Sirius Red/Fast Green Collagen Analysis

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Tradename: Phyto-Biotics Perilla®

Code: 40600

CAS #: 90082-61-4 & 56-81-5 & 57-55-6 & 1686112-36-6 (or) 68333-16-4

Test Request Form #: 5878

Lot #: 71710

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Michael Hovis*

Test Performed:
Sirius Red/Fast Green Collagen Assay

Introduction

Collagen is the main protein of connective tissues, such as skin, bone, tendon and ligament, and the most abundant protein in mammals. Collagen accounts for nearly 25% to 35% of the total human protein content. Collagen is a long, fibrous protein that forms bundles called fibers giving structure and support to cells and tissues. Collagen has great tensile strength and is responsible for skin's elasticity and, therefore, its degradation leads to wrinkles that accompany aging.

Sirius Red/Fast Green Collagen Assay was conducted to assess the changes in collagen synthesis by **Phyto-Biotics Perilla®** treated *in vitro* cultured human dermal fibroblasts.

Assay Principle

Sirius Red is a unique dye that binds specifically to the helical structure of types I through V collagen, while Fast Green binds to non-collagenous proteins. These two dyes work in conjunction to provide a semi-quantitative method of determining amounts of collagen and non-collagenous proteins in a sample. After staining a sample the dyes are easily extracted and have optical density (OD) absorptions at 540nm (Sirius Red) and 605nm (Fast Green). Collagen concentrations are calculated through equations with OD values.

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Materials

A. Kit:	Sirius Red/Fast Green Collagen Kit (Chondrex; 9046)
B. Incubation Conditions:	37°C at 5% CO_2 and 95% Relative Humidity (RH)
C. Equipment:	Forma Humidified Incubator, ESCO Biosafety Laminar Flow Hood, Synergy HT Microplate Reader; Pipettes
D. Cell Line:	Normal Human Dermal Fibroblasts (Invitrogen; C-004-5C)
E. Media/Buffers:	Complete and Serum-Free Dulbecco's Modified Eagle Medium (DMEM); Phosphate Buffered Saline (PBS)
F. Culture Plate:	Falcon Flat Bottom 24-Well Tissue Culture Treated Plates
G. Reagents:	Ascorbic Acid-2-Glucose(AA2G) (100µM); Insulin Growth Factor-1 (IGF-1) (50ng/mL); Glacial Acetic Acid, Ethanol
H. Other:	Sterile Disposable Pipette Tips; Wash Bottles

Methods

Human dermal fibroblasts were seeded into 24-well tissue culture plates and allowed to grow to confluency in complete DMEM. 1%, 0.1%, and 0.01% concentrations **Phyto-Biotics Perilla®** were added to the serum-free DMEM and incubated with fibroblasts for 24 hours. AA2G and IGF-1 were used as positive controls.

Media was removed from wells containing adherent fibroblasts and the cells were washed with PBS. 500µl of a cooled 95% ethanol/5% glacial acetic acid solution was added to the wells and incubated for 10 minutes at room temperature. 200µL of the Sirius Red/Fast Green dye solution was added to the fixed cell layer and incubated at room temperature for 30 minutes. The dye solution was removed from the cell layer and washed with water. 1mL of extraction solution was added for color extraction. The optical density was read at 540nm and 605nm on the Synergy HT Microplate Reader.

The collagen concentration of **Phyto-Biotics Perilla®** treated-fibroblasts was determined by calculations based on the optical density measurements and expressed in µg.

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Results

The data obtained from this study met criteria for a valid assay and the positive controls performed as anticipated.

Phyto-Biotics Perilla® at all concentrations was able to promote collagen synthesis compared to complete media. Additionally, **Phyto-Biotics Perilla®** at 0.1% was able to promote collagen synthesis compared to our positive controls.

Collagen concentration is calculated by the following formula:

$$Collagen\ (\mu g) = \frac{[OD\ 540 - (OD\ 605\ \times 0.291)]}{0.0378}$$

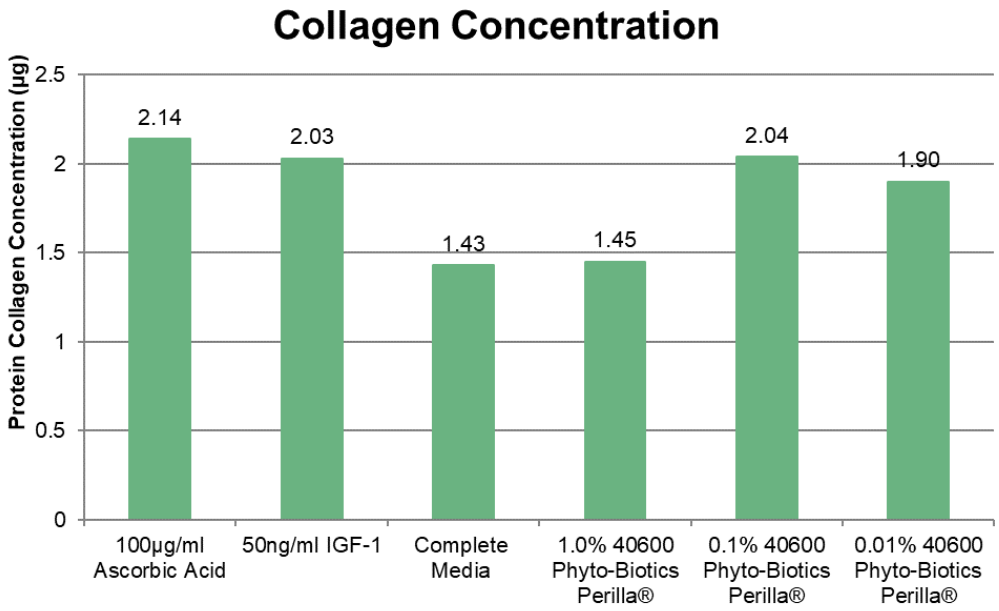

Figure 1: Collagen concentration

*Please note that when dealing with in-vitro studies, 1.0% dose wise is comparable to 100% in application. This high dosage can account for slightly decreased viability and efficacy and is simply used for a comparison range.

Discussion

As shown in figure 1, **Phyto-Biotics Perilla®** exhibited potent collagen synthesis activity. As expected, the rate of increase for collagen synthesis using **Phyto-Biotics Perilla®** appears to be dose dependent. The increase in collagen production may lead to improvement in the dermal-epidermal junction integrity as well as an improved scaffolding matrix. For these reasons, we can assume **Phyto-Biotics Perilla®** is suitable for cosmetic applications designed to boost collagen synthesis to aid in providing a younger and healthier complexion.