Gene Expression Changes in the Skin of Patients Undergoing Medial Thigh Liposuction With Pre-Surgical and Post-Surgical Application of Topical Products

Mary E. Ziegler, PhD; Brannon Claytor, MD, FACS; Michaela Bell, BS, MBA; Laurie Casas, MD, FACS; and Alan D. Widgerow, MBBCh, MMed, FCS, FACS

Abstract

Background: Skin topical preconditioning before and after surgical procedures is a relatively new concept, particularly in relation to the efficient removal of tissue breakdown products. Clinical trials demonstrate improvements, such as less induration, when surgery is combined with topical product preconditioning and with usage post-surgery. Objectives: This trial aimed to assess the efficacy of such a regimen at the molecular level through gene expression studies in combination with clinical assessments. Methods: Six women who underwent medial thigh liposuction administered either a bland moisturizer or the experimental topical products to each side of the surgical area twice daily. Biopsies were taken before any topical application, at 2 and 4 weeks after liposuction. An inflammation-related gene expression analysis was conducted to compare the different conditions. In addition, the degree of induration was assessed in a blinded manner. Results: Compared with the bland moisturizer, the experimental group demonstrated a hastened immune inflammatory response moving more rapidly to an anti-inflammatory reversal at 2 weeks followed by a wound healing extracellular remodeling effect at 4 weeks. This matched the clinical picture depicting less induration with the treatment. Conclusions: For patients undergoing body procedures, a topical treatment with the Alastin induces an accelerated healing response, inducing the clearance of “waste” products and the induction of anti-inflammatory genes. Furthermore, this topical treatment stimulates extracellular matrix remodeling, which ultimately leads to less induration.

Level of Evidence: 5

The concept of topically preconditioning the skin before surgery and the maintenance of topical applications post-surgery is attracting much interest. Several clinical observations and biopsy results have demonstrated improvements due to the use of topicals in the peri-procedure period in the skin’s extracellular matrix (ECM) and the resolution of skin induration, fibrous banding, and ecchymosis.1,2 This is thought to be due to an improved cross-talk between fibroblasts and collagen and elastin fibers in the ECM as a preconditioning before surgery,2 coupled with an

Corresponding Author:
Dr Alan D. Widgerow, 3129 Tiger Run Court Suite #109, Carlsbad, CA 92010, USA.
E-mail: awidgerow@alastin.com; Instagram: @alanwidge

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improvement in macrophage absorption of lipid droplets following surgeries involving the disruption of fat tissue.

From the standpoint of wound healing, for chronic wound management, it is necessary to prepare the wound bed for healing before applying any therapeutics as the buildup of byproducts from dysfunctional healing and excess inflammation can create a wound bed milieu that averts healing. Eliminating these byproducts from the wound bed is a priority in wound bed preparation, encouraging accelerated endogenous healing and facilitating the effectiveness of the therapeutic measures.

Using topical applications to provide “skin bed” preparation has yielded significant improvements based on histological and clinical observations. However, an objective molecular measurement of the changes seen within skin layers that could impact healing and patient comfort is lacking. To this end, a split-body study was designed to compare comprehensive gene expression changes in patients following bilateral extensive medial thigh liposuction with and without the use of a topical treatment.

**METHODS**

**Study Population**

This was a split-body, randomized, double-blinded study that occurred over 8.5 months from August 8, 2019 to March 17, 2020. The study was approved by the Integreview IRB (Austin, TX). A total of 6 patients participated in this study. The eligible patients included women undergoing extensive liposuction of the medial thighs. The purpose of selecting liposuction patients was due to the fact that this procedure greatly disrupts the fat, allowing for the determination of whether the treatment improved healing by clearing the debris caused by the fat disruption. The participants agreed to apply the topical products twice daily for 2 weeks before and after surgery. Patients who were not good candidates for the surgery, as determined by the physician, were excluded from participating in the study. Pregnant or lactating patients were excluded as well as those planning to become pregnant during the study duration. This study adhered to the guiding principles of the Declaration of Helsinki, and written informed consent was obtained from all patients.

**Liposuction Procedure**

The liposuction ports were identical in each case. Two incisions on each leg. The superior incision along the bikini line in the upper medial groin and the second along the medial aspect of the thigh 10 cm superior to the medial condyle of the knee. The liposuction access was always remote from the skin biopsy sites. The liposuction volume was consistent at 250 ml per medial thigh with similar suction techniques.

**Topical Application**

The patients were randomized to receive Regenerating Skin Nectar (RSN) with TriHex Technology and TransFORM Body (TFB) treatment with TriHex Technology either on the right or left treatment area. On the opposite side, they received a mild unscented moisturizer (Cetaphil Lotion, Galderma Laboratories, Fort Worth, TX), which was applied from 2 separate bottles to ensure that the patients were blinded to the experimental condition. The kits and patients numbers were randomized in excel, and the investigator was blinded. The patients were preconditioned with RSN 2 weeks before the elected surgery on the designated side. Immediately after the procedure, they applied RSN and then TFB to the designated side, around the incision and procedure area for up to 10 weeks or longer as determined by the physician. On the opposite side, the patients applied the moisturizer 2 weeks before surgery and after the surgery for up to 10 weeks. Cetaphil was used as a comparative moisturizer. Our goal was to ensure that general moisturizing was not accounting for the effects we observed. The Cetaphil was meant to counter the effects of the increased hydration that was caused by the use of the treatment products.

**Punch Biopsies**

Among the 6 patients, 5 consented to punch biopsies (3 mm) in the surgical area of both treatment sides. Three patient biopsies were subjected to gene expression analysis, while the remaining 2 were submitted for histological examination (1 patient refused biopsies). The biopsy sites were located along a line drawn from the insertion of the adductor brevis on the inferior ramus of the pubis to the medial condyle of the femur at the knee. The first biopsy was 8 cm inferior to the adductor insertion, the second biopsy was 10 cm, and the third was 12 cm inferior. The biopsies were performed at the pretreatment stage and at weeks 2 and 4 post-surgery. All biopsies were performed by a board-certified plastic surgeon. Upon collection, the biopsies for gene expression were immediately placed in RNAlater (Invitrogen, Carlsbad, CA) stabilization solution and stored overnight at room temperature. Then, the samples were stored at ~20°C until they were shipped to Genemarkers (Kalamazoo, MI) for RNA processing. A total of 18 clinical skin biopsies were obtained from 3 patients assigned for gene expression studies.

**RNA Isolation**

RNA isolation and subsequent RNA analyses described below were performed by Genemarkers. Briefly, total RNA was isolated from each biopsy using an RNeasy Mini kit (Qiagen, Germantown, MD) following the manufacturer’s instructions for fibrous tissues. RNA concentration and
purity were determined using a Nanodrop 2000 spectrophotometer (ThermoFisher, Waltham, MA). RNA integrity was assessed using an Agilent Bioanalyzer 2100 (Santa Clara, CA). All samples showed high-quality RNA metrics and similar yields were obtained for all samples, with the exception of one sample. This sample had a low RNA yield and required vacuum concentration.

**Endogenous Control Gene Selection**

An endogenous control gene was selected that was consistently expressed in all of the samples for comparison. The Thermo Fisher (TF) Human Inflammation Panel includes 21 endogenous controls tested across 29 separate assays. Three algorithms (Normfinder algorithm, Minimum Variance Median algorithm [minvarmed], and the coefficient of variability [CV]) were used to calculate the stability scores in order to determine the most consistent endogenous control gene. Lower stability scores represented a more consistent expression between samples in the study. Based on the stability scores and average rankings of the endogenous controls, hypoxanthine phosphoribosyltransferase 1 was identified as the most stable endogenous control gene.

**Reverse Transcription, Product Preparation, and Preamplification**

The TF Human Inflammation Panel contains 586 validated TaqMan Assays related to human inflammation. The reverse transcription (RT) and preamplification steps were carried out in an Applied Biosystems 2720 Thermal Cycler (Foster City, CA), according to the manufacturer’s instructions for Low Sample Input on TaqMan OpenArray Pathway Panels (Life Technologies, Carlsbad, CA). Two separate gene-specific RT products were generated from 100 ng of each RNA sample using a SuperScript Vilo RT Kit (Invitrogen, Carlsbad, CA) and 2 custom Taqman PreAmp Pools (Applied Biosystems), labeled pool A or pool B (10 minutes at 25°C, 60 minutes at 42°C, and 5 minutes at 85°C). Each RT product was then preamplified using Taqman Preamplification Master Mix (Applied Biosystems), the same custom Taqman PreAmp Pool (A or B), and 14 cycles of preamplification (15 seconds at 95°C and 4 minutes at 60°C). At the end of the preamplification, the products from pools A and B for each sample were mixed thoroughly and then diluted 1:20 with nuclease-free water.

**qPCR Processing and Analysis**

The qPCR reactions were performed in an OpenArray format in the Life Technologies QuantStudio 12K Flex instrument. Each gene was assayed in duplicate. The qPCR data quality was assessed and exported from the raw data files using Expression Suite software (Applied Biosystems). The qPCR data were then imported into the “OmicsOffice for qPCR” tool of TIBCO Spotfire Analyst software. Statistical data analysis was performed using the relative quantitation (RQ) method. In the first step of an RQ analysis, the cycle threshold (CT) value of the target gene was normalized to the CT value of an endogenous control gene to generate the delta CT (dCT). The dCT values were calculated to normalize the variability between the samples that might occur during the experimental procedures. (See the Endogenous Control Gene Selection section for more details).

**SkinFibroMeter Measurements**

The patients were assessed using SkinFibroMeter (Delfin Technologies, Miami, Fl) measurements. The SkinFibroMeter assesses induration in absolute units of stiffness. The patients underwent a pretreatment visit, a baseline (Day 0) visit, and follow-up visits at every week initially, then at 2 weeks, and then every 3 weeks until 10 weeks post-procedure/surgery. An increased value over the baseline reading was indicative of stiffness/induration. The SkinFibroMeter data are presented for the 1-week and 2-week time points for the 6 patients for both the treated and untreated sides. The readings at 4 weeks and beyond leveled out and are not presented.

**Investigator Assessment**

To assess induration, the investigators were blinded to the treatments. The level of induration was assessed using a graded scale as follows: 0 is none; 1 is barely perceptible; 2 is slight; 3 is moderate; and 4 is severe. All 6 patients were assessed at the follow-up visits at every week initially, then at 2 weeks, and then every 3 weeks until 10 weeks post-procedure/surgery. The data presented are from the 2-week and 4-week follow-ups, which correspond to the biopsy time points.

**Statistical Analysis**

For the gene expression data, paired t-tests \((N = 3, P < 0.05)\) were performed using TIBCO Spotfire software (Palo Alto, CA). The statistical comparison generated delta delta CT (ddCT) values (the mean dCT of the treated group – the mean dCT of the control group). The statistical software converted the ddCT values into log and linear RQ values for export (RQ = 2^ - ddCT). The linear RQ values were converted to linear fold-change values to simplify data interpretation. The linear fold-change data were calculated from the exported linear RQ values using Microsoft Excel.
as follows: for the RQ values > 1.0, the linear fold-change value = RQ value, and for the RQ values < 1.0, the linear fold-change value = −1/RQ value. The percent change was also provided and calculated as the RQ value minus 1. For the clinical assessment, Student’s t-tests were performed using Microsoft Excel. P < 0.05 was considered significant.

RESULTS

Assessing Overall Gene Expression Changes

The biopsies used for gene expression were all from female patients with a mean age of 36 years (range, 33-38 years). The gene expression changes for the 2-week and 4-week biopsies were compared with the pretreatment biopsies for the treated and untreated samples separately. The results are presented in Table 1. The genes included in the table have a change in gene expression ≥1.5-fold in at least one comparison. Based on these results, the gene expression results were analyzed using the different groups as described below.

Comparison of the Biopsies Collected at 2 Weeks From the Untreated and Treated Groups

To assess how the treatment affected healing after 2 weeks, the gene expression data from the biopsies collected at 2 weeks from the untreated and treated groups were analyzed. Among the significantly upregulated genes in each group, 5 genes were shared between the untreated and treated groups (Figure 1). A closer examination of the fold-change differences between the 5 genes for the treated and untreated groups revealed that the biggest fold-change difference between the groups was for the gene interleukin (IL)-6. This difference was 1.94-fold for the untreated group and 3.34-fold for the treated group. The gene expression data from the biopsy samples collected at 2 weeks from the treated and untreated sides showed strikingly different patterns of gene expression. The untreated side revealed typical inflammatory signaling that was typical after a surgical procedure, while the treatment group had an altered immune response in a manner that promoted clearance and healing.

Finally, we examined reactome pathways (https://reactome.org/) that were enriched in the groups after 2 weeks. The untreated group showed reactome pathways related to mitogen and interleukin stimulation as well as PI3K/Akt activation (Table 4). Together, these pathways are considered pro-inflammatory regulators. In contrast, the unique pathways enriched in the treated group triggered anti-inflammatory, immunomodulatory, and clearance responses. Taken together, the biopsies collected at 2 weeks from the treated and untreated sides showed strikingly different patterns of gene expression. The untreated side revealed typical inflammatory signaling that would be expected in response to the procedure. In contrast, the treated side revealed that the inflammatory response favored pro-clearance and preparation for healing.

Comparison of the Biopsies Collected at 2 Weeks and 4 Weeks From the Treated Group

The gene expression data from the biopsy samples collected from the 4-week-untreated group revealed that only 2 genes (CHRNA7 and IL-11) were differentially upregulated in comparison to the pretreatment sample. These results indicated that by 4 weeks, the untreated group had
Table 1. Assessing Gene Expression Changes Compared With the Pretreatment Biopsies

| Gene-assay       | 2W vs pre | 4W vs pre |
|------------------|-----------|-----------|
|                  | UT        | T         | UT       | T         |
| AGTR1-Hs00258937_m1 | n.s.      | 2.09      | n.s.     | n.s.     |
| BDKRB2-Hs00176121_m1 | n.s.      | n.s.      | n.s.     | −1.54    |
| BMP7-Hs00233476_m1  | −1.61     | n.s.      | n.s.     | n.s.     |
| BMP8A-Hs00257330_s1 | n.s.      | −1.50     | −1.04    | n.s.     |
| C1R-Hs00354278_m1   | n.s.      | 2.50      | n.s.     | 1.39     |
| C15-Hs01043795_m1   | 3.22      | n.s.      | n.s.     | n.s.     |
| C3-Hs0100879_m1     | n.s.      | 1.73      | n.s.     | n.s.     |
| CCL17-Hs0017074_m1  | 2.00      | n.s.      | n.s.     | 3.30     |
| CCL19-Hs0017149_m1  | n.s.      | n.s.      | n.s.     | 3.52     |
| CCL20-Hs0101368_m1  | n.s.      | 3.85      | n.s.     | 3.12     |
| CCL22-Hs00171080_m1 | n.s.      | n.s.      | n.s.     | 2.19     |
| CCL27-Hs00171157_m1 | −2.33     | −2.86     | n.s.     | n.s.     |
| CCR11-Hs00664347_s1 | 2.42      | n.s.      | n.s.     | n.s.     |
| CCR2-Hs00243702_s1  | n.s.      | 1.88      | n.s.     | 1.37     |
| CD40LG-Hs00163934_m1 | n.s.      | n.s.      | n.s.     | 1.70     |
| CD55-Hs00892618_m1  | n.s.      | n.s.      | n.s.     | −1.55    |
| CFD-Hs00157263_m1   | 1.88      | n.s.      | n.s.     | n.s.     |
| CHRNA7-Hs01063373_m1 | 2.83      | 1.41      | 1.81     | n.s.     |
| CLCF1-Hs00757942_m1 | n.s.      | n.s.      | n.s.     | −1.63    |
| CLU-Hs00156548_m1   | n.s.      | 1.55      | n.s.     | n.s.     |
| CRLF1-Hs00191064_m1 | −1.91     | n.s.      | −1.91    | −2.05    |
| CYBB-Hs001656163_m1 | n.s.      | 1.64      | n.s.     | n.s.     |
| EBI3-Hs00194957_m1  | n.s.      | n.s.      | n.s.     | 2.62     |
| FIGF-Hs01128659_m1  | n.s.      | 3.19      | n.s.     | n.s.     |
| FLT3LG-Hs00181740_m1 | n.s.      | n.s.      | 1.86     | n.s.     |
| GDF15-Hs00171132_m1 | n.s.      | n.s.      | n.s.     | 2.47     |
| HGF-Hs00300159_m1   | 2.48      | n.s.      | n.s.     | n.s.     |
| IL11-Hs00174148_m1  | n.s.      | n.s.      | n.s.     | 2.06     |
| IL1F9-Hs00219742_m1 | n.s.      | n.s.      | −1.66    | −1.79    |
| IL21R-Hs00222310_m1 | n.s.      | 1.57      | n.s.     | 1.57     |
| IL27RA-Hs00945029_m1 | n.s.      | n.s.      | n.s.     | 1.52     |
| IL32-Hs00170403_m1  | n.s.      | n.s.      | n.s.     | 1.70     |
| IL33-Hs01125943_m1  | 2.53      | n.s.      | n.s.     | n.s.     |
All of the values are the fold change relative to the pretreatment biopsy samples. Fold changes ≥1.5 were used for further analyses. 2W vs pre, biopsy results at 2 weeks post surgery compared to pre-surgical biopsy; 4W vs pre, biopsy results at 4 weeks post surgery compared to pre-surgical biopsy; CCL, chemokine ligand; CYBB, cytochrome b beta chain; HGF, hepatocyte growth factor; NLRP, Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing; n.s., not significant; TGM, transglutaminase; T, treated; UT, untreated.
essentially returned to baseline. However, in the 4-week-treated group, 18 genes were significantly upregulated compared with the pretreatment group. Given this observation, our next comparison was between the 2-week- and 4-week-treated groups, with the goal of understanding more about what the treatment was doing to further activate the healing patterns that were displayed at 2 weeks. Among the significantly upregulated genes in the 2-week- and 4-week-treated groups (Table 1), there were only 4 genes in common, including chemokine ligand (CCL20), IL21R, LY86, and PCLB2 (Figure 3). All of these genes showed a pattern of decreased expression between 2 and 4 weeks, with the exception of IL21R, which remained the same (Table 1).

The String Database analysis of the 4-week-treated biopsy samples revealed protein–protein interactions that centered around CD40LG (Figure 4). To compare these results to the 2-week-treated biopsy group, the top 10 biological process terms from each group are provided in Table 5. As mentioned above, the 2-week-treated group showed signs of immunomodulatory action based on the unique biological process terms. This was also true for the 4-week-treated group, but there was also an enrichment for terms related to rebuilding and remodeling after injury. For molecular function, the terms were similar but were decreasing with the time of treatment. For the cellular component terms, again, there was a decrease in the number of terms over time, indicating more stability in the tissue (Table 5).

Finally, we examined the reactome pathways that were enriched in the 2 treated groups (Table 6). The unique pathways in the 2-week-treated group showed evidence of the activation of anti-inflammatory, immunomodulatory, and clearance signaling processes. In contrast, the 4 unique pathways enriched in the 4-week-treated group were related to not only anti-inflammatory signaling but also macrophage regulation and ECM remodeling. Together, these data provide evidence that the treatment initially activated a gene signature that stimulated clearance and protected the tissue from harm. As time went on, the treatment abolished the pro-inflammatory signature and stabilized an anti-inflammatory environment for the promotion of new ECM and further healing.

**Induration Assessment**

To assess induration, first, the SkinFibroMeter readings at 1 week and 2 weeks after the procedure for the treated and untreated sides were compared with the baseline (before the procedure) reading. Compared with the baseline reading, the 1-week-untreated group showed a 23% increase, which was a sign of more induration, whereas the 1-week-treated group decreased by 5% compared with the baseline, indicating a lower level of induration. These differences were statistically significant ($P < 0.05$) (Figure 5A). Two weeks after the procedure, the untreated group showed a 32% increase, while the treated group increased by only 12%. These differences were not statistically significant. Taken together, the treated group showed less induration compared with the untreated group.

To further evaluate induration, we used blinded investigator assessments. The data from the 2 and the 4 weeks groups were compared between the treatment groups. At 2 weeks, there was a statistically significant lower
induration grade for the treated group. By 4 weeks, the grade was lower for both groups but was approximately 0 for the treated group (Figure 5B). This time point was not statistically significant. These data support that the treatment results in a lower level of induration after the procedure.

**DISCUSSION**

Normal everyday living expose patients’ skin to extrinsic damage, mainly in the form of photodamage, which causes ongoing changes within the ECM. Surgical procedures also violate cellular and extracellular structures that impact healing and recovery, which manifest in skin changes. In both these scenarios, one common issue is “waste product” accumulation. In the ECM, waste takes the form of fragmented collagen, elastin, and glycation end products, and can lead to impaired cellular recycling mechanisms involving the proteasome and autophagic processes. In surgical body contouring procedures, the destruction of fatty tissue releases lipid droplets from the adipose cells, which are highly inflammatory and create localized pockets of “inflammasomes” that can present as skin induration, hardened fibrous banding, and even fat necrosis, where phagocytic processes are overwhelmed. In an effort to deal with these waste products and to optimize their elimination, certain peptides and active agents have been incorporated into topical preparations (RSN with TriHex Technology and TFB Treatment with TriHex Technology, Alastin Skincare Inc., Carlsbad, CA) to improve autophagic processes and macrophage efficiency.

The mechanism of action of these agents have been tested through in vitro assays and clinical studies. However, further objective validation was sought in surgical cases in an effort to merge the clinical impressions with the changes taking place at a molecular level through an examination of gene expression in real-time.

Here, we evaluated the inflammatory gene expression profiles of skin biopsy samples from patients before and after liposuction from areas of the skin that were treated with different topical preparations. Biopsies were taken from the pretreated skin and from the treated and untreated areas at 2 and 4 weeks after the procedure. All gene expression comparisons were made in relation to the pretreatment biopsy. Expression changes predominantly included upregulated genes among the groups.
Table 3. Unique Gene Ontology Results from the 2-Week Untreated vs Treated Group Comparison

| 2-Week-untreated group                     | 2-Week-treated group                     |
|--------------------------------------------|------------------------------------------|
| Biological process                         | Biological process                       |
| GO:0071345       Cellular response to cytokine stimulus | GO:0032940   Secretion by cell           |
| GO:0001818       Negative regulation of cytokine production | GO:0002443   Leukocyte mediated immunity |
| GO:0032675       Regulation of interleukin-6 production | GO:0051716   Cellular response to stimulus |
| GO:0045079       Negative regulation of chemokine biosynthetic process | GO:0045087   Innate immune response       |
| GO:0002526       Acute inflammatory response | GO:0032103   Positive regulation of response to external stimulus |
| GO:0032642       Regulation of chemokine production | GO:0002699   Positive regulation of immune effector process |
| GO:0045778       Positive regulation of ossification | GO:0002824   Positive regulation of adaptive immune response |
| GO:0001976       Neurological system process involved in regulation of systemic arterial blood pressure | GO:0070887   Cellular response to chemical stimulus |
| GO:0002830       Positive regulation of type 2 immune response | GO:0002705   Positive regulation of leukocyte mediated immunity |
| GO:0033135       Regulation of peptidyl-serine phosphorylation | GO:0006958   Complement activation, classical pathway |
| Molecular function                           | Molecular function                        |
| GO:0004896       Cytokine receptor activity | GO:0005102   Signaling receptor binding |
| GO:0005164       G protein-coupled receptor binding | GO:0005164   Tumor necrosis factor receptor binding |
| GO:0048020       CCR chemokine receptor binding | GO:0005515   Protein binding |
| GO:0038023       Signaling receptor activity | GO:0001540   Amyloid-beta binding |
| GO:0005488       Binding | GO:0005488   Protein heterodimerization activity |
| GO:0046982       Protein dimerization activity | GO:0046983   Protein dimerization activity |
| Cellular component                           | Cellular component                        |
| None                                        | GO:0005615   Extracellular space         |
| GO:0005576       Extracellular region        | GO:0005576   Secretory granule           |
| GO:0030141       Secretory granule          | GO:0031093   Platelet alpha granule lumen |
| GO:0016020       Membrane                    | GO:0071944   Cell periphery              |
| GO:0034774       Secretory granule lumen    | GO:0005783   Endoplasmic reticulum        |
| GO:0005886       Plasma membrane            | GO:0005886   Plasma membrane              |
IL-6 is a pleiotropic regulator of inflammation and immunity. Its expression initially functions as a warning signal to the entire body in the event of tissue damage. In comparison to the pretreatment biopsy, both the 2-week-treated and untreated biopsies showed an upregulation of IL-6, which was at the center of their respective protein–protein interaction networks. However, the expression of IL-6 in the treated biopsies at 2 weeks was 1.7-fold higher than that in the untreated group, suggesting that the treatment may produce a different response with respect to IL-6.

The untreated group at 2 weeks revealed that IL-6 interacted with CCL17, IL-33, and IL3RA. These interactions are indicative of the regulation of T regulatory cells, the polarization of a Th2 response to activate macrophages, and the modulation of leukocyte production, proliferation, and survival, which work to enhance acute inflammation. Furthermore, the network analysis showed an interaction between IL-6 and HGF. HGF balances the inflammatory action caused by the acute phase response by suppressing it. Together, these gene interactions demonstrated that the 2-week-untreated group displayed a gene pattern that followed the typical inflammatory cascade after surgery, with an enhanced pro-inflammatory signal and the marking of the beginning stages of anti-inflammatory modulation, providing an excellent comparison for the treatment group.

In the 2-week-treated samples, IL-6 showed interactions with IL21R, CCL20, CCR2, and C3. These connections trigger Th17 cell differentiation, T and B cell differentiation, and the regulation of immune tolerance. In addition, the activation of both innate and adaptive immune responses are part of these pathways as well as local protection against invading agents. Thus, compared with the untreated group, these gene expression patterns revealed that the immune response at 2 weeks in the treated group shifted toward an immunomodulatory pattern, indicating a possible anti-inflammatory and M2 macrophage activation profile. Thus, the treatment appeared to have initiated the start of debris clean up at this time point.

This was further substantiated by the interaction of IL-6 with NLRP3 (Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing) and cytochrome b beta chain in the 2-week-treated group. The NLRP3 inflammasome is activated in primary macrophages and acts as a broad sensor of cell homeostasis rupture. Upon activation, NLRP3 assembles a multiprotein platform that leads to caspase-1 activation, which controls, by direct cleavage, the maturation of cytosolic pro-cytokines. NLRP3-induced caspase-1 activation also activates nicotinamide adenine dinucleotide phosphate oxidase, playing a role in host defense. The increase in NLRP3 expression in the 2-week-treated samples may be indicative of a quickened inflammatory and wound healing response. Wounds lacking NLRP3 exhibit reduced growth factor and macrophage infiltration. There is also a link between the activation of autophagy and NLRP3. Inflammasome activation triggers autophagy induction, and autophagy eliminates activated inflammasomes, which are important for immune homeostasis.

The surgical procedure the patients in this study underwent results in the presence of debris that must be cleared.

Table 3. Continued

| 2-Week-untreated group | 2-Week-treated group |
|------------------------|----------------------|
| Biological process     | Biological process   |
| GO:0016021 Integral component of membrane |
| GO:0044433 Cytoplasmic vesicle part |
| GO:0005887 Integral component of plasma membrane |
| GO:0012505 Endomembrane system |
| GO:0070821 Tertiary granule membrane |
| GO:0044432 Endoplasmic reticulum part |
| GO:0044425 Membrane part |
| GO:0035579 Specific granule membrane |
| GO:0005788 Endoplasmic reticulum lumen |
| GO:0030667 Secretory granule membrane |
Part of this debris includes lipid droplets. These large particles, which are released by adipocytes, are digested through autophagy (lipophagy), which alters these large lipid droplets into smaller products that can be taken up by macrophages, thus providing clearance.\textsuperscript{25,26} The treatment used in this study is designed to stimulate macrophage clearance, offering more efficient removal of lipid droplets, allowing for remodeling of the ECM.\textsuperscript{2,7,27} The gene expression patterns for the 2-week treatment clearly showed that treatment enhanced gene expression patterns related the initial immune response with an M1 macrophage stimulation and the transformation to an anti-inflammatory response, and the 4-week treatment, as described below, finalizes the story by shifting the gene expression pattern to reveal the activation of ECM remodeling.

There were only 2 upregulated genes in the 4-week-untreated group. Overall, the 4-week-untreated group appeared to have returned to the baseline gene expression. In contrast, the 4-week-treated group showed a unique pattern of upregulation. The upregulation of NLRP3 observed in the 2-week-treated group was no longer seen in the 4-week-treated group. Long-term overexpression of NLRP3 is associated with chronic, non-healing wounds, possibly due to the prevention of M2 macrophage polarization.\textsuperscript{3,28} A “switch” from M1 type macrophages to M2 is generally considered a sign of inflammation resolution and is associated with properly

**Table 4. Unique Reactome Pathway Results from the 2-Week Untreated vs Treated Group Comparison**

| 2-Week-untreated group | Reactome pathway | 2-Week-treated group | Reactome pathway |
|------------------------|------------------|----------------------|------------------|
| HSA-449147             | Signaling by interleukins | HSA-168249          | Innate immune system |
| HSA-5684996            | MAPK1/MAPK3 signaling | HSA-198933          | Immunoregulatory interactions between a lymphoid and a nonlymphoid cell |
| HSA-6811558            | PI3P, PP2A, and IER3 regulate PI3K/AKT signaling | HSA-977606          | Regulation of Complement cascade |
| HSA-446652             | Interleukin-1 family signaling | HSA-375276          | Peptide ligand-binding receptors |
| HSA-6798695            | Neutrophil degranulation | HSA-8957275         | Post-translational protein phosphorylation |
| HSA-381426             | Regulation of insulin-like growth factor (IGF) transport and uptake by insulin-like growth factor-binding proteins (IGFBPs) | HSA-6783783         | Interleukin-10 signaling |
| HSA-380108             | Chemokine receptors bind chemokines | HSA-1280218         | Adaptive immune system |
| HSA-449147             | Interleukin signaling | HSA-109582          | Hemostasis |
| HSA-194138             | Signaling by VEGF | HSA-194138          | Signaling by VEGF |

**Figure 3.** Venn diagram comparing the upregulated genes in the 2-week-treated vs the 4-week-treated groups. The numbers are based on the genes that were significantly upregulated (≥1.5-fold) in comparison to the pretreatment biopsies. The data are based on Table 1. 2W T, 2-week-treated group; 4W T, 4-week-treated group.
The data support that the upregulation of NLRP3 at week 2 was resolved by week 4. However, at 4 weeks, the treated group showed upregulation of NLRP12. NLRP12 is expressed primarily by myeloid-monocytic lineage cells, including monocytes, granulocytes, and eosinophils, functioning as a negative regulator of the inflammatory response. Taken together, the 4-week-treated group revealed a gene pattern that showed that pro-inflammation was diminishing, and anti-inflammation was in full force.

Furthermore, CD40LG was at the center of the 4-week-treated protein–protein interaction network, and it was linked to CCL22. This interaction is important for the recruitment of TH2 cells into inflammatory regions in order to regulate the TH-2-mediated immune response. M2 macrophages are the primary source of CCL22 production. This chemokine response was unique to the 4-week-treated group, further supporting activation of the anti-inflammatory response.

Finally, a decrease in fibrosis and/or skin hardening, which is known as induration was observed with the use of this product as measured by 2 clinical assessments. This was further supported by the gene expression data. For the 4-week-treated samples, matrix metalloproteinases 25 (MMP25) and transglutaminase (TGM2) were upregulated; these genes are associated with tissue remodeling. MMPs, including MMP25, are responsible for degrading ECM proteins, and this is an important part of wound healing. TGM2 is an important epidermal barrier protein that contributes to adhesion and epidermal–dermal integrity. Together, these data support that at 4 weeks, with the treatment, the gene expression pattern showed that ECM remodeling was activated, which was not observed in the 2-week-treated samples or in the 4-week-untreated group.

This study has limitations that should be addressed. First, while this is technically a randomized double-blinded study, the small sample size significantly undermines the power of the study. However, the data from the patients were consistent, statistically significant, and we did not observe any outliers, which suggests that the findings will hold up in a larger study in the future. In addition, we used Cetaphil as the bland moisturizer for the control. This was done to ensure that the effects we were observing were actually not just due to improved hydration of the area. To more accurately assess the effects of our treatments, we could have used the base material for the Alastin products. Finally, in order to verify our gene expression findings, in the future, we plan to explore protein expression as well, which will further provide evidence of the functional outcomes observed as a result of the treatment.
Table 5. Unique Gene Ontology Results from the 2-Week-Treated vs the 4-Week-Treated Group Comparison

| #term id   | Biological process                                      | #term id   | Biological process                                      |
|------------|---------------------------------------------------------|------------|---------------------------------------------------------|
| GO:0006950 | Response to stress                                     | GO:0071345 | Cellular response to cytokine stimulus                  |
| GO:0002673 | Regulation of acute inflammatory response               | GO:0048247 | Lymphocyte chemotaxis                                   |
| GO:0032940 | Secretion by cell                                       | GO:0048583 | Regulation of response to stimulus                       |
| GO:0032101 | Regulation of response to external stimulus             | GO:0050900 | Leukocyte migration                                     |
| GO:0050727 | Regulation of inflammatory response                     | GO:0050670 | Regulation of lymphocyte proliferation                  |
| GO:0050778 | Positive regulation of immune response                  | GO:1902533 | Positive regulation of intracellular signal transduction |
| GO:002443  | Leukocyte mediated immunity                             | GO:0031640 | Killing of cells of other organism                      |
| GO:0051716 | Cellular response to stimulus                           | GO:0043408 | Regulation of mapk cascade                               |
| GO:002252  | Immune effector process                                 | GO:0061844 | Antimicrobial humoral immune response mediated by antimicrobial peptide |
| GO:0045765 | Regulation of angiogenesis                              | GO:0070372 | Regulation of erk1 and erk2 cascade                      |
|            | Molecular function                                       |            |                                                         |
| GO:005102  | Signaling receptor binding                              | GO:0008009 | Chemokine activity                                      |
| GO:0048018 | Receptor ligand activity                                | GO:004896  | Cytokine receptor activity                              |
| GO:0001664 | G protein-coupled receptor binding                      |            |                                                         |
| GO:0005164 | Tumor necrosis factor receptor binding                  |            |                                                         |
| GO:0038023 | Signaling receptor activity                             |            |                                                         |
| GO:0001540 | Amyloid-beta binding                                    |            |                                                         |
| GO:0005488 | Binding                                                 |            |                                                         |
| GO:0048888 | Transmembrane signaling receptor activity               |            |                                                         |
| GO:0046982 | Protein heterodimerization activity                     |            |                                                         |
| GO:0046983 | Protein dimerization activity                           |            |                                                         |
|            | Cellular component                                       |            |                                                         |
| GO:0005576 | Extracellular region                                    | GO:0044421 | Extracellular region part                               |
| GO:0030141 | Secretory granule                                       | GO:0009897 | External side of plasma membrane                        |
| GO:0031093 | Platelet alpha granule lumen                            | GO:0009986 | Cell surface                                            |
| GO:0016020 | Membrane                                                |            |                                                         |
| GO:0071944 | Cell periphery                                          |            |                                                         |
| GO:0034774 | Secretory granule lumen                                 |            |                                                         |
| GO:0005783 | Endoplasmic reticulum                                   |            |                                                         |
| GO:0005886 | Plasma membrane                                         |            |                                                         |
| GO:0016021 | Integral component of membrane                          |            |                                                         |
Table 5. Continued

| Biological process                      | Biological process                      |
|----------------------------------------|----------------------------------------|
| Cytoplasmic vesicle part               | Integral component of plasma membrane  |
| Endomembrane system                    |                                        |
| Tertiary granule membrane              |                                        |
| Endoplasmic reticulum part             |                                        |
| Membrane part                          |                                        |
| Specific granule membrane              |                                        |
| Endoplasmic reticulum lumen            |                                        |
| Secretory granule membrane             |                                        |

Table 6. Unique Reactome Pathway Results from the 2-Week-Treated vs the 4-Week-Treated Group Comparison

| Reactome pathways                      | Reactome pathways                      |
|----------------------------------------|----------------------------------------|
| Innate immune system                   | Interleukin-35 signaling                |
| TNFs bind their physiological receptors| Interleukin-27 signaling                |
| Immunoregulatory interactions between a lymphoid and a nonlymphoid cell | G alpha (i) signaling events |
| Regulation of complement cascade       | Degradation of the extracellular matrix |
| Peptide ligand-binding receptors       |                                        |
| Neutrophil degranulation               |                                        |
| Initial triggering of complement       |                                        |
| Post-translational protein phosphorylation |                                    |
| Platelet degranulation                 |                                        |
| Regulation of insulin-like growth factor (IGF) transport and uptake by insulin-like growth factor-binding proteins (IGFBPs) | |
| Adaptive immune system                 |                                        |
| Hemostasis                             |                                        |
| Signaling by VEGF                      |                                        |
| Interleukin-4 and interleukin-13 signaling |                                |
CONCLUSIONS

For patients undergoing body procedures, a topical treatment of Alastin RSN and TFB Treatment with TriHex Technology induces an accelerated healing response that involves the clearance of “waste” products and the induction of anti-inflammatory genes. Furthermore, this topical treatment stimulates ECM remodeling, which ultimately improves the long-term results of the healing process, leading to less induration. A full clinical evaluation of this treatment and histological biopsy results will be presented in the near future in order to translate the gene expression data to patient outcomes.

Disclosures

A.D.W. is the Chief Medical Officer, Alastin Skincare Inc. M.B. is the Director of Clinical Studies, Alastin Skincare Inc. M.E.Z. is a Consultant for Alastin Skincare Inc. L.C. is a Consultant and shareholder for Alastin Skincare Inc. B.C. declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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