Personal Cell Sciences Corp.

Multi Center Clinical Study Summary

Autologous adipose-derived adult stem cell conditioned media and its topical effects on facial skin aging

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Abstract

As individuals age, the skin undergoes changes, such as irregular pigmentation, thinning and loss of elasticity that are due to both genetic and environmental factors. Various medical treatments and topical cosmeceuticals have been used to treat some symptoms of photo-aging; however, the results have been less than satisfactory. Research has demonstrated that Mesenchymal stem cells within the stromal-vascular fraction of subcutaneous adipose tissue (fat), Adipose-Derived Stem Cells (ADSCs), display multi-lineage developmental plasticity and secrete growth factors that control and manage the damaged neighboring cells. Conditioned Medium (CM) created from ADSCs (ADSC-CM) stimulated both collagen and elastin synthesis, which improved the appearance of wrinkles and accelerated wound healing.

A 8 week multi center controlled clinical study was undertaken to test the effects of a topical face formulation incorporating Conditioned Medium (CM) made from patients' own adipose derived stem cells and growth factors. Objective measurements of skin appearance (both clinically and microscopically) and structural skin analysis were performed. Additionally, patient perception and satisfaction surveys regarding use of the formulation were conducted. The objective and subjective measurements showed an overwhelmingly positive effect on the skin of this unique, cutting edge topical anti-aging formulation.

Introduction

A large body of scientific evidence has demonstrated that adipose-derived growth factors (cytokines) may stimulate collagen and elastin synthesis, repairing damaged skin as well as protecting the skin from photo-damage and injury. Attempts have been made to create topical skin care products incorporating animal, plant or synthetic generic human growth factors in order to improve the health of the skin; however the results have been less than satisfactory. Further, by combining current scientific methodologies and the ability to commercially extract fat from individuals, mesenchymal stem cells can be isolated from adipose tissue, grown and stimulated to produce growth factors. No commercially available anti aging product currently exists that incorporates an individual's own adult stem cell derived growth factors in order to stimulate the skin's natural healing abilities. This study is the first to attempt to demonstrate the effectiveness of such a treatment.

Personal Cell Sciences conducted an 8 week multi center clinical study with 19 patients using the topical formulation containing Autokine-CM on the crows feet area of the face. One center of the study had 9 patients, (8 female, 1 male, age range 42-68, average age 49.1 years) with Dr. Fredric Stern, Bellevue, Washington www.sternctr.com and the second center had 10 patients with Dr. Richard Goldfarb, Langhorne, Pennsylvania www.centerforsmartlipo.com. Additionally, to further validate the effectiveness of the product, at baseline a biopsy punch was performed to collect a skin sample. At the conclusion of the 8 week study a second biopsy punch was performed in the area where participants applied the formulation twice a day for 8 weeks. All patients were photographed on day 1, at 2 weeks, 4 weeks and 8 weeks. The biopsy punch analysis was performed by Dr. Robert Kellar, PhD at Development Engineering Sciences, LLC, Flagstaff, AZ. www.des-company.com .

Materials and Methods

- Nine (9) volunteer patients at The Stern Center for Aesthetic Surgery in Bellevue, Washington underwent a "mini-liposuction" procedure under local anesthetic, performed by Fredric A. Stern, MD, FACS. A 60 cc (4 tablespoons) sample of fat was extracted under sterile conditions from each patient.

- Ten (10) volunteer patients at the Center for Smart Lipo in Langhorne, PA. underwent a "mini-liposuction" procedure under local anesthetic, performed by Richard Goldfarb, MD, FACS. A 60 cc (4 tablespoons) sample of fat was extracted under sterile conditions from each patient.

- The fat sample was placed into a collection bag containing media provided by American CryoStem Corporation (www.americancryostem.com). This sample was sent overnight to American CryoStem's clinical laboratory, where it was processed and the adipose-derived stem cells (ADSCs) isolated from the fat. In conjunction with Personal Cell Sciences, the stem cells were grown in a proprietary medium for a period of several days to stimulate secretion of various cytokines, growth factors and matrix proteins creating (Autokine-CM™). The Autokine-CM™ was then blended into a proprietary topical eye formulation branded U Autologous, unique for each of the 19 patients.

- Approximately 4 weeks after the fat extractions, the individualized, U Autologous eye formulation was formulated and distributed to the study patients. In Dr. Stern's "split-face" study, 9 patients were instructed to continue their normal skin care regimen on the right side of their face and use the U-Autologous eye formulation on the left side of the face and behind the left ear, twice a day for 8 weeks.

- Photographs using a Nikon D60 camera system were taken on 9 patients under controlled lighting with uniform background and Computerized Skin Care analysis was performed (using the Canfield Visia™ Skin Care Analysis System) at baseline, 2 weeks, 4 weeks and 8 weeks.

- 3.0 mm punch biopsies behind the right ear were performed on 9 patients at the onset of the study. These were placed in media for histological and biochemical (RNA) analyses at the conclusion of the study.

- 3.0 mm punch biopsies behind the left (treated) ear were performed on the same 9 patients after 8 weeks of using the U-Autologous product on that side.

- Throughout the 8 week treatment period, the study patients completed questionnaires regarding the perception of the health of their skin and their satisfaction, or lack thereof, with the product.

Results

Photographic and Visiometric Analysis

As determined by the objective visiometric analysis utilizing Canfield Visia™ Skin Care Analysis system the patients in the first center of the study demonstrated 78% of the patients benefited from the treatment. These patients demonstrated an **average measured reduction in the volume of wrinkles of 25.6%** on the treated side after 8 weeks of use of the U-Autologous formulation. One patient demonstrating as much as an **87% improvement.** These dramatic results are clearly evident in the accompanying photographs.

In addition to this dramatic 87% wrinkle reduction, an overall improvement in signs of photo-aging of the treated skin were noted.

Histolopathology and Immunohistochemistry Findings

"See attached final report"

Patient Perception and Satisfaction Surveys

Data was compiled from patient perception and satisfaction surveys completed during the clinical study. The data indicated:

- 87% of patients experienced a more youthful appearance after just 4 weeks of use
- 95% of patients experienced positive changes in their skin's texture after 4 weeks
- 81% of patients experienced dramatic reduction of fine lines after 4 weeks

Discussion/Summary

A significant body of research has demonstrated that the application of growth factors (cytokines) topically to the skin may result in accelerated wound healing and repair of damaged skin. This study was intended to determine whether the application of a formulation containing growth factors (Autokine-CM™) secreted by an individual's own Adipose Derived Stem Cells (ADSC's) have a similar or enhanced effect on improving the structural health and appearance of the skin.

Three parameters were measured to determine efficacy of the U-Autologous formulation: biometric visiometry using the Canfield Visia™ Skin Analysis system, thereby eliminating subjective interpretation of the investigator; skin biopsies before and after treatment with histological and biochemical assays performed; and patient perception questionnaires. The first two parameters were objective measures, the third parameter subjective, but nonetheless important. **All three parameters demonstrated significant, dramatic improvement in the appearance of the skin, with wrinkle reduction and improved skin tone, as well as documented improvement in the extracellular matrix of the subjects' skin**.

This groundbreaking study demonstrates that Autokine-CM™ when applied to the skin in the U-Autologous formulation can reduce the appearance of wrinkles and improve the skin's health after only 8 weeks of use.

PCS001
Final Report

Development Engineering Sciences, LLC

708 N. Fox Hill Rd. Flagstaff, AZ 86004

Title: PCS001 “Clinical Evaluation of PCS Formulation as a Topical Treatment.

Final Report on microscopic observations of human skin biopsy samples and real-time RT-PCR analysis from a clinical evaluation sponsored by Personal Cell Sciences.

INTRODUCTION

Histopathology and real time RT-PCR analysis at the 8 week timepoint (baseline vs. 8 weeks) was the focus of the following analysis and evaluations.

The goal of the current study was to determine if active treatment of healthy, intact skin resulted in biologically relevant differences.

HISTOPATHOLOGY & IMMUNOHISTOCHEMISTRY FINDINGS

Methods:

Histopathology evaluations were assessed using hematoxylin and eosin stained serial sections from paraffin blocks that were digitally scanned using the Aperio ScanScope CS system. Similarly, immunohistochemistry evaluations were performed using serial sections from paraffin blocks, processed using standard immunohistochemistry techniques. Sections were reacted with three different primary antibodies, collagen I, filaggrin, and elastin. Slides were also digitally scanned using the Aperio ScanScope CS system. Digital, whole-slide scans were used for all evaluations.

Results: Histopathology Findings

Histopathology comparisons between baseline and the 8 week timepoint did not reveal any noticeable differences with respect to acanthosis, spongiosis, chronic inflammation, hyperkeratosis, epidermal mononuclear infiltration, focal acantholysis, or dermal edema. Subtle differences exist in epidermal thickness, vascular prominence, and occasional perivascular mononuclear cells. However, all differences were within normal range for histologic (not histopathologic) characteristics of normal human skin. No trend change existed for prominence of any of these characteristics between active and control samples. Representative histopathology is shown in figure 1.

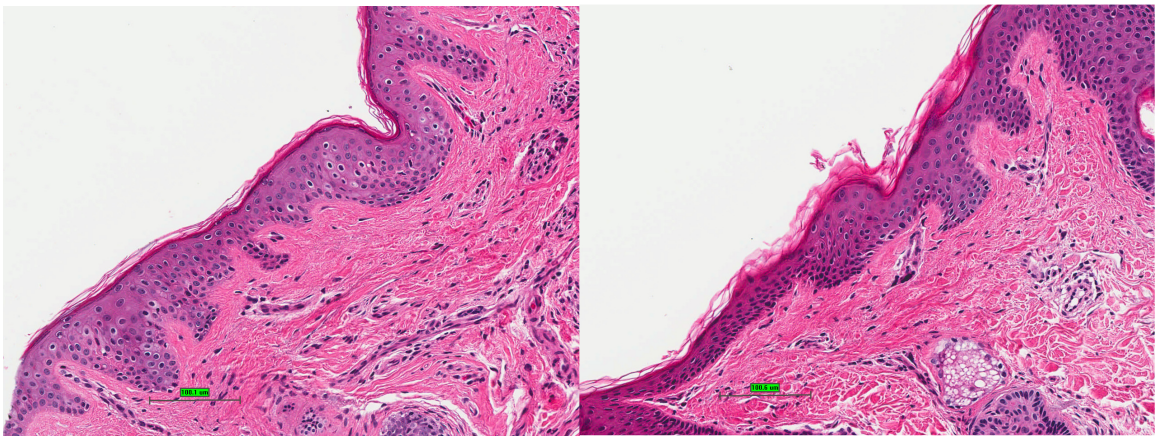

Figure 1. Representative histopathology (hematoxylin & esosin stain). Left, patient #01, baseline. Right, patient # 01, 8 week timepoint. Scale bars = 100um.

Results: Immunohistochemistry Findings

Immunohistochemistry (IHC) analysis revealed no significant change in collagen I levels from baseline to the 8 week timepoint. IHC revealed an *increase in filaggrin and elastin levels from baseline to the 8 week timepoint*.

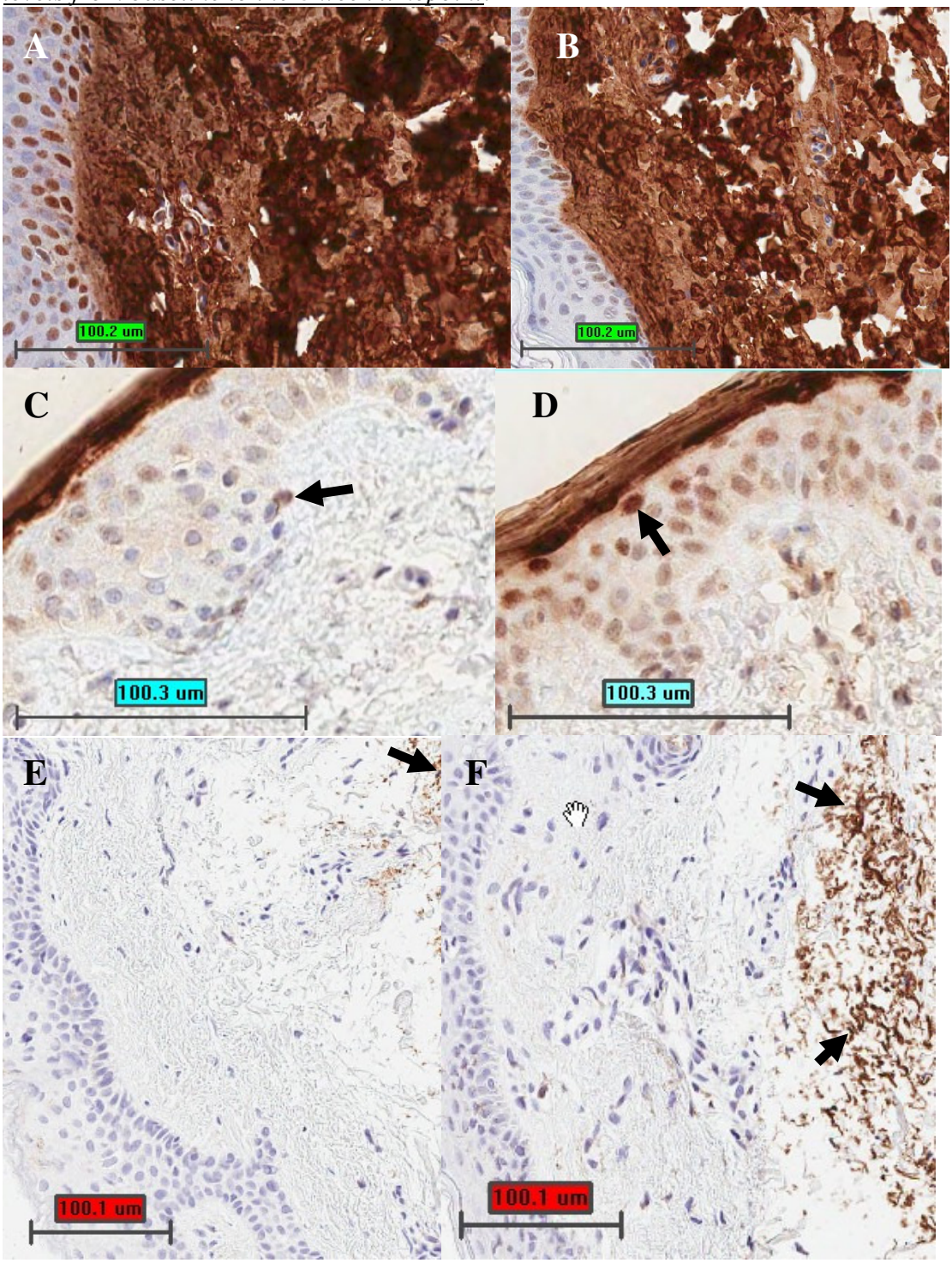

Figure 2. Representative IHC images. A) Baseline collagen, B) 8 wk collagen, C) Baseline filaggrin, D) 8 week filaggrin, E) Baseline elastin, F) 8 wk elastin. Positive stain is in DAB brown color. Arrow heads highlight positive stain.

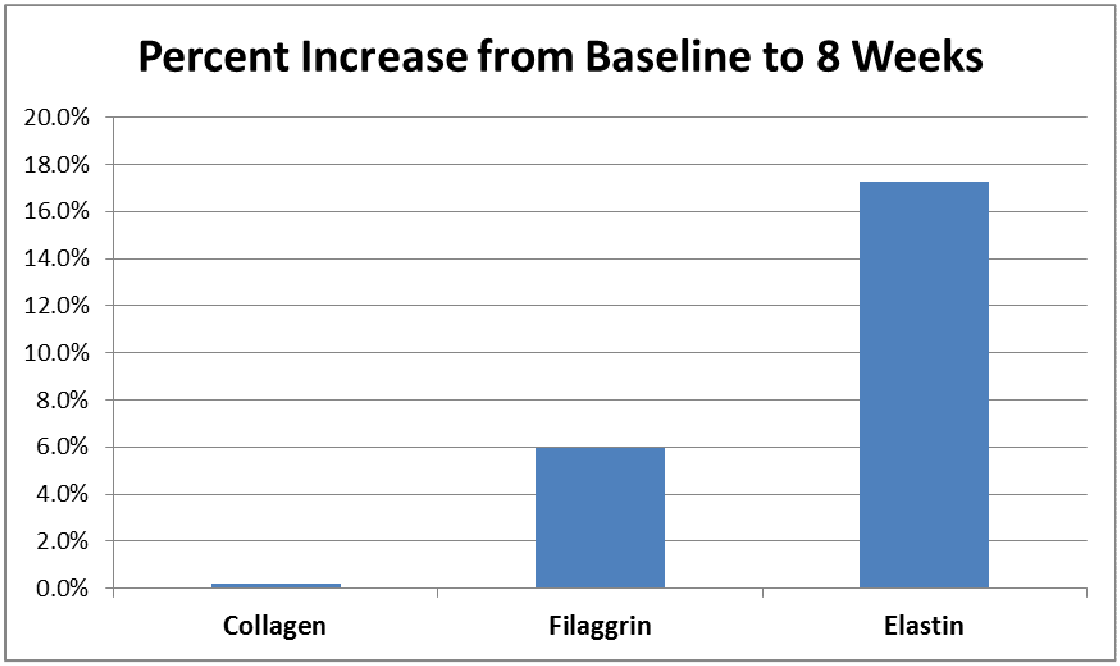

Figure 3. Percent Increase in key structural proteins from Baseline to 8 Weeks.

Histopathology & Immunohistochemistry Summary:
No safety issues were seen (histopathology H&E data) between active vs. control sites. Additionally, key structural proteins within the integument (skin): filaggrin and elastin were found to positively increase in expression over an 8 week treatment period.

REAL TIME RT-PCR FINDINGS

Methods:
For RNA isolation from tissue the RNEasy kit (Qiagen) was used as directed in the protocol. Tissue was processed in Lysis solution using an Omni mechanical tissue disruptor. The Superscript VILO kit (Invitrogen) was used for generating cDNA. Protocol reaction conditions were followed for 20uL reactions. The RNA isolation elutions were not diluted prior to being used for RT (cDNA) reactions. cDNA reaction products were all diluted 2:1 prior to being run in 20 uL real time RT-PCR reactions using Gene expression assays. All real time RT-PCR reactions were run on an Applied Biosystems 7900 Real Time PCR System. cDNA was reacted with the following probes:

- Collagenase
- Elastase
- ß-Actin (Housekeeping Gene)
- GAPDH (Housekeeping Gene)

Results: Real Time RT-PCR Findings

GAPDH was selected as the housekeeping gene to normalize for sample to sample variations. Real time RT-PCR analysis demonstrates the following findings in mRNA signal for the following probes in baseline vs. 8 week samples:

- collagenase (collagen reducer; breaks down collagen) is *down-regulated*
- elastase (elastin reducer; breaks down elastin) is *down-regulated*

Both collagenase and elastase are enzymes that work to break down key structural proteins normally found in the skin; collagen and elastin, respectively.

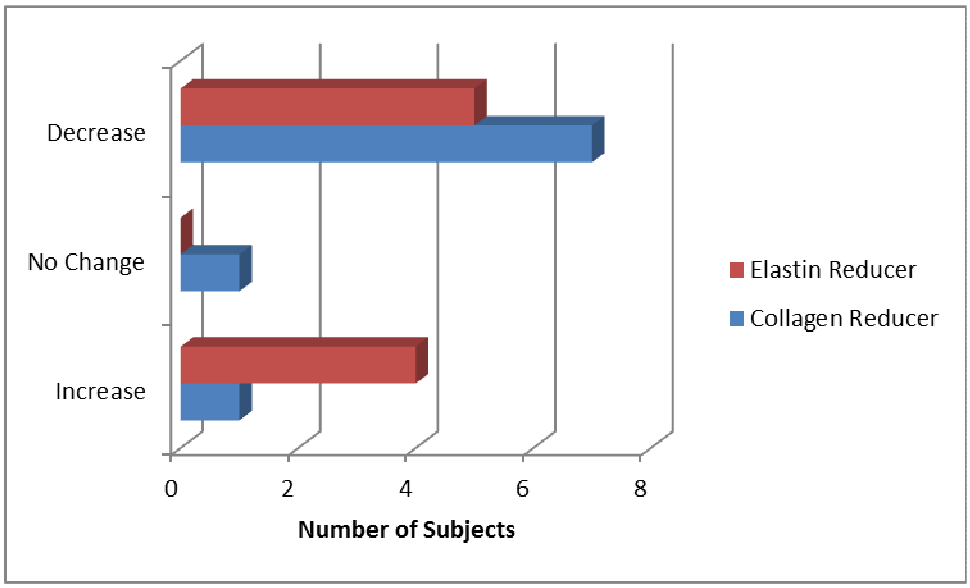

Figure 4. Real Time RT-PCR Summary of collagenase (collagen reducer) and elastase (elastin reducer) results.

Real Time RT-PCR Summary:

These data suggest that following 8 weeks of treatment with the topical cream, a vast majority of the patients had positive responses in the amount of enzyme activity in their skin. These responses are not just at the skin surface as the message for collagenase and elastase were measured from the biopsy punches (this includes deep tissue). The patients in this study benefited from topical treatment in that they decreased the expression of collagenase and elastase, thus preserving the resident amounts of key structural proteins in their skin.

- In 7 of 9 patients, the amount of collagenase (MMP-1) was down regulated. This means less harmful breakdown of collagen (a key structural protein).
- In 5 of 9 patients, the amount of elastase (MMP-12) was down regulated. This means less harmful breakdown of elastin (a key protein that contributes to skin elasticity or stretchiness).

STUDY DISCUSSION

Filaggrin
Filaggrin is a structural protein in the skin which facilitates the organization of keratinocytes and helps promote the formation of a densely packed, impenetrable barrier known as the stratum corneum. During epidermal terminal differentiation, the ~400 kDa profilaggrin polyprotein is dephosphorylated and rapidly cleaved by serine proteases to form monomeric filaggrin (37 kDa), which binds to and condenses the keratin cytoskeleton, contributing to the cell compaction process that is required for the squamous cell phenotype of the stratum corneum. Loss of profilaggrin or filaggrin leads to a poorly formed stratum corneum with flaky skin (ichthyosis), which is also prone to water loss (xerosis).

What does this mean in the current study?
Filaggrin levels appear to be higher in patients after 8 weeks of topical treatment. This suggests that active treatment increases the production of filaggrin expression in the skin, potentially contributing to a more structurally-sound barrier function of the stratum corneum.

Collagen I
Collagen I is a structural protein found in the dermis. It provides the framework to the skin and represents the major extracellular matrix protein within the integument.

What does this mean in the current study?
Collagen I protein levels are relatively unchanged in the current study (IHC results). However, RT-PCR results indicate that 8 weeks of topical treatment decreases the expression level of the enzyme that naturally breaks down collagen (collagenase). This may indirectly allow for greater collagen presence in the dermis which may provide skin with greater structural integrity. The consistency in the data lies in the fact that three major structural skin proteins (filaggrin, elastin, and indirectly collagen via collagenase) all increase in their expression after 8 weeks of topical treatment. Because these proteins trend together, we can conclude with a higher level of confidence that active treatment is stimulating structural changes within the underlying skin.

Elastin
Elastin is a structural protein found in the dermis as well as other critical tissues such as blood vessels, heart, bladder and ligaments where it provides physiologically relevant elasticity.

What does this mean in the current study?
Elastin levels appear to be higher after 8 weeks of treatment (IHC results). Additionally, elastase, an enzyme which breaks down elastin is down regulated in a majority of patients. Greater elastin presence in the dermis may provide skin with greater structural integrity and elasticity. These findings should be compared to clinical observations

within the current study to understand if they translate to greater skin elasticity values in active treatment sites.

STUDY CONCLUSIONS

In the current study, topical delivery of custom formulation was evaluated in consenting human subjects. Biopsy samples were taken from patients at baseline and following 8 weeks of treatment. Biopsy samples were coronally cut in half, with one half processed for histopathology and the second half processed for real time RT-PCR diagnosis.

Overall study conclusions indicate no significant safety issues in active-treated sites. These conclusions are reached from hematoxylin & eosin (H&E) data, where no safety concerns were seen within the age-appropriate, normal healthy skin.

Additionally, histopathology revealed structurally significant differences in active-treated sties. Specifically, IHC and RT-PCR data from collagen, filaggrin, elastin, collagenase, and elastase demonstrate that all three structural proteins are up-regulated in their presence following 8 weeks of active therapy.

In summary, data from the current study (histopathology and real time RT-PCR) suggest active mechanisms are in play with the use of the topical therapy to intact, healthy skin. No safety issues were seen in the current study and structurally significant and biologically relevant differences were detected as a result of active treatment.

SIGNATURES

July 21, 2012

Robert S. Kellar, Ph.D.