TrueScience® Liquid Collagen Activates 6 Collagen Genes in Fibroblasts

Objective: To evaluate the benefits of TrueScience® Liquid Collagen on targeted gene expression in fibroblasts.

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Reference: LifeVantage Corp., Lehi, Utah, USA (LV-56)

Introduction

We investigated a collagen/botanical blend in a ready-to-drink shot that contains 10 different types of hydrolyzed fish-collagen peptides together with a red quinoa grain and a blend of blueberry and young Ponkan citrus. Various ingredients found in the collagen/botanical blend, such as red quinoa and blueberry/young Ponkan citrus blend, have been evaluated *in vitro* for their capabilities to boost collagen secretion with excellent results.

Red quinoa has previously shown to activate and upregulate skin-barrier genes as well as significantly increase the gene activation of the *COL1A2* gene, which codes for a protein that is a key part of Type 1 collagen production. Collagen Type 1 is the most abundant type found in the human body.

Efficacy evaluation of red quinoa was also performed in human subjects and showed significant improvements in skin moisturization, skin brightening, skin smoothness, the appearance of crow's feet, and skin collagen density.

To further our understanding of the benefits from the complete combination of ingredients of the collagen/botanical blend, not just of the individual ingredients, more targeted gene expression studies were conducted. Additional collagen producing genes, structural support genes, as well as barrier genes were investigated.

Fibroblasts

Selecting the correct cell type was a key first step in developing a protocol for this gene expression study. Fibroblasts were chosen first because they were used in the original studies on red quinoa, and we wanted to have a clear comparison between the results on red quinoa compared to the entire collagen/botanical blend. This cell type was also chosen because fibroblasts are found throughout the body. Their primary role is to provide support to tissues. Fibroblasts' role specifically in skin is to produce and organize components of the skin that act as the scaffold or support for the skin through proteins, including collagen, elastin, and laminin.

Fibroblasts produce the collagen and extracellular matrix proteins for skin and organs across the body, including the intestines, spleen, brain, lungs, liver, kidneys, and blood vessels - making them essential to overall structural health. They provide support to the structure of capillaries. Cardiac fibroblasts in the walls of the heart help support heartbeats. Muscles contain three layers of fibroblasts that support skeletal muscles. They are also important for tendons and bones.

As a result, fibroblasts have the functional versatility to allow for the investigation of a wide array of possible effects when treated with the collagen/botanical blend.

Targeted gene expression was used to investigate these potential influences.

What is targeted gene expression?

Targeted gene expression refers to the deliberate activation or expression of a specific gene in a particular tissue, cell type, or under specific conditions.

Essentially, targeted gene expression allows us precise control over experimental conditions and to study gene functions of interest in a simplified, isolated environment. By using fibroblast cells, we can regulate variables like timing and dosage, making it easier to understand how a specific gene affects fibroblast behavior. In vitro studies are also faster, less expensive, and ethically less complex than in vivo experiments, making them ideal for high-throughput testing before moving to other models.

Gene expression changes as we age, and in many cases, it may slow down or decrease in fibroblasts, particularly for genes involved in maintenance, repair and regeneration of collagen production. This results in skin wrinkles, weaker bones, reduced skin elasticity, and skin thinning.

In this study we looked for genes directly involved in collagen-building processes as well as collagen-degrading genes and epidermal barrier-function genes. Collagen Type 1 (COL1A1, COL1A2) is a major component of skin, bones, tendons, ligaments and teeth. It forms thick collagen fibers to provide tensile strength and structural support. Collagen Type 2 (COL2A1) also provides tensile strength and structural support, and it is primarily found in elastic cartilage and hyaline – a type of translucent cartilage found primarily in joints – to allow cartilage to resist compression and maintain shape. Collagen Type 3 (COL3A1) is found together with Type 1 in skin, lungs, and blood vessels. It gives these tissues the flexibility and strength they need for proper function, which is important in organs that need to stretch and retract. Collagen Type 7 (COL7A1) anchors the top layer of skin to the dermis below, supporting elasticity and keeping the skin strong and intact. Collagen Type 13 (COL13A1) is important in maintaining the integrity of the epithelial layer found throughout the body. Elastin (*ELN*) was also a gene of interest because it allows for cross-linking of elastic fibers so they can stretch and contract.

Other genes investigated were *MMP1* and *MMP9*, two genes that code for matrix metalloproteinase 1 and 9, enzymes highly involved in the degradation processes of collagen.

The last set examined were genes for key structural proteins involved in skin epidermal-barrier formation and function. Claudin 1 (*CLDN1*) codes for a tight junction protein and is an integral part in controlling skin permeability and skin barrier to water loss. Filaggrin (*FLG*) codes for an aggregate keratin filament, also crucial for skin hydration, barrier integrity and controlling skin pH. Both Loricrin (*LOR*) and Involucrin (*IVL*) lead to the production of structural skin proteins that provide mechanical strength and barrier to the cornified skin layer—the outermost part of the epidermis.

METHODS

Targeted Gene Expression in Fibroblasts (CCD-986Sk cells)

The study was conducted in three parts:

1. A dose finding study
2. A simple gene expression study to verify dose timing and concentration
3. Targeted gene expression of all genes of interest using RT-qPCR

After determining the proper dosage and time points for evaluation, the fibroblasts were treated with and without Liquid Collagen.

Targeted Gene Expression Real-Time qPCR

CCD-986SK cells were procured through ATCC (ATCC Cat. # CRL-1947). Cells were cultured in IMDM culture medium with L-glutamine, supplemented with 10% fetal bovine serum and 1% P/S anti-biotic solution.

For gene expression studies, cells were seeded for 70% confluence in 10cm tissue culture dishes and exposed to test agent the following day. At the indicated times, RNA was extracted using the PureLink RNA Mini Kit (Thermo-Fisher Scientific, Waltham MA, Catalog #12183018A). Cells were lysed on the plate using 600µL of lysis buffer; otherwise, all manufacturer's instructions were followed. RNA was eluted with 45µL of RNAse-free water and quantified on a UV5 Nano (Mettler Toledo).

First-strand cDNA synthesis was carried out on 0.5µg of RNA, using the SuperScript VILO Master Mix (Thermo-Fisher Scientific, Catalog # 11755050) according to manufacturer's instructions. A 6-fold dilution was made of each cDNA in PCR-grade water, and 6.5µL of this solution was carried forward into qRT-PCR. (Table 1)

PCR primers were purchased from Thermo Fisher Scientific as follows:

Table 1.

Symbol	Identifier	Label
COL1A1	Hs00164004_m1	FAM
COL1A2	Hs01028956_m1	FAM
COL2A1	Hs00264051_m1	FAM
COL3A1	Hs00943809_m1	FAM
COL7A1	Hs00164310_m1	FAM
COL13A1	Hs01103890_m1	FAM
ELN	Hs00355783_m1	FAM

Symbol	Identifier	Label
MMP1	Hs00899658_m1	FAM
MMP9	Hs00957562_m1	FAM
CLDN1	Hs00221623_m1	FAM
FLG	Hs00856927_g1	FAM
LOR	Hs01894962_s1	FAM
IVL	Hs00846307_s1	FAM
ACTB	Hs01060665_g1	VIC

Reactions were carried out in 15µL total volume, made up as follows: 6.5µL cDNA, 6µL PCR-grade water, 0.5µL gene-of-interest primer (FAM label), 0.5µL actin primer (VIC label), 7.5µL Taqman Fast Advanced Master Mix (Thermo Fisher Scientific, Catalog #4444963). Reactions were run on an Applied Biosystems QuantStudio Real-Time PCR Instrument (Thermo Fisher Scientific) under the following conditions: 50°C - 2 minutes, 95°C – 20 seconds, 40 cycles of (95°C – 3 seconds, 60°C – 30 seconds). Threshold cycle (CT) was determined by the instrument software. Differences in threshold cycle between the gene of interest and actin (ΔC_T) were determined for each sample and used to determine fold induction of each gene of interest, compared to untreated controls (ΔΔCt [Delta Delta Ct] method).

RESULTS AND DISCUSSION

From earlier studies we knew red quinoa alone increased *COL1A2* gene expression by 43% and decreased *MMP9* expression by 33%. This study showed that the collagen/botanical blend with its synergistic ingredients, including red quinoa, boosted *COL1A2* expression by 130% and decreased *MMP9* expression by 79%. We also saw a decrease in another collagen degrading enzyme gene, *MMP1*, by 57% (Table 2).

Table 2. Changes in *COL1A2*, *MMP9* and *MMP1* gene expressions after treatment with the collagen/botanical blend.

Gene	Red Quinoa Alone	Liquid Collagen
COL1A2	43% increase	130% increase
MMP9	33% decrease	79% decrease
MMP1	-	57% decrease

Furthermore, 5 additional collagen genes were activated together with other structural genes (Table 3).

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Gene	Collagen/Botanical Blend
COL1A1	33%
COL2A1	169%
COL3A1	59%
COL7A1	31%
COL13A1	15%
ELN	18%
CLDN1	208%
FLG	27%
LOR	33%
IVL	42%

CONCLUSION

Fibroblasts are an integral part of our skin and structural organs, such as intestines, spleen, brain, skin, joints, lung, liver, kidney, and blood vessels. They provide the backbone to the vascular system and skeletal muscles, bones, and tendons and so this was an excellent cell type to study the beneficial effects of the collagen/botanical blend.

The collagen/botanical blend combines marine collagen peptides with botanicals that have individually been shown to stimulate collagen-building and support the skin barrier and reduce *MMP9*, an enzyme that breaks collagen down. From a consumer study on red quinoa, subjective testimonials reported beneficial effects on skin appearance, joint health, and general well-being.

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The goal of the new research was to show the synergistic benefits of the entire collagen/botanical blend, building on the foundation of the prior data on individual ingredients. We chose to investigate fibroblasts due to their importance throughout the body. This allowed us to target genes involved in Type I, II, III, VII, and XIII collagen production, along with other skin structural proteins.

We saw gene expression increases in all the collagen and structural protein components tested. It was especially exiting to see the collagen/botanical blend activated both genes required to create the 2 alpha1(I) and 1 alpha2(I) proteins fibers that make a complete Type 1 Collagen protein, the most abundant protein in the human body. Both *COL1A1* and *COL1A2* genes were activated by 33% and 130%, respectively. The other collagen genes investigated *COL2A1, COL3A1, COL7A1*, and *COL13A1* also increased by 169%, 59%, 31%, and 15%, respectively. The collagen/botanical blend increased the expression of *COL1A2* by 3x as compared to the red quinoa ingredient alone. This synergy was also observed with the decrease in *MMP9* gene expression from -33% to -79%. Together with the decrease in the other matrix metalloproteinase gene MMP1 by 57%, the collagen/botanical blend allows for an additional 5 types of collagen genes to be activated and produced.

Other structural and barrier genes were activated to support the production of skin barrier and structural skin proteins. Elastin (*ELN*) gene expression increased by 18%, Claudin-1 (*CLDN1*) by 208%, Filaggrin (*FLG*) by 27%, Loricrin (*LOR*) by 33%, and Involucrin (*IVL*) by 42%. Each of these genes contribute to the activation of peptides and proteins that structurally support healthier skin from the inside and outside.

This demonstrates that the synergistic effects of the components found the collagen/botanical blend could make a significant impact on your health, both internally and externally.

