The Effects of a New Transdermal Hydrating and Exfoliating Cosmetic Face Mask in the Maintenance of Facial Skin

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Hydration of the stratum corneum plays a critical role in all aspects of cosmetic dermatology as well as in certain skin diseases. Water content is of crucial importance to maintain a healthy, supple, and soft-appearing epidermal surface and barrier function. This article is the first in a series that investigates the effectiveness of the hydrodynamic significance of a new skin therapy utilizing a combination of lyophilized botanicals to prepare the facial surface for any type of resurfacing procedure and continued maintenance of the resurfacing results, or as a device to increase hydration of the epidermis when needed. The water content of stratum corneum is essential and critical to healthy skin maturation and desquamation. Conventional in vivo noninvasive test methods do not provide direct information about water penetration profiles or the effect of water on stratum corneum components that hold water. This research investigates moisture absorption rates of stratum corneum and the effects of skin hydration on the ultrastructural and microscopic changes in dermal type III collagen. It also evaluated heat shock protein (HSP) distribution through immunohistochemical analysis because the stratum corneum loses its capacity to bind water with age. The absorption tests were performed at differing times on women aged 38 to 64 years after initial face mask test material application and removal. Comparing both treated and untreated skin samples from mastectomy tissue specimens, researchers observed that treated skin samples absorbed and retained as much as 30% of the moisture presented to the surface of the skin as compared to untreated skin samples. The following observations were made between treated and untreated skin samples: substantial dermal microvascular changes, thickening of epidermal stratum corneum, flattening of dermal papillae, and water retention in collagen. The observed thickening of the epidermal stratum corneum may provide an exceptional pretreatment milieu for desquamation with any type of facial resurfacing procedure. Utilizing this technology on a weekly basis could provide an

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excellent posttreatment maintenance program for continued removal of dead surface skin cells and cell fragments, as well as the protein debris that tends to dull the appearance of the skin. The lyophilized botanicals provided by the face mask supply beneficial medicinal properties not found in many dermaceutical preparations.

BACKGROUND

Before the mid-1970s, the stratum corneum was thought to be biologically inert, like a thin plastic sheet protecting the more active lower layers of the skin. In the past 30 years, and especially the past 5 years, scientists have discovered that the biological and chemical activity of the stratum corneum is very intricate and complex. Among the various functions of the skin, the most fundamentally important one is carried out by the stratum corneum and barrier function. It effectively protects the body from desiccation, even in a dry environment. Cells of the stratum corneum contain keratin, a protein that helps keep the skin hydrated by preventing water evaporation.

An additional function is the prevention against external invasion of injurious agents. These keratin cells can absorb large amounts of water, aiding in skin hydration which explains why humans experience wrinkling of the skin on the fingers and toes when immersed in water for prolonged periods.

Despite the general decline of various bodily functions as individuals advance in age, the barrier function of the stratum corneum does not deteriorate but rather seems to improve with aging, which reflects the reduced epidermal proliferation associated with slower desquamation of the stratum corneum. Although the intercellular lipid production crucial for maintaining the stratum corneum is reduced in the aging epidermis, it is compensated by a thickening of the stratum corneum by larger corneocytes covering the aged skin surface due to the retardation of the desquamating process.

A decrease in the amount of water-binding and water-retaining capacity in the stratum corneum in aging skin is most likely due to a deficiency in the numerous lipid lamellar sheets in the matrix of stratum corneum. These cross-linked cornified cell envelopes of proteins and lipids provide one of the most important functions of the stratum corneum that keeps the skin surface soft and smooth. The most likely contributing factor that accounts for decreased water retention is large areas of skin that are exposed and subjected to continual UV radiation. Sun-damaged skin is most evident in elderly individuals who often display the unique features of photoaging on their exposed skin surfaces, especially the face and hands. Chronic exposure to UV radiation destroys the integrity of the skin (the true appearance of non–sun-damaged skin of any individual can be seen on the hips during bathing). Photoaging of the face and hands most frequently is treated with various resurfacing modalities. The question arises whether there is a simple pretreatment hydrating modality that will enhance the effects of facial resurfacing and help maintain the results of the procedure over a prolonged period of time.

Historically, cutaneous delivery of medications with patch technology is not new to medicine, but it is relatively new to dermatology. Several pharmaceutical compounds delivered by cutaneous patches have enjoyed success in recent years, including nitroglycerine, scopolamine, estradiol, clonidine, glycol salicylate, and methyl salicylate.[1-3] The active ingredient is applied to the skin on a simple adhesive substrate, while different materials are employed to obtain a controlled uniform release over time of the subject medications.

A new transdermal hydrating and exfoliating cosmetic face mask (Indige) achieves improvements in treated skin areas with compounds not typically used to mediate release of substances known for their therapeutic effects. It is worth noting that the face mask contains ingredients with active principle ingredients that both penetrate the skin barrier and retain moisture. The transdermal face mask utilizes a unique formulation of low pH emulsifiers, surfactants, antioxidants, and botanicals that are known for their medicinal qualities as tissue revitalizers and anti-inflammatories.

The Indige transdermal face mask consists of a mixture of polyvinyl alcohol; linoleamidopropyl PG-diammonium chloride; vitamins C, A, and E; sodium salt of pyroglutamic acid; and the botanical extracts aloe vera, common comfrey, geranium, calendula, elderberry flower, burdock, ephedra, cucumber, plantain, and tormentil in a honey-based vehicle. Modern medicine is rooted in ethnobotanical traditions using indigenous flora to treat symptoms of human diseases or to improve specific aspects of the body condition.

Unfortunately, the American medical community generally lacks knowledge of the function, metabolism, interaction, adverse reactions, and preparation of herbal products. There are over 60 botanicals now marketed in dermaceutical formulations, and physicians need to gain a working knowledge of the major botanicals. Unlike many pharmaceutical compounds that often are designed to block enzyme reactions, botanicals are more active with a wide range of functions. The medicinal effects of the face mask formulation cover a multitude of actions, such as stimulating circulation; encouraging cell division; improving skin elasticity; enhancing the lymphatic system; providing anti-inflammatory properties; balancing sebum production; and stimulating the regeneration of wound tissue by intensifying the metabolism of glycoproteins, nucleoproteins and collagen during the healing process. Other botanicals provide fungicidal, antimicrobial, antiviral, and moisturizing activity. One of the botanical extracts used in the face mask, geranium, has 250 different molecules belonging to many chemical families.

All these compounds were mixed in proper proportions in a reactor at a controlled temperature, then quick frozen at −35°C and later stabilized at 10°C. The mixture was then applied to a porous, lightweight, permeable scrim fabric and lyophilized until the moisture content reached approximately 6%. When ready for facial application, the face mask was activated with distilled water and applied directly to a remoistened facial skin surface.

The following experiments were performed with the aim of determining the mode of action of the mask material and its effects on the cutaneous surface. Clinical research was carried out by Laguens & Associate Consultants, La Plata, Argentina.

MATERIALS AND METHODS

Participants

Twenty-four women between the ages of 38 and 64 years with carcinoma of the breast scheduled to undergo mastectomy for their disease volunteered and were selected for the study. All participants were informed about the nature of the tests and each participant gave written consent. None of the test participants had any dermatologic condition that would disqualify them from participation. The participants were divided into 4 groups of 6 individuals each. Prior to the surgery a test piece of the face mask material (4×3 cm) was placed under the areola and activated with distilled water then left in place for 45 minutes.

Group A received the test face mask material immediately before surgery, during induction of anesthesia; group B received the test face mask material 1 hour before surgery; group C received the test face mask 3 hours before surgery; and group D received the test face mask 6 hours before surgery.

The average estimated time of the surgical procedure was approximately 45 minutes. Immediately upon completion of the resection of each breast, skin samples of the treated area were extracted along with skin samples of the same size from an adjacent untreated area, which acted as an untreated control. Both treated and untreated skin samples were cut into fragments for light and electron microscopy studies. Samples of skin from each group were frozen in CO_2 for cutaneous absorption testing. The remaining test material was used to determine moisture absorption rates.

Determination of Moisture Content

Each sample of treated and untreated skin was weighed separately and then placed in an oven at 100°C for 30 minutes. The samples were again weighed and moisture content was determined by measuring the evaporated water with the following formula: (X1−X2)/X1×100, where X1 is the weight of the sample before the evaporation and X2 is the weight of the sample after heat exposure.

Light Microscopy Examinations

Each sample was fixed in a formalin-buffered solution (pH 6.8) for 20 hours. The samples were then dehydrated by passage into increasing graduated alcohol solutions, embedded in Paraplast, and cut into 5-mm thickness and stained with routine hematoxylin and eosin, Giemsa, periodic acid-Schiff, Shorr, and methylene blue stains. The stratum corneum, as well as the whole epidermal thickness, was then measured together with the luminal diameters of the dermal plexus capillaries by using an image processing computer with a morphometry program and the Leica Quantimet 500 plus camera. Vascular surfaces were measured in a similar manner only if the vessels were cut perpendicular to their axis. Vessels with ovoid or elongated shapes were not measured.

Ultrasound Studies

One sample of treated as well as untreated skin was embedded for a period of 24 hours in glutaraldehyde solution 2% and postfixed with an osmium tetroxide solution 1%. They were both then dehydrated in increasingly graduated alcohol, including Araldite. Next, they were cut with an ultramicrotome, contrasted with lead citrate and uranyl acetate, and examined with a Phillips EM 200 transmission electron microscope.

Immunohistochemical Studies

Sections of both treated and untreated skin samples processed for light microscopy were deparaffinated, rehydrated, and incubated for 30 minutes with commercial monoclonal antibodies HSPs of different molecular weights: HSP27 (BioGenex); HSP70, HSP84, and HSP104 (Affinity BioReagents); type IV collagen antibodies (BioGenex) and reactions were developed with commercial kits containing alkaline phosphate for HSP (APAAP quick staining kit) and with a peroxidase antiperoxide system (PAP kit).

Absorption Studies

In order to assess the absorption capacity of the skin stimulated by the test face mask material, the presence of one of the botanical components was measured at different depths of the stratum corneum using the following method: sections of frozen skin were cut with a cryostat into 100-mm thickness. Cuts were parallel to the cutaneous surface and each section was numbered successively according to depth of cut. Every specimen underwent a dialysis process through membranes in distilled water for 24 hours at 4°C. The dialysis liquid was concentrated in Merck Extrelut filling columns and eluted with 40 mL of dichloromethane and 40 mL ethyl ether. The eluate was dried and rehydrated with absolute alcohol. The final product was inoculated in GF254 Merck silica gel plates for thin layer chromatography with methanol:ammonia mobile phase (100:1.5). Development was achieved by exposing the plates to UV light with wavelengths of 254 to 366 nm. This method was chosen after several tests were designed to determine which botanical extract would best fit the analytical method used. As a result, the botanical calendula extract was selected. This extract is rich in flavonoids, especially the flavonoid fraction methylated aglycons.[4,5]

RESULTS

Assessment of the Moisture Content

Results in Table 1 demonstrate that after 45 to 60 minutes, the mean difference in moisture between treated and untreated skin samples was 30.1873%. These differences gradually decreased in value with longer time intervals of application with the lowest values in samples 6 hours after face mask material removal.

Light Microscopy Studies

Evident morphologic changes were observed in all cases of the epidermal stratum corneum in the group of participants where the study was performed immediately after removal of the face mask material. The stratum corneum showed more thickening in treated skin than untreated skin. As the time interval between face mask material removals grew larger, the stratum corneum thickness was reduced, tending to resemble the controls (Table 2).

The observable morphologic changes in the stratum corneum were evident by a gap between the layers, whereas control skin samples showed compact corneum layers. The same was true of the dermal papillae in the treated skin, which showed enlargement of their bases. In the groups of participants with the greatest interval between face mask material application and removal, these changes were less evident.

Vascular diameters progressively decreased between treated skin and untreated skin and were no longer evident 6 hours after material removal. A marked difference in vascular diameter measurements was evident between treated skin and controls. Vascular surface values correlated exactly with the vascular diameter values obtained (Table 3).

Ultrasound Studies

In the treated skin samples obtained immediately after face mask material removal, disorganization was observed in the distribution of the collagen fibers in the surface dermal connective tissue. This disorganization was due to perifibrillar deposits of amorphous materials, most likely water, penetrating the fundamental structure of connective tissue. In samples obtained later, no substantial changes were observed in the collagen fascicles disposition or in the connective tissue matrix.

Immunohistochemical Study of Type III Collagen

Both the treated and untreated skin samples showed uniform dermal type III collagen distribution in the dermal papillae with no substantial differences in amounts when evaluated per surface unit. Nonetheless, differences found in the total amounts of type III collagen were due to a modification in the shape of the dermal papillae in treated skin: the papillae were flat and had a large base, whereas in untreated skin samples their height was 2-fold greater than the width of their base. These changes were found only in the samples obtained immediately after test face mask material removal. Over time, the samples tended to lower until becoming practically indistinguishable due to the loss of fluid.

Immunohistochemical Study: HSP Expression

Treated skin samples obtained immediately after the removal of the test face mask material showed a marked HSP27 overexpression in the malpighian stratum. The expression of HSP70, HSP84, and HSP104 was similar between treated and untreated skin samples. In both cases there were few areas in the malpighian layers. These

Table 1

Moisture Differences in Treated vs Untreated Skin Samples[a]

Group	Sample	X1	X2	Moisture, %	Difference T–U, %	Mean, %
A	1 U	69.2400	67.6693	2.26848		
	1 T	28.7910	27.9366	2.96759	30.818	
	2 U	34.2510	33.4638	2.29832		
	2 T	38.0090	36.8795	2.97166	29.297	
	3 U	47.0150	45.9476	2.27033		
	3 T	49.8266	48.3413	2.98093	31.230	
	4 U	26.8114	26.1972	2.29081		
	4 T	34.0050	32.9980	2.96132	29.230	
	5 U	52.5963	51.5250	2.22235		
	5 T	50.4250	48.9575	2.91026	30.954	
	6 U	41.1126	40.1862	2.25325		
	6 T	39.9902	38.8228	2.91921	29.555	30.1873
B	7 U	49.0455	47.9460	2.92325		
	7 T	53.3911	51.9302	2.81319	22.673	
	8 U	61.4834	60.1227	2.26316		
	8 T	56.5042	54.9527	2.82325	24.748	
	9 U	44.0725	43.1104	2.23179		
	9 T	52.4364	51.0026	2.81127	25.965	
	10 U	28.4220	27.8039	2.22231		
	10 T	30.1325	29.3020	2.83428	27.502	
	11 U	33.1629	32.4289	2.26336		
	11 T	39.3624	36.1122	2.90002	28.129	
	12 U	51.0445	49.9260	2.24038		
	12 T	52.8135	51.3254	2.89932	29.416	26.4055

Group	Sample	X1	X2	Moisture, %	Difference T–U, %	Mean, %
C	13 U	44.3105	43.3251	2.23451		
	13 T	42.4091	41.3262	2.62043	15.209	
	14 U	50.0341	48.9265	2.26386		
	14 T	49.0470	47.8321	2.53992	12.194	
	15 U	61.3695	60.0232	2.22430		
	15 T	63.5528	62.0034	2.49896	10.592	
	16 U	29.6925	29.0439	2.23332		
	16 T	28.5751	27.8672	2.54028	13.745	
	17 U	30.1085	29.4446	2.25480		
	17 T	31.2193	30.4251	2.61036	15.769	
	18 U	42.9726	42.0380	2.22315		
	18 T	49.0546	47.8352	2.54922	14.667	13.6960
D	19 U	54.2025	53.0231	2.22438		
	19 T	54.1537	52.0280	2.31589	4.119	
	20 U	43.2831	42.3265	2.26006		
	20 T	46.4729	45.3880	2.39032	5.763	
	21 U	30.0833	29.4267	2.23723		
	21 T	28.0377	27.3990	2.33121	4.201	
	22 U	61.3610	60.0340	2.21039		
	22 T	63.2612	61.8262	2.32105	5.006	
	23 U	34.0944	33.3428	2.25425		
	23 T	33.2511	32.4760	2.38384	5.882	
	24 U	48.4446	47.3828	2.23080		
	24 T	47.1677	46.1114	2.29072	2.228	4.5323

Abbreviations: X1, weight in grams before evaporation; X2, weight in grams after evaporation; T, treated; X1; U, untreated.

[a]Moisture rates of treated vs untreated skin at different times after patch removal. Group A samples were obtained immediately after patch removal. Group B samples were obtained 1 hour after patch removal. Group C samples were obtained 3 hours after patch removal. Group D samples were obtained 6 hours after patch removal.

Table 2

Stratum Corneum Thickness In Treated vs Untreated Skin Samples[a]

Group	Participant	1	2	3	4	5	Sum	Mean	SD	Range	
										Max	Min
A	1 T	0.11	0.13	0.11	0.14	0.10	0.59	0.12	0.01	0.14	0.10
	1 U	0.06	0.06	0.06	0.07	0.09	0.34	0.07	0.02	0.09	0.06
	2 T	0.14	0.11	0.13	0.16	0.11	0.65	0.13	0.02	0.16	0.11
	2 U	0.06	0.07	0.06	0.07	0.07	0.33	0.07	0.01	0.07	0.06
	3 T	0.11	0.13	0.15	0.10	0.11	0.60	0.12	0.02	0.15	0.10
	3 U	0.06	0.07	0.08	0.06	0.07	0.34	0.07	0.02	0.08	0.06
	4 T	0.10	0.11	0.14	0.13	0.12	0.60	0.12	0.01	0.14	0.10
	4 U	0.05	0.06	0.06	0.07	0.07	0.31	0.06	0.01	0.07	0.50
	5 T	0.12	0.11	0.13	0.10	0.12	0.58	0.12	0.01	0.13	0.10
	5 U	0.06	0.05	0.06	0.06	0.05	0.28	0.06	0.01	0.06	0.05
	6 T	0.11	0.12	0.12	0.13	0.13	0.61	0.12	0.01	0.13	0.11
	6 U	0.06	0.07	0.07	0.05	0.06	0.31	0.06	0.01	0.07	0.50
B	7 T	0.11	0.10	0.11	0.11	0.10	0.53	0.11	0.01	0.11	0.10
	7 U	0.06	0.05	0.06	0.06	0.05	0.28	0.06	0.01	0.06	0.05
	8 T	0.11	0.11	0.10	0.09	0.10	0.51	0.10	0.01	0.11	0.09
	8 U	0.06	0.07	0.06	0.06	0.06	0.31	0.06	0.01	0.07	0.06
	9 T	0.10	0.11	0.10	0.10	0.11	0.52	0.10	0.01	0.11	0.10
	9 U	0.07	0.07	0.07	0.06	0.06	0.33	0.07	0.01	0.07	0.06
	10 T	0.10	0.10	0.09	0.09	0.10	0.48	0.10	0.01	0.10	0.09
	10 U	0.07	0.06	0.06	0.06	0.06	0.31	0.06	0.01	0.07	0.06
	11 T	0.11	0.09	0.11	0.10	0.11	0.52	0.10	0.01	0.11	0.09
	11 U	0.07	0.07	0.06	0.07	0.06	0.33	0.07	0.01	0.07	0.06
	12 T	0.11	0.11	0.10	0.10	0.09	0.51	0.10	0.01	0.11	0.09

Group	Participant	1	2	3	4	5	Sum	Mean	SD	Range	
										Max	Min
	12 U	0.06	0.05	0.06	0.05	0.06	0.28	0.06	0.01	0.06	0.05
C	13 T	0.09	0.09	0.08	0.10	0.09	0.45	0.09	0.01	0.10	0.08
	13 U	0.06	0.07	0.06	0.05	0.06	0.30	0.06	0.01	0.07	0.05
	14 T	0.10	0.10	0.09	0.09	0.09	0.47	0.09	0.01	0.10	0.08
	14 U	0.06	0.06	0.07	0.06	0.06	0.31	0.06	0.01	0.07	0.05
	15 T	0.10	0.08	0.08	0.10	0.09	0.45	0.09	0.01	0.10	0.09
	15 U	0.07	0.06	0.06	0.07	0.05	0.31	0.06	0.01	0.07	0.06
	16 T	0.09	0.08	0.09	0.09	0.08	0.43	0.09	0.01	0.09	0.08
	16 U	0.05	0.05	0.06	0.06	0.06	0.28	0.06	0.01	0.06	0.05
	17 T	0.09	0.09	0.08	0.09	0.09	0.44	0.09	0.01	0.09	0.08
	17 U	0.06	0.06	0.07	0.05	0.06	0.30	0.05	0.01	0.07	0.05
	18 T	0.09	0.09	0.07	0.08	0.10	0.43	0.09	0.01	0.10	0.07
	18 U	0.07	0.07	0.06	0.07	0.07	0.33	0.07	0.01	0.07	0.06
D	19 T	0.06	0.07	0.06	0.05	0.06	0.30	0.06	0.01	0.07	0.05
	19 U	0.06	0.05	0.05	0.06	0.06	0.28	0.06	0.01	0.06	0.05
	20 T	0.05	0.07	0.07	0.06	0.06	0.31	0.06	0.01	0.07	0.05
	20 U	0.06	0.05	0.04	0.06	0.05	0.26	0.05	0.01	0.06	0.04
	21 T	0.07	0.06	0.06	0.07	0.07	0.33	0.07	0.01	0.07	0.06
	21 U	0.06	0.06	0.06	0.06	0.06	0.30	0.06	0.01	0.06	0.06
	22 T	0.06	0.06	0.07	0.06	0.06	0.31	0.06	0.01	0.07	0.06
	22 U	0.06	0.05	0.05	0.06	0.06	0.28	0.06	0.01	0.06	0.05
	23 T	0.50	0.06	0.06	0.06	0.07	0.31	0.06	0.01	0.07	0.05
	23 U	0.06	0.05	0.05	0.06	0.06	0.29	0.06	0.01	0.06	0.05
	24 T	0.06	0.07	0.07	0.07	0.06	0.32	0.06	0.01	0.07	0.06
	24 U	0.07	0.06	0.05	0.06	0.07	0.32	0.06	0.01	0.07	0.06

Abbreviations: SD, standard deviation; Max, maximum; Min, minimum; T, treated; U, untreated.

[a]Stratum corneum thickness (mm) for treated and untreated skin. Five measurements were performed per sample. Group A samples were obtained immediately after patch removal. Group B samples were obtained 1 hour after patch removal. Group C samples were obtained 3 hours after patch removal. Group D samples were obtained 6 hours after patch removal.

Table 3

Vascular Diameters For Treated vs Untreated Skin Samples[a]

Group	Participant	1	2	3	4	5	Sum	Mean	SD	Range	
										Max	Min
A	1 T	0.11	0.07	0.07	0.06	0.10	0.41	0.08	0.02	0.11	0.06
	1 U	0.05	0.06	0.06	0.06	0.06	0.28	0.06	0.00	0.06	0.05
	2 T	0.10	0.07	0.09	0.07	0.10	0.43	0.09	0.01	0.10	0.07
	2 U	0.05	0.06	0.05	0.05	0.06	0.27	0.05	0.00	0.06	0.05
	3 T	0.07	0.08	0.11	0.07	0.06	0.39	0.08	0.02	0.11	0.06
	3 U	0.06	0.05	0.06	0.06	0.04	0.27	0.05	0.01	0.06	0.04
	4 T	0.08	0.10	0.09	0.08	0.07	0.42	0.08	0.02	0.10	0.07
	4 U	0.05	0.05	0.06	0.06	0.05	0.27	0.05	0.00	0.06	0.05
	5 T	0.09	0.09	0.07	0.10	0.07	0.42	0.08	0.02	0.10	0.07
	5 U	0.06	0.06	0.05	0.06	0.05	0.28	0.06	0.00	0.06	0.05
	6 T	0.10	0.09	0.09	0.07	0.10	0.45	0.09	0.02	0.10	0.07
	6 7	0.05	0.06	0.06	0.05	0.06	0.28	0.06	0.00	0.06	0.05
B	7 T	0.07	0.07	0.08	0.08	0.08	0.38	0.08	0.01	0.08	0.07
	7 U	0.06	0.04	0.05	0.05	0.06	0.26	0.05	0.01	0.06	0.04
	8 T	0.06	0.08	0.08	0.06	0.10	0.38	0.08	0.20	0.10	0.06
	8 U	0.05	0.05	0.06	0.06	0.06	0.28	0.06	0.00	0.06	0.05
	9 T	0.07	0.07	0.06	0.08	0.07	0.35	0.07	0.01	0.08	0.06
	9 U	0.06	0.04	0.05	0.05	0.05	0.25	0.05	0.01	0.06	0.04
	10 T	0.09	0.06	0.06	0.07	0.08	0.36	0.07	0.02	0.09	0.06
	10 U	0.05	0.05	0.06	0.06	0.05	0.27	0.05	0.00	0.06	0.05
	11 T	0.08	0.07	0.07	0.08	0.08	0.36	0.07	0.01	0.08	0.07
	11 U	0.06	0.06	0.05	0.05	0.05	0.26	0.05	0.00	0.06	0.05
	12 T	0.10	0.07	0.09	0.06	0.06	0.38	0.08	0.02	0.10	0.06

Group	Participant	1	2	3	4	5	Sum	Mean	SD	Range	
										Max	Min
	12 U	0.05	0.05	0.07	0.06	0.06	0.29	0.06	0.01	0.07	0.05
C	13 T	0.06	0.07	0.06	0.08	0.05	0.32	0.06	0.02	0.08	0.05
	13 U	0.05	0.05	0.06	0.06	0.05	0.27	0.05	0.00	0.06	0.05
	14 T	0.07	0.05	0.06	0.06	0.07	0.31	0.06	0.01	0.07	0.05
	14 U	0.04	0.06	0.06	0.05	0.05	0.26	0.05	0.01	0.06	0.04
	15 T	0.06	0.06	0.07	0.07	0.07	0.33	0.07	0.00	0.07	0.06
	15 U	0.05	0.06	0.06	0.06	0.06	0.29	0.06	0.00	0.06	0.05
	16 T	0.06	0.06	0.07	0.06	0.06	0.31	0.06	0.00	0.07	0.06
	16 U	0.07	0.05	0.06	0.06	0.04	0.28	0.06	0.02	0.07	0.04
	17 T	0.06	0.06	0.06	0.06	0.05	0.29	0.06	0.00	0.06	0.05
	17 U	0.06	0.05	0.05	0.06	0.05	0.26	0.05	0.00	0.06	0.05
	18 T	0.07	0.08	0.07	0.06	0.06	0.34	0.07	0.01	0.08	0.06
	18 U	0.06	0.06	0.05	0.06	0.05	0.28	0.06	0.00	0.06	0.05
D	19 T	0.06	0.07	0.05	0.06	0.06	0.30	0.06	0.01	0.07	0.05
	19 U	0.06	0.05	0.06	0.06	0.06	0.29	0.06	0.00	0.06	0.05
	20 T	0.04	0.06	0.05	0.06	0.04	0.25	0.05	0.01	0.06	0.04
	20 U	0.06	0.05	0.05	0.05	0.05	0.26	0.05	0.00	0.06	0.05
	21 T	0.07	0.04	0.05	0.06	0.06	0.28	0.06	0.01	0.07	0.04
	21 U	0.06	0.05	0.06	0.06	0.07	0.30	0.06	0.01	0.07	0.05
	22 T	0.07	0.06	0.07	0.06	0.07	0.33	0.07	0.00	0.07	0.06
	22 U	0.06	0.05	0.07	0.05	0.06	0.29	0.06	0.01	0.07	0.05
	23 T	0.05	0.05	0.05	0.06	0.04	0.25	0.05	0.01	0.06	0.04
	23 U	0.06	0.06	0.05	0.05	0.04	0.26	0.05	0.01	0.06	0.04
	24 T	0.05	0.05	0.06	0.05	0.06	0.27	0.05	0.00	0.06	0.05
	24 U	0.05	0.06	0.05	0.05	0.05	0.26	0.05	0.00	0.06	0.05

Abbreviations: SD, standard deviation; Max, maximum; Min, minimum; T, treated; U, untreated.

[a]Surface dermal plexus vascular diameters (mm) for treated vs untreated skin. Five measurements were performed per sample. Group A samples were obtained immediately after patch removal. Group B samples were obtained 1 hour after patch removal. Group C samples were obtained 3 hours after patch removal. Group D samples were obtained 6 hours after patch removal.

Table 4

Expression of Heat Shock Proteins in Treated vs Untreated Skin Samples[a]

		HSP27		HSP70		HSP84		HSP104	
	Participant	T	U	T	U	T	U	T	U
A	1	+++	+/−	+	+	+	+/−	+/−	+
	2	++	−	+/−	+/−	+/−	−	+/−	−
	3	+++	+/−	+/−	+/−	+/−	+/−	+/−	+/−
	4	+++	+	−	+/−	+/−	−	−	−
	5	+++	+/−	+/−	+/−	+/−	+/−	−	−
	6	+++	+	+	+	+/−	+	+/−	+/−
B	7	++	+/−	+/−	+/−	+/−	+	−	−
	8	++	+/−	−	+/−	+	+/−	+/−	+/−
	9	+++	+	+	+/−	+/−	+/−	−	+/−
	10	++	−	+/−	+/−	+/−	−	+/−	−
	11	++	+	+/−	+/−	+/−	+/−	+/−	+/−
	12	++	+/−	+	+	+/−	+/−	−	−
C	13	++	+/−	+	+/−	+/−	+	+/−	+/−
	14	+	+/−	+/−	+/−	+/−	+/−	+/−	+/−
	15	+	−	+/−	+/−	+/−	+/−	+/−	+/−
	16	++	+	+/−	−	+/−	+/−	+/−	+/−
	17	++	+/−	−	−	+/−	+/−	+/−	−
	18	+	−	+/−	+/−	+/−	+/−	−	+/−
D	19	+/−	−	+/−	−	+/−	+	−	+/−
	20	+	+/−	+/−	−	−	+/−	+/−	+/−
	21	+/−	+/−	−	+/−	+	+/−	+/−	−
	22	−	+/−	−	+/−	+/−	−	−	−
	23	+/−	−	+/−	+/−	+/−	+/−	+/−	+/−
	24	−	−	+/−	+/−	+/−	+/−	−	−

Abbreviations: HSP, heat shock protein; T, treated; U, untreated; −, no expression; +/−, minimal expression; +, low expression; ++, regular expression; + + +, marked expression.

[a]Heat shock protein expression in epidermis treated with the transdermal patch at different times after being removed vs untreated skin. Group A samples were obtained immediately after patch removal. Group B samples were obtained 1 hour after patch removal. Group C samples were obtained 3 hours after patch removal. Group D samples were obtained 6 hours after patch removal.

differences in HSP27 expression remained in the sample 1 hour after test material removal, whereas in samples obtained later HSP27 overexpression was no longer detected (Table 4).

Absorption Studies

Thin-layer chromatography revealed a greenish spot that fluoresced when exposed to UV light at wavelengths 254 to 366 nm with a response factor of 0.2+/−0.04 in all elutes from sections made at different skin tissue depths. Its intensity decreased in sample n°2 from medium skin section. (Sample identified as n°1 corresponds to surface skin section of 0 to 100 mm in thickness and sample n°3 corresponds to deep section from 200 to 300 mm thickness.) This chromatography profile could only be observed in samples obtained immediately after test material removal. No spot was detected in sections made at deeper depths in subsequent samples. Most likely the calendula was metabolized. It has been shown for the first time that [3-3H]oleanolic acid glycosides formed in the cytosol of *Calendula officinalis* are transported to the extracellular space in the form of pentaglucoside VI (44%), whereas glucuronides derived from [3-3H]oleanolic acid 3-O-monoglucuronide (29%) as well as a part of glucosides (24%) were transported into the cell walls.[6]

COMMENT

In view of these results, this type of topical delivery system may have the potential to deliver a multitude of ingredients to improve cutaneous texture. One of the major skin changes observed in this study included the enlargement of the stratum corneum due to rehydration and water retention in all treated skin samples. The stratum corneum thickening observed was not caused by an increase in the number of corneum layers, but by absorption of fluid by dead keratin cells causing the stratum corneum to swell and begin to separate. Deeper keratin cell layers in the skin do not exhibit this phenomenon. As the dead cell layer expands it begins to take up more surface area, but it still stays connected to the living tissue beneath it. This phenomenon is seen more easily when hands and feet wrinkle when immersed in water for a long period of time. The wrinkling gives a temporary new surface area for this unique expansion. The stratum corneum separation is caused by the buildup of fluid between the layers of tissue. Furthermore, it should be noted that the stratum corneum surface layers are exfoliated when the test material is removed. The face mask material is designed to provide an adhesive quality upon removal. This exfoliative action was notable and may be evidence that preprocedure hydration will make the corneum layer easier to remove with traditional resurfacing procedures. The exfoliative action of the face mask material will be the subject of a future clinical article because it is too extensive to cover in this study. The second article will attempt to explain the smoothing action of the exfoliation process and the fine line disappearance on the cutaneous surface by supplemental facial hydration and exfoliation.

The smoothing modification of the cutaneous texture could be caused by changes in the shape of the dermal papillae, the broadening of their base, as well as the lowering of the height. This modification also could be due to water accumulation between collagen fiber fascicles, incidental to the ultrastructural and immunochemical studies of type IV collagen distribution. Papillae flattening may be due to the broadening of their bases, which would account for a reduction in height, though the pressure created by the adhesiveness of the test material may be a contributing factor to the flattening of the papillae. The dermal papillae, which provide oxygen and nutrition to the epidermis, are of great importance in maintaining the integrity of the skin and protecting the body from external forces. However, there is little information available about morphological changes in the papillae and how the collagen fibers beneath the papillae change.

Another important change was found in the vascular diameters of the treated skin in the surface dermal plexus. The vascular diameters in the treated skin increased from 30% to 50% as compared to untreated skin.

These vascular changes could be the result of medicinal effects of several of the botanical compounds, which stimulate both vascular and lymphatic circulation. These changes in increase in vessel diameter lead to an increased blood flow with decrease in intravascular pressure. This vasodilatation leads to local increases in temperature which favors an increase in cellular metabolic processes. This phenomenon has been observed in breast cancer development during the advancement from atypical hyperplasia to cancer in situ.[7]

The skin has a temperature-dependent metabolism differing from the rest of the body. A change in ambient temperature influences the skin's metabolism. These conditions do not hold true for internal tissues that are thermally isolated by adipose tissues in the hypodermis and usually function at temperature close to 37°C.[8] Increased vascular diameter and subsequent rises in local temperature were measured thermographically and will be reviewed in a subsequent clinical article.

Local thermal control of skin blood flow includes important roles of local androgenic activity, sensory nerves, and nitric oxide. Nitric oxide is critical for vasodilation of vessels and it is a clinically established fact that ascorbate stimulates nitric oxide production. Ascorbate is one of the triad of antioxidant vitamins in the face mask formulation. There also are several botanical extracts in the face mask formulation known to stimulate vascular as well as lymphatic circulation.

In addition, there is an additional physical process to be considered. Although the face mask material is permeable, it will function as a limited isolating barrier slowing down the evaporation of water that would create an interface to preserve moisture and generate a homogeneous microclimate not subject to ambient temperatures. The water retained in the stratum corneum layer also may function as an isolating barrier that prevents or reduces heat diffusion to the exterior.

A possible consequence of the latter is the overexpression of HSP27 in the treated epidermis while different molecular weights of HSP70, HSP84, and HSP104 show no significant expression. Small HSPs have the ability to oligomerize and form intracellular polymer aggregates. Oligomerization pattern is governed by the phosphorylation state of the protein that may influence its ability to protect against cellular stresses.

Heat shock proteins usually are activated by a series of stimuli, including heat, heavy metals, methanol, and stress. Heat shock protein 84 and HSP27 expression occurs in both stress and nonstress conditions because these proteins are constitutively expressed in healthy cells, particularly in association with other structural proteins, such as tubulin and actin.[9] Both proteins, such as keratin and/or its synthesis system, may be an overexpression due to unmasked HSP27 antigenic determinates associated with the tubulin-keratin protein complex.[10]

The possibility also exists that the overexpression of these HSPs is caused by the absorbed botanical constituents of the face mask material. This overexpression indicates the existence of a change in keratinocyte homeostasis. These studies also reveal that skin permeability is unaltered since calendula flavonoids were detected in deep as well as surface sections of skin. Results tend to prove that the skin remains permeable to the flavonoids as observed in the surface layers. They are most likely retained in the keratin while the excess flavonoids observed in the deeper layers could be due to a passage of excess flavonoids after the saturation of epidermal retention capacity is reached. The absence of flavonoids in median sections of the skin is interpreted as a mere question of timing in absorption kinetics. If the face mask materials were applied for 20 minutes or 3 hours instead of 45 minutes, the chromatogram profile may have been different.

In view of these results achieved at different times after application of the face mask test materials, it is evident that the intensity of changes described decrease over time even though these effects last for several hours. This evidence brings about 3 new working hypotheses: (1) Would the hydration effect of the face mask material as a preprocedure preparation improve overall exfoliation of a resurfacing technique? (2) Would the application of the face mask material improve the absorption and effectiveness of topical dermatologic topical compounds, such as an anesthetic, or compounds such as retin-A? and (3) Will the face mask material effectively help preserve the effect of postresurfacing procedures long-term if humectants were applied immediately after the face mask removal?

SUMMARY

The results of this study produced clinically measurable evidence that the mechanism of action of the Indige face mask appears to be potentially valuable in the maintenance of facial skin. When the face mask material was activated and placed in contact with wet skin, the occlusive layer appeared to interfere with the process of air exchange, increasing the local temperature, blocking the evaporation, and creating a new microenvironment over the occluded skin. This action leads to capillary dilation, creating a pushing/pulling effect that literally pushes water under the surface strata, adding to water accumulation due to the subsequent vascular phenomena.

Aside from the physical phenomena related to the structure of the face mask material, the constituents in the formulation helped produce the observed effects. The presence of sodium salt of pyroglutamic acid in the formula helps increase the process of water capture, contributing to the overall water retention in the tissues by the keratin. The presence of phospholipids and flavonoids complement the mechanism of action in the face mask material by providing moisture and critical proteins to the cell structure, thus promoting faster absorption of the other active ingredients. Separate evaluation of the action of mechanisms of each botanical constituent exceeds the objective of this study; related information can be found in specialized literature.[11-15]

Finally, the exfoliative effect of the face mask is worth noting. Even during clinical testing, a distinct peeling of the skin surface was noticed and will be the subject of a subsequent clinical article

reviewing the confirmation of the exfoliating effect of the cells adhering to the face mask material upon removal. Exfoliation was more notable than expected. Even though facial skin is clinically regarded as the most sensitive skin, its evolutive function makes it most resilient. It should be remembered that facial skin has a higher metabolic rate than other types of skin. This factor is apparent especially when considering sebaceous hypersecretion localized on the face and neckline triangle that provides special lubrication and thermal protection to these skin areas.

The increased exfoliative effect of the face mask on facial areas allows removal of the outermost surface stratum corneum layers of the epidermis, removing with them the lipid layer where all the metabolic detritus of the skin is deposited, along with saprophytic bacteria and environmental contaminants. These elements have an oxidizing effect on lipids, which gives the skin a dull and opaque look. This effect could be related to a deep cleansing of the epidermis. The thinning out of the treated seborrheic keratoses also is related to this exfoliative mechanism. It is not surprising that these exfoliative effects are more noticeable in seborrheic skin. The local temperature increase observed could have a favorable effect on metabolic reactivation of treated skin. This exfoliative mechanism is possibly no more than a physical phenomenon of the adherence of the surface layers of the skin to the face mask material, though this is not altogether convincing, given that this effect is generalized and uniform throughout the treated area. For this reason, the possibility that one of the botanical components of the face mask material could contain natural substances with exfoliative properties should not be disregarded. Discussion of the biological effects of the many extracts that constitute the face mask material exceeds the objectives of this study, even though it is known that many of them have an exfoliative effect. Further information regarding the mechanism of action of the botanical extracts can be found in specialized literature and will be discussed in a subsequent paper.

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