture, and dryness after a single RF/microneedling treatment followed by daily usage of the biocellulose mask for 1 week. Skin tone evenness, red/blotchiness, and overall appearance were trending toward significant improvement by Day 7. Adverse events were not observed in any subject. Seventy percent of subjects would recommend use of the mask after RF/micro needling treatment.

Conclusion. The results demonstrate the effectiveness of the biocellulose mask in soothing skin and accelerating its healing post a RF/microneedling procedure. The mask may be used directly on compromised skin immediately post microneedling, without objective or subjective irritation. Improvement and conditioning of the facial skin using the mask daily for one week after a treatment has been shown.

INTRODUCTION

Dermatologic procedures are often associated acute erythema, edema, post-procedure discomfort and slow healing. An unbranded, sterile, serum-infused, biocellulose mask (Topix Pharmaceuticals, Inc., West Babylon, NY) has been developed to relieve patient discomfort, while accelerating rates of healing, and minimizing inflammation post dermatologic procedures.

Components of the mask include short and long molecular weight hyaluronic acids, matrix repair peptides, green tea polyphenols, resveratrol and other antioxidants. The short chain fractions of HA readily penetrate the skin to deeply moisturize and prevent transepidermal water loss. The high molecular weight HA fractions retain moisture on the surface. The anti-inflammatory, anti-oxidant blend of green tea polyphenols, resveratrol, caffeine, ectoin and ergothioneine promote recovery and support healing. The biocellulose substrate saturated with the serum, comfortably adheres to the face, enveloping post-procedure skin with deep and sustained hydration and healing agents, while providing cooling and soothing comfort.

A non-insulated microneedle radiofrequency (RF) device (EndyMed PRO™, Intensif Handpiece, EndyMed Medical, Cesarea, Israel) has been shown to create microzones of coagulation in the papillary and reticular dermis with minimal epidermal damage, and bulk volumetric heating (Harth 2013, Harth 2014). This technology has shown efficacy in the treatment of acne scars (Harth 2014) and, in combination with non-ablative RF skin tightening and ablative RF fractional skin resurfacing, in full-face skin rejuvenation (Kaplan 2016). RF microneedling can be used on all skin types without concerns over dyschromia

RF microneedling for aesthetics is rapidly increasing in popularity and is a useful model to evaluate the safety and efficacy of the post-procedure treatment mask. The present study assesses the healing benefit and comfort enhancement of the aforementioned biocellulose mask immediately after a single RF/microneedling treatment and for the following week.

METHODS

Subjects. Ten healthy females aged 35 to 60 years, Fitzpatrick skin type II (n=7) and III (n=3), and mild to moderate wrinkles (Glogau grade II[n=8] or III [n=2]) enrolled in the open-label, single-site pilot study. Inclusion criteria were willingness to (1) use only their current skin care regimen during the study; (2) use no products other than the dispensed skin cleanser, sunscreen, and biocellulose masks; and (3) willingness to wear a hat or apply SPF 30 or greater sunblock if sun exposure is necessary. Grounds for exclusion were pregnancy, breast feeding, plans for pregnancy, or unwillingness to use appropriate methods of birth control; excessive facial exposure to UV radiation (sunlight or artificial); presence or recent history of a facial condition (e.g., moderate to severe acne vulgaris, atopic dermatitis, psoriasis, rosacea, seborrheic dermatitis, excessive facial hair or coloration) which, in the opinion of the principal investigator, might interfere with evaluation of study parameters; presence of implanted metal devices in the treatment area; invasive or non-invasive skin treatments (e.g., hair removal, injectable fillers or toxins) in the target area within the previous 3 months; permanent makeup, tattoos, body piercing, or excessive hair in the treatment area; history of squamous cell carcinoma or melanoma in the treatment area within the previous 5 years; allergy or hypersensitivity to any of the product ingredients; current or recent (past 30 days) participation in another research study; or per the principal investigator's

discretion, any other mental or physical condition that might make it unsafe for the subject to participate in this study. All subjects consented to photography and provided signed informed consent to participate in the study.

Study Design. The protocol is outlined in Table 1. Subjects were screened and qualified subjects were enrolled during Visit 1 (Day 1). Screening and baseline evaluations occurred during Day 1 unless a washout period was required. Subjects were instructed to stop using topical astringents and abrasives (1 week), antibiotics (2 weeks), retinoids (2 weeks), and glycolic or lactic acids (2 weeks) on the face. Photographs (full front, 45° left and right) were obtained with a mounted digital camera (Canfield Scientific, Parsippany, NJ).). For photography, subjects wore no makeup, including foundation, blush, eye shadow, lipstick and mascara. They were required to remove all jewelry, use headbands to keep hair back away from face, and keep their eyes closed for all photographs.

Table 1. Protocol outline

Procedure	Day		
	1	3 (± 1)	7 (± 1)
Investigator global assessments*	x	x	x
Subject tolerability assessments	x	x	x
Glogau grading	x	x	x
RF/Microneedling treatment	x		
Mask Posttreatment for 15-20 minutes	x		
Dispense diary	x		
Reconcile/collect diary		x	x
Concomitant medications query	x	x	x
Adverse event query		x	x
Subject satisfaction questionnaire			x
Clinical photography*	x	x	x

*Before and after treatment.
RF = radiofrequency

Pretreatment. The face of each subject was washed thoroughly with soap and lukewarm water and dried. The target area was photographed and assessed visually to determine initial RF/microneedling treatment parameters. Topical anesthetic was applied and later removed with a gauze pad moistened with 70% medical grade alcohol. Care was taken to remove all anesthetic before RF/microneedling treatment.

Treatment device. The FDA-approved RF/microneedling device uses a consumable sterile tip containing a matrix of tiny RF micro-needle (300-micron diameter) electrodes arranged to deliver RF energy. Using the microprocessor control, the operator can modify the treatment parameters to achieve specific tissue effects directly related to the subject 's skin condition.
Treatment area: Describe how the full face was treated before getting into detail about the test spot, otherwise readers may think only a test spot was treated.

Determining treatment parameters. A test spot was done in the target area of each subject to define the optimal treatment parameters. Low parameters were used initially and adjusted according to observed skin reactions and needs of the subject. For example, if the skin had mild to moderate redness and edema and the subject felt mild to supportable discomfort for a given set of parameters, the operator did not alter the settings. If erythema and edema were absent and the subject felt no discomfort at the

initial settings, the pulse duration was increased by 30 msec or the power was increased by 2 watts. If the jaw, zygomatic arch, or temples were treated and the skin was edematous and the subject felt great discomfort, the pulse duration was reduced by 30 msec or the power was decreased by 2 watts. The recommended initial parameters for each facial area are shown in Table 2.

Table 2: Recommended initial parameters for radiofrequency/microneedling treatment.

Area Of Treatment	Energy (watts)	Needles Depth (mm)	Pulse Duration
Forehead	13	1.2-1.4	110
Periorbital	12	1-1.2	110
Cheeks	14	2.2-2.8	110-140
Perioral	12	1.2-1.5	110

Mask. When the appropriate treatment parameters were determined each subject was treated with 2 passes of the RF/microneedling device over the whole face. Treatment was immediately followed by application of the biocellulose mask to the entire face for 15 to 20 minutes. Subjects were given an additional six masks for daily home use and asked to return to the office on 3 and 7 days later for evaluation and maintain daily treatment diaries. Compliance was evaluated by notes in the diary.

Study objectives. The primary objective was to show the efficacy and safety of using the biocellulose mask to accelerate healing after the RF/microneedling procedure. The secondary objective was to evaluate improvement and conditioning of the facial skin using the mask once daily for one week after a single RF microneedling treatment.

Assessments. Wrinkles were evaluated at each visit using the Glogau scale (Type I, no wrinkles with minimal to no discoloration and no keratoses; Type II, wrinkles in motion, slight lines near the mouth or eyes, no keratoses; Type III, wrinkles at rest, always visible, noticeable discolorations, visible keratoses; Type IV, only wrinkles throughout, makeup appears to cake and crack when applied, gray or yellow discoloration of skin, history of skin cancer). Full-face Investigator Global Assessments (Table 3) were made at each visit, before and after treatment and before and after application of the mask, using a scale of 0 to 4.

Table 3. Global assessments of skin attributes

Skin Attribute	Rating	
	0	4
Radiance	dull	glowing
Tone (evenness)	Even, healthy color	Uneven, discolored
Smoothness	smooth appearance	severe, rough appearance
Texture	smooth, even feeling	rough, uneven feeling
Red/Blotchy	clear	severe redness
Dryness/Flakiness	smooth	rough and dry
Overall Appearance	Healthy, youthful appearance	poor appearance

Full-face investigator objective tolerability was as
erythema, edema, dryness, and peeling using the
scale: 0=none, 1=minimal, 2=mild, 3=moderate, ar
Full-face subjective tolerability was assessed as
tingling, itching, burning, and itching using the fol
0=none, 1=minimal, 2=mild, 3=moderate, 4=sever

Data analysis. The investigators hypothesized that the use of mask immediately after the RF/microneedling procedure would result in (1) improved scores in the Investigator Global Assessments (a measure of efficacy) and (2) acceptable scores in the Investigator Objective and Subjective Tolerability Assessments (indicators of safety). The investigators further hypothesized that facial skin condition would be improved by the healing components of the mask. Since the scales are discrete whole numbers, comparisons with baseline were made using the non-parametric Wilcoxon Signed Rank Test. Corrections for multiple comparisons were not made.

Adverse events. Subjects were asked to record adverse events in their diaries. Severity and relationship to the study treatment were assessed and recorded.

RESULTS

Adverse events were not observed in any subject during the study. Efficacy, tolerability, and subject satisfaction are shown below.

Radiance. The median pretreatment assessment for all skin attributes, including radiance, was 2.0 initially (Table 1). For radiance, a higher score indicated improvement and the highest score for any subject was 3.0 throughout the study. The number of subjects achieving 3.0 at each time point is shown in Figure 1. Median improvement compared to baseline was significant at Day 7 (Table 1) in which 8/10 subjects achieved improvement at 3.0.

Table 1. Investigator global assessments (median [IQR]) with comparisons to baseline

	Day 1			Day 3	Day 7
Skin Attribute	PreT, PreM*	PostT, PreM	PostT, PostM	PostT, PostM	PostT, PostM
Radiance	2.0 (0.2)	2.0 (0.1)	2.0 (0.0)	2.0 (1.0)	3.0 (0.1) (P = 0.0313)
Evenness	2.0 (0.0)	2.0 (1.0)	2.0 (1.0)	2.0 (0.1)	2.0 (1.0)
Smoothness	2.0 (0.0)	3.0 (0.1)	2.0 (0.0)	1.5 (1.0)	1.0 (0.0) (P = 0.0195)
Texture	2.0 (0.0)	3.0 (0.1)	2.0 (0.1)	2.0 (1.1)	1.0 (0.0) (P = 0.0078)
Blotchy, Red	2.0 (0.0)	3.0 (0.0)	2.0 (0.0)	2.0 (1.0)	1.0 (1.0)
Dryness	2.0 (0.0)	2.0 (0.1)	2.0 (1.0)	1.5 (1.0)	1.0 (0.0) (P = 0.0078)
Overall Appearance	2.0 (0.0)	2.5 (1.0)	2.0 (0.0)	2.0 (1.0)	2.0 (1.0)

*Baseline.
IQR = interquartile range; PreT = pretreatment; PreM = premask; PostT = posttreatment; PostM = postmask.

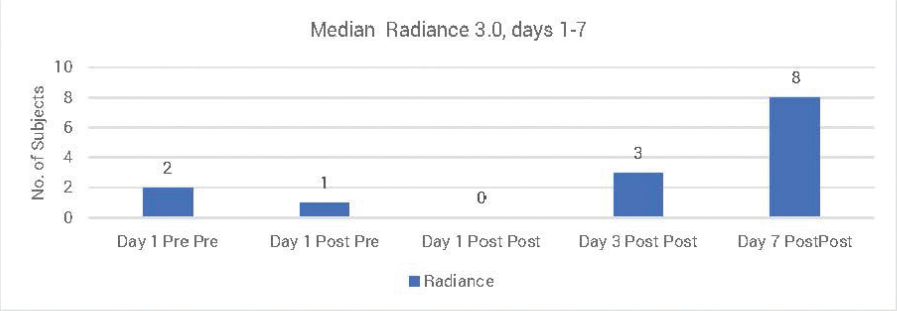


Figure 1. Number of subjects who achieved a score of 3.0 for radiance 1 to 7 days after a single radiofrequency/microneedling treatment and application of the biocellulose mask.

Other Assessments. For the remaining skin attributes, a lower score indicated improvement and the lowest score for any subject was 1.0 throughout the study. The number of subjects achieving 1.0 at each time point is shown in Figure 2 for each attribute. Improvement became apparent for all attributes by day 3. At Day 7, median improvement compared to baseline was significant for smoothness, texture, and dryness (Table 1). Trends (non-significant) toward improvement were evident for evenness ($p = 0.1250$), red blotchy ($p = 0.0547$), and overall appearance ($p = 0.1250$) at Day 7. On Day 1 (PosT, PreM), 10 subjects were scored 2 or 3 for evenness, red/blotchiness, and overall appearance. On the third day, 9, 7, and 8 subjects were scored 2 or 3 and 1, 3, and 2 subjects were scored 1, respectively. On the seventh day, 6, 4, and 6 subjects were scored 2 or 3 and 4, 6, and 4 subjects were scored 1, respectively. These data clearly indicate a trend toward improvement for these attributes with daily usage of the mask.

Figure 2. Number of subjects who achieved a score of 1.0 for skin attributes 1 to 7 days after a single radiofrequency/microneedling treatment and application of the biocellulose mask

Tolerability. Objective and subjective tolerability for all subjects during the study are shown graphically in Figures 3 and 4. Erythema (Figure 3) was resolved in all subjects by Day 7 and edema (Figure 3) was absent in all subjects by Day 3.

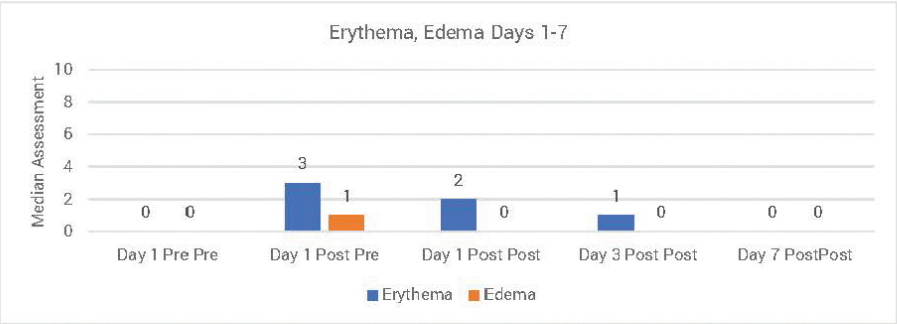


Figure 3. Median assessment score (all subjects) for erythema and edema 1 to 7 days after radiofrequency/microneedling treatment and application of the biocellulose mask

Median tolerability scores for stinging, tingling, and burning during the study are shown in Figure 4. Stinging (Figure 4) was either 1 or 2 at Day 1 (PosT, PreM); either 1 or 0 at Day 1 (PosT, PosM); and 0 in all subjects thereafter. On Day 1 (PosT, PreM), tingling (Figure 4) was either 0 or 1 in 9 subjects and 2 in a single subject. On Day 1 (PosT, PosM), tingling was 0 in 9 subjects and 1 in a single subject. Tingling was 0 in all subjects thereafter. Itching was 0 or 1 on Day 1(PosT, PreM); 0 in all subjects on Day 1 (PosT, PosM); 0 or 1 on Day 3, and 0 in all subjects on Day 7. Burning (Figure 4) was 0 to 2 on Day 1 (PosT, PreM); 0 in 9 subjects on Day 1 (PosT, PosM), and 0 in all subjects for the remainder of the study.

Enhanced post-procedure healing and reduced discomfort with use of a sterile treatment-serum infused biocellulose mask.

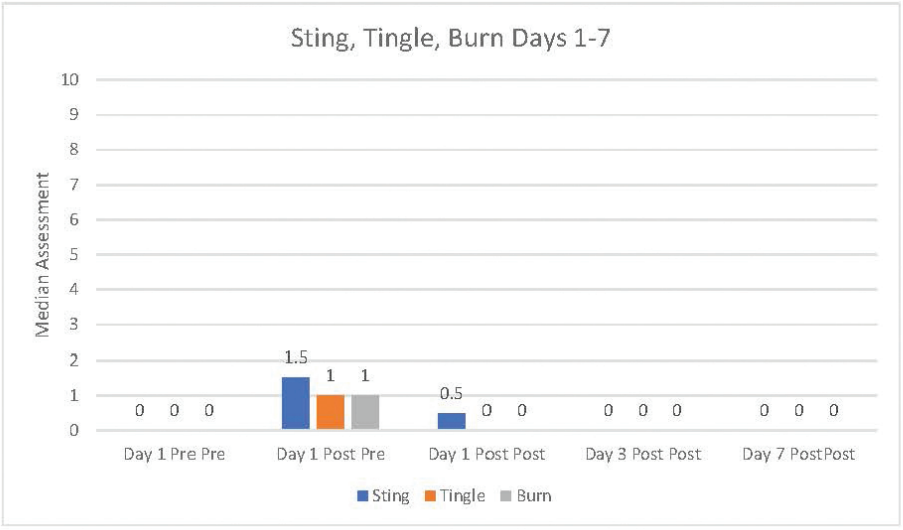
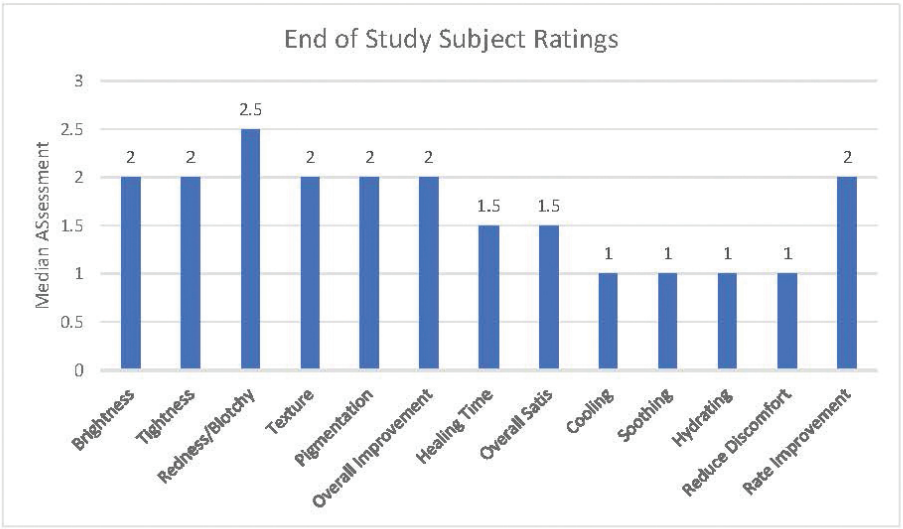


Figure 4. Median assessment score (all subjects) for stinging, tingling, and burning 1 to 7 days after a single radiofrequency/microneedling treatment and application of the biocellulose mask.

Dryness was scored 0 in most subjects at each time point. The remaining subjects were scored 1 (n=1, Day 1 [PosT, PreM]; n=1, Day 3; and n=2, Day 7). Peeling was scored 0 in all subjects at each time point.



Enhanced post-procedure healing and reduced discomfort with use of a sterile treatment-serum infused biocellulose mask.

Figure 5. Median assessment score (all subjects) for end-of-study parameters 1 to 7 days after a single radiofrequency/microneedling treatment and application of the biocellulose mask.

Subject Self-Assessments. Subjects were asked to assess the results (0-4 with 4 the most favorable) for the parameters shown in Figure 5. The median value was highest (2.5) for redness/blotchy followed by brightness, tightness, texture, pigmentation, overall improvement, and rate improvement (all 2.0), and then healing time and overall satisfaction (all 1.5) and cooling, soothing, hydrating, and reducing discomfort (all 1.0). The minimum value of all parameters was 1.0 and the maximum values were 4.0 (redness blotchy, hydrating, reducing discomfort, rate improvement), 3.0 (brightness, tightness, texture, pigmentation, overall improvement, healing time, overall satisfaction) and 2.0 (cooling, soothing).

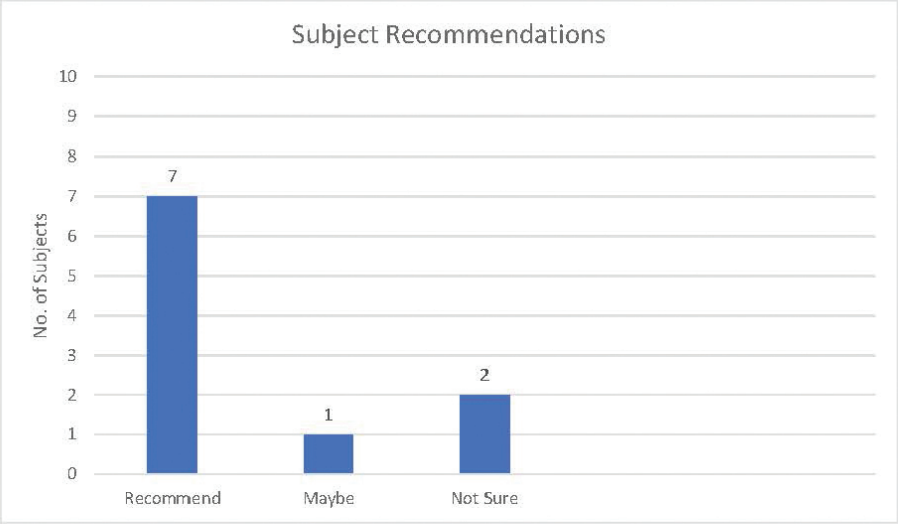


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Figure 6. Willingness of subjects to recommend the RF/microneedling procedure with face mask to friends and family.

Discussion

There is no set protocol for post procedure in today's world. We have been recommending moist environments for many years – which started with petrolatum and has progressed to include products with various growth factors or silicone-based products to help wounds heal faster than what we have with just plain petrolatum. The advantage of the mask is first – the initial soothing effect on the skin which works faster than most anything I know. And with its novel combination of anti-inflammatory ingredients, it also helps reduce redness very quickly – and was very accepted by everyone that used it.

This is the first study to evaluate the efficacy and safety of a biocellulose mask to accelerate healing, enhance improvement, and reduce discomfort up to 1 week after a single RF/microneedling procedure of the face.

Subjects achieved statistically significant improvement in skin radiance, smoothness, texture, and dryness after a single RF/microneedling treatment and daily usage of the biocellulose mask for 1 week (Table 1). The earliest noticeable improvements were in smoothness and dryness as shown by

the decrease in median scores from 2.0 to 1.5 on Day 3 for both skin attributes. Data for evenness, red/blotchiness, and overall appearance suggested trends toward improvement in these attributes by Day 7.

Adverse events were not observed in any subject. Tolerability was indicated by favorable trends in both objective and subjective parameters. Erythema was resolved in 4 subjects by Day 3 and in all subjects by Day 7 while edema was resolved in 6 subjects on Day 1 (PosM) and in all subjects by Day 3 (Figure 3). Stinging, tingling, and burning were gone by Day 3 (Figure 4) while itching was absent by day 7. Minimal dryness was noted in only a few subjects during the study. Peeling was not observed in any subject during the study.

Seventy percent of subjects would recommend the treatment and mask and the remaining subjects were not sure. No subject stated that he or she would not recommend the mask. Subjects were most satisfied with improvement in skin redness/blotchiness, brightness, tightness, texture, pigmentation, overall improvement, and rate of improvement.

These encouraging results justify additional studies with more subjects, longer duration, and a control group undergoing RF/microneedling treatment without the biocellulose mask.

CONCLUSIONS

The results demonstrate the efficacy and safety of the biocellulose mask to accelerate healing post RF/microneedling. Improvement and conditioning of the facial skin using the mask daily for one week has been shown.

References

Kaplan H, Kaplan L. Combination of microneedle radiofrequency (RF), fractional RF skin resurfacing and multi-source non-ablative skin tightening for minimal-downtime, full-face skin rejuvenation. *J Cosmet Laser Ther*. 2016 Dec;18(8):438-441.

International Cosmetic Ingredient Dictionary and Handbook, Sixteenth Edition, Volume 1, Editors: Beth Lange, Ph.D., et al, Published by Personal Care Products Council, 1620 L Street, NW, Suite 1200, Washington, D.C. 20036

Harth Y, Frank I. In vivo histological evaluation of non-insulated microneedle radiofrequency applicator with novel fractionated pulse mode. J Drugs Dermatol. 2013 Dec;12(12):1430-3.

Harth Y, Elman M, Ackerman E, et al. Depressed acne scars—effective, minimal downtime treatment with a novel smooth motion non-insulated microneedle radiofrequency technology. J Cosmet Dermatol Sci Appl. 2014; 4: 212-218.