To order reprints or e-prints of JDD articles please contact sales@jddonline.com

doi:10.36849/JDD.6401

Copyright © 2021 ORIGINAL ARTICLE Journal of Drugs in Dermatology

Rationale and Preclinical Evaluation of a Multimodal Topical Body Skincare Product for Toning and Tightening

Kevin Smith MD,[a] Patrick Nido BS,[b] Prithwiraj Maitra PhD,[b] Tsing Cheng PhD,[b] Kuniko Kadoya PhD[b]
[a]Charlotte Plastic Surgery, Charlotte, NC
[b]Allergan Aesthetics, an AbbVie Company, Irvine, CA

ABSTRACT

A novel tightening and toning cream (TTC) was designed to improve body skin quality at multiple levels by engaging several key pathways that contribute to skin function, strength, and integrity. Evaluation of gene expression in both human in vitro 3D skin and ex vivo skin treated with TTC demonstrated changes reflecting improved extracellular matrix and dermal integrity, lymphatic drainage, mitigation of inflammation, cellular clearance and recycling, and adipocyte metabolism. This study provides the rationale and preclinical support for the use of TTC as a standalone agent to improve body skin quality or in combination with body contouring procedures.

J Drugs Dermatol. 2021;20(10):1041-1044. doi:10.36849/JDD.6401

INTRODUCTION

As skin ages, naturally or prematurely, it gradually loses its firmness, wrinkles, and/or sags due to deterioration of the dermal extracellular matrix (ECM) that provides support, elasticity, and strength to the skin. Many topical cosmeceuticals are offered as an accessible and consumer-friendly option purported to enhance skin quality by preventing further damage to the ECM, and/or stimulating regeneration, yet the effectiveness is limited with much room for improvement.[1] In addition, many products are heavily focused towards rejuvenating facial skin whereas differences in body skin physiology require different needs.[2]

Our growing knowledge about the biological processes underlying skin damage and homeostasis within the cutaneous microenvironment has yielded new targets for skin rejuvenation. For instance, the microvasculature that shuttles fluids, nutrients, and immune cells is reduced in density and function in chronologically or extrinsically damaged skin.[3] Persistent inflammation resulting from an imbalance of pro- and anti-inflammatory cytokines is proposed to contribute to the degradation of the ECM. Moreover, a decline in cellular recycling mechanisms allows damaged organelles and macromolecules to accumulate, further propagating inflammation and promoting senescence.[4] Therefore, modalities that address multiple aspects of the skin microenvironment in tandem may produce superior results.

Dermal adipocytes represent another compartment for improving skin quality, however, the excess of fat poses as a distinct concern for many individuals. While minimally invasive body contouring procedures can shrink or breakdown fat, a depletion of subcutaneous adipocytes, which provide skin volume and fullness, can leave undesired loose, excess skin. Patients with this concern may achieve a better overall aesthetic result with the addition of a topical product that addresses skin firmness and tightness. Moreover, support to the lymphatic, immune, and intracellular machinery that regulate skin health may also complement body sculpting by clearing the resulting adipocyte debris and edema associated with these procedures. Dermal adipose tissue can also contribute to skin texture, such as with cellulite, a bothersome dimpling of the skin that manifests from enlarged adipocytes extruding through the fibrous septae that anchor the dermis to the subcutaneous muscle. Vascular dysfunction and interstitial fluid buildup are proposed drivers of cellulite when combined with tortuous dermal architecture and adipose hypertrophy; therefore, resolution of cellulite also demands a solution that integrates multiple aspects of skin biology.

With this rationale, a tightening and toning cream (TTC) was formulated using a proprietary blend of bioactive botanicals that target multiple key processes that control overall skin quality including ECM integrity, lymphatic drainage, mitigation of inflammation, cellular clearance and recycling, and adipocyte metabolism. In the following report, we describe select ingredients and mechanisms of action leveraged by TTC, as well as provide preclinical proof-of-concept for its use to improve skin health and appearance, and to complement body contouring procedures.

This document contains proprietary information, images and marks of Journal of Drugs in Dermatology (JDD).
No reproduction or use of any portion of the contents of these materials may be made without the express written consent of JDD. If you feel you have obtained this copy illegally, please contact JDD immediately at support@jddonline.com

To order reprints or e-prints of JDD articles please contact sales@jddonline.com

MATERIALS AND METHODS

Tissue Models

EpiDermFT™ 3D full thickness in vitro human skin models (MatTek Corp, Ashland, MA) were cultured with EpiDerm^{FT} Assay Media (MatTek Corp). Tissues were irradiated with 200mJ/cm^2 ultraviolet (UV) light with UV-B filter lamp (Honle, Germany) followed by application of 15 µL of TTC or dH_2O (control) and incubated at 37°C and 5% CO_2 for 24 hours.

Ex vivo studies were performed using Hyposkin® human skin explants (Genoskin, Salem, MA). After equilibrating tissues with Hyposkin® Medium (Genoskin), 15µL of TTC or dH_2O was applied and tissues were incubated at 37°C and 5% CO_2. The topical composition was re-applied, and media changed every 24 hours for an additional two days.

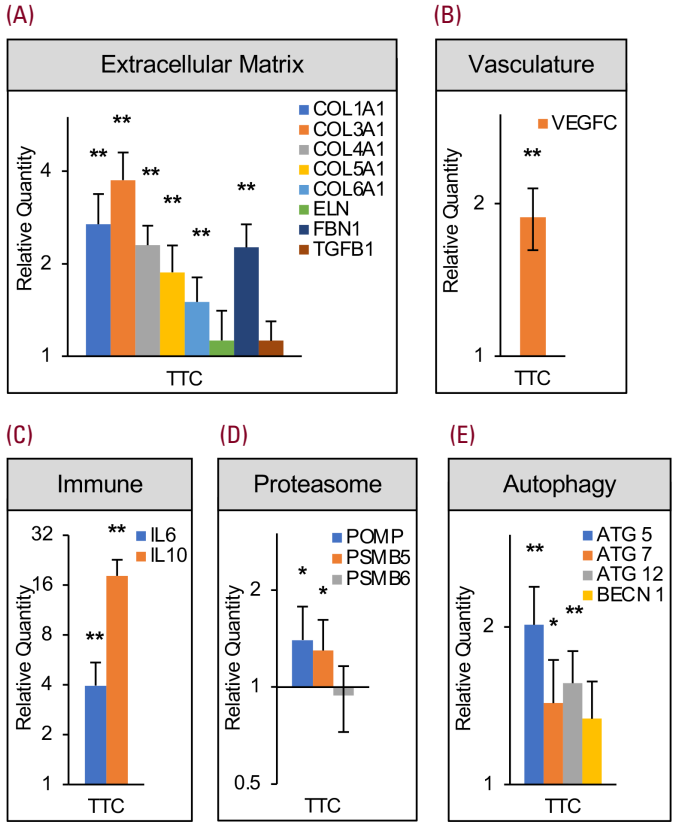
Quantitative Real-Time PCR

After incubation, tissues were collected into Invitrogen™ RNAlater® solution (ThermoFisher Scientific, Waltham, MA). mRNA was extracted using the Maxwell® RSC simplyRNA Tissue Kit (Promega, Madison, WI) followed by cDNA synthesis using the Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific). Gene expression was evaluated using Applied Biosystems™ Fast Advanced Master Mix and pre-designed TaqMan Gene Expression Assays on the QuantStudio7 Flex instrument (ThermoFisher Scientific). Relative expression was calculated compared to UV irradiated control treated samples (in vitro), or to control treated skin (ex vivo), and was normalized to GAPDH.

RESULTS

Key ingredients and mechanisms for TTC are presented in Table 1. The utility of TTC was first tested using in vitro UV-damaged 3D-reconstructed human skin, which contains both dermis and epidermis. Application of TTC resulted in significant upregulation of all ECM-associated genes tested including collagens, elastic fiber proteins and TGFβ1 (Figure 1A), Treatment with TTC selectively upregulated pro-lymphangiogenic *VEGFC* (Figure 1B), and significant induction of anti-inflammatory IL-10 was observed compared to pro-inflammatory IL-6 (Figure 1C). In addition, expression of key genes involved in major cellular

FIGURE 1. Effects of TTC on gene expression in human in vitro 3-D skin. (A-E) Gene expression for the indicated pathways was measured 24 hours after UV-B irradiation and application of TTC. Relative quantities were calculated compared to UV irradiated, non-treated control tissues.



n=5, data is presented as mean +/- SD. *P<0.05, **P<0.01

TABLE 1.

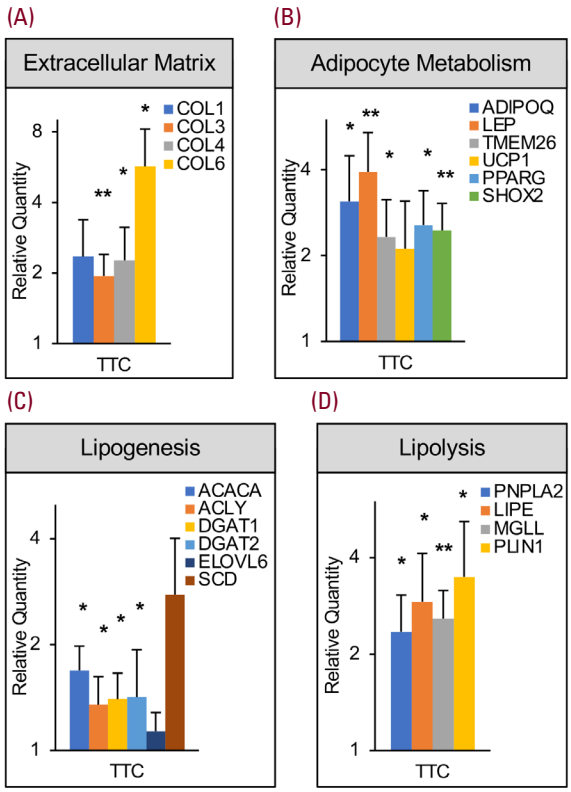
Key ingredients in TTC and the mechanisms engaged by them.

Pathway	Ingredient	Mechanism
Extracellular Matrix	Chlorella vulgaris extract, Lentinus edodes extract	Stimulates collagen and elastin biosynthesis; inhibits collagenases and elastases; improves the structure, organization, and quality of collagen
Lymphatic Drainage	Coleus forskohlii root Extract	Dilates blood and lymph vessels; accelerates the removal of released fatty acids and cellular waste; promotes the discharge of excess tissue fluid
Autophagy	Melissa officinalis leaf extract	Promotes autophagy, which allows for clearance & recycling of damaged cellular components & organelles; helps to maintain regenerative stem cells and promotes resistance to stress
Proteasome	Hydrolyzed rice protein	Preserves ubiquitin-proteasome activity that degrades and recycles damaged and misfolded proteins; helps to maintain cellular protein homeostasis
Lipolysis	Coffea arabica seed oil, Brassica campestris sterols	Induces lipolysis and free-fatty acid release from adipocytes
Lipogenesis	Oenanthe javanica extract	Repressor of adipogenesis and lipogenesis

This document contains proprietary information, images and marks of Journal of Drugs in Dermatology (JDD).
No reproduction or use of any portion of the contents of these materials may be made without the express written consent of JDD. If you feel you have obtained this copy illegally, please contact JDD immediately at support@jddonline.com

To order reprints or e-prints of JDD articles please contact sales@jddonline.com

FIGURE 2. Effects of TTC on gene expression in human ex vivo skin. (A-D) Gene expression for the indicated pathways was measured after 3 days of treatment with TTC. Relative quantities were calculated compared to non-treated control tissues.



n=3, data is presented as mean +/- SD. *P<0.05, **P<0.01

recycling mechanisms, the proteasome and autophagy, were increased by TTC application (Figure 1D, E).

Next, TTC was also evaluated on ex vivo human abdominal skin (from a 34-year-old female, Fitzpatrick skin type II), which included hypodermis with adipose tissue. Similar to what was observed in the in vitro 3D model, TTC induced the expression of collagens (Figure 2A). Adipose markers of a thermogenic phenotype (UCP1 and TMEM26), adipokines (LEPT and ADIPOQ), and regulators of adipocyte function (SHOX2 and PPARG) were all increased with TTC application (Figure 2B). While lipogenesis targets were upregulated by TTC, this was matched by a larger increase in lipolytic genes (Figure 2C, D).

DISCUSSION

An ideal topical body contouring agent would simultaneously address multiple etiologies that affect skin quality. The primary goal of this report was to provide rationale and pre-clinical evidence for the bioactivity of a multimodal topical product intended to firm and rejuvenate skin, by boosting various ECM and DEJ components as well as supporting systems that promote lymphatic vessel quality, anti-inflammatory responses, cellular clearance and recycling, and adipocyte metabolism. Using a select gene panel in two different skin models, it was demonstrated that TTC can produce biological effects that are consistent with skin health, maintenance, and repair. Upregulation of ECM-associated genes in both models suggests that TTC stimulates dermal ECM component synthesis and improves ECM integrity (Figure 1A, 2A). VEGFC, a driver for lymphangiogenesis, was also upregulated by TTC which may translate into enhanced lymphatic drainage (Figure 1B). Rebalancing pro-inflammatory and anti-inflammatory cytokines, as was observed with TTC, can promote overall skin health (Figure 1C). In addition, key proteasome and autophagy related genes were also induced, indicating stimulation of cellular recycling mechanisms (Figure 1D, E). Finally, when TTC was applied on ex vivo skin containing adipocytes, changes in gene expression were observed that reflect an increase in adipocyte function and a metabolic state favoring lipolysis over lipogenesis (Figure 2B-D). In summary, these results indicate that TTC may benefit overall skin quality and aesthetic.

Comprehensive treatments for skin quality require that all components of the skin be considered since virtually every aspect of skin biology is affected by aging.[3] The dermal microenvironment is highly vascularized, allowing fluids, nutrients, and immune cell traffic to maintain proper homeostasis. The cutaneous microvasculature declines with age, and it is proposed that improving the quality of blood and lymphatic vessels can support skin rejuvenation. Since cellulite etiology involves interstitial fluid buildup, the benefits of improving the lymphatic microvasculature can be two-fold; restoring dermal homeostasis as well as facilitating fluid drainage and metabolite export from the enlarged fatty pockets in cellulite. In sum, TTC targets multiple biological pathways that can influence skin appearance; thus, it can be applied to multiple skin concerns.

Because keratinocytes and fibroblasts make up much of the skin, other cell types such as local dermal adipocytes have been largely overlooked. Considering recent findings that enlarged dermal adipocytes can affect the function of fibroblasts, coupled with the large potential for paracrine signaling through the plethora of secreted "adipokines," modulation of this depot may have dramatic implications for overall skin quality beyond cellulite and cosmetic fat reduction.[5,6]

While this study only looked at gene expression using in vitro and ex vivo skin, the encouraging results provide impetus to evaluate TTC in randomized and controlled clinical studies that may uncover synergies from each pathway unable to be observed or measured in the employed test systems. For example, lymphatic circulation can help clear released free fatty acids from the tissue and prevent re-uptake. Lymphatic vessels are also heavily involved in the regulation of inflammation

This document contains proprietary information, images and marks of Journal of Drugs in Dermatology (JDD).
No reproduction or use of any portion of the contents of these materials may be made without the express written consent of JDD. If you feel you have obtained this copy illegally, please contact JDD immediately at support@jddonline.com

To order reprints or e-prints of JDD articles please contact sales@jddonline.com

by facilitating immune cell trafficking. Recently, a topical body firming moisturizer containing adipose targeting agents was shown to induce lipolysis in vitro and reduce upper arm circumference.[7] Another topical body product improved clinical outcomes when combined with cryolipolysis.[8] Both of these topical body treatment products employ mechanisms of action that partially overlap with TTC (eg, ECM stimulation, lipolysis), but neither product targets the full spectrum of key biological pathways that TTC addresses. This combinatorial approach is key to achieving optimal outcomes in skin quality, whether used as a standalone body treatment or when combined with body contouring procedures.

DISCLOSURES

KL Smith is a member of the Speaker's Bureau and Advisory Board for Allergan Aesthetics, an AbbVie Company. P Nido, P Maitra, T Cheng, and K Kadoya are employees of AbbVie, Inc, and may own stock in the company.

This study was funded by Allergan Aesthetics, an AbbVie company, Irvine, CA. Writing assistance and editorial support was provided by Liza Selwan-Lewis, PhD, an employee of AbbVie, Inc. All authors met the ICMJE authorship criteria. Neither honoraria nor compensation was provided for authorship.

Funding: This research was supported by Allergan Aesthetics, an AbbVie Company. Employees of AbbVie participated in the research, interpretation of data, review of the manuscript, and the decision to submit for publication.

ACKNOWLEDGMENT

The authors gratefully acknowledge Carlos Alvarado for technical support.

REFERENCES

1. Zouboulis CC et al. Aesthetic aspects of skin aging, prevention, and local treatment. *Clin Dermatol*. 37, 365-372 (2019).
2. Gopaul, R. Body beauty: insights into non-facial skin aging. In *Cosmetics & Toiletries*. Vol 136 26-32 (2021).
3. Rittie L, Fisher GJ. Natural and Sun-Induced Aging of Human Skin. *Cold Spring Harb Perspect Med*. 5, a015370-a015370 (2015).
4. Eckhart L, Tschachler E, Gruber F. Autophagic control of skin aging. *Front Cell Dev Bio*. 7(2019).
5. Kruglikov IL, Scherer PE. Skin aging: are adipocytes the next target? *Aging*. 8, 1457-1469 (2016).
6. Kovács D, Fazekas F, Oláh A, Törőcsik D. Adipokines in the skin and in dermatological diseases. *Int J Mol Sci*. 21, 9048 (2020).
7. Kavali CM, Nguyen TQ, Zahr AS, Jiang LI, Kononov T. A randomized, double-blind, split-body, placebo-controlled clinical study to evaluate the efficacy and tolerability of a topical body firming moisturizer for upper arm rejuvenation. *Aesthet Surg J*. 41, NP472-NP483 (2020).
8. 8. Widgerow AD, Moradi A, Poehler J. A double-blind randomized controlled trial evaluating the efficacy and tolerability of a topical body treatment in combination with cryolipolysis procedures. *J Drugs Dermatol*. 18, 342-348 (2019).

AUTHOR CORRESPONDENCE

Kuniko Kadoya PhD
E-mail:..... kuniko.kadoya@abbvie.com

This document contains proprietary information, images and marks of Journal of Drugs in Dermatology (JDD).
No reproduction or use of any portion of the contents of these materials may be made without the express written consent of JDD. If you feel you have obtained this copy illegally, please contact JDD immediately at support@jddonline.com