

Tradename: AC CytoPure PF

Code: 20757PF

CAS #: 999999-99-4

Test Request Form #: 1286

Lot #: NC130424-B

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*

Study Director: *Maureen Danaher*

Principal Investigator: *Jennifer Goodman*

Test Performed:

High Resolution Ultrasound Skin-Imaging Study

Introduction

Collagen is the most abundant protein found in the skin and other areas of the body. Collagen connects other tissues to bone, muscles, tendons, cartilage, and skin. Within the skin, collagen aids in the youthful appearance by providing an anchor point. With age, collagen naturally decreases and results in sagging, wrinkles, and fine lines, which can be accelerated by excessive sun exposure. Collagen encourages the top layer of skin to regenerate which in return keeps skin looking healthy and youthful.

Accordingly, a High-Resolution Ultrasound Skin Imaging Study was conducted to assess the ability of **AC CytoPure PF** to improve Collagen Fiber Density.

Study Principle

Participants applied specific products to designated areas on their forearms twice a day for four weeks. Ultrasound measurements were collected once a week during the four-week study period. Ultrasound skin imaging is based on measuring reflections of an emitted acoustic pulse that are transmitted into the skin. After processing the reflected signals, a cross-sectional image is generated based on the intensity of the reflected signals (Figure 1).

The signal intensity is converted to a color scale with dark colors representing areas of low reflection. This means that there are no changes or very small changes in density between the structures in the skin. Bright colors represent areas with strong reflections, indicating substantial changes in density between structures. The epidermis is characterized by a high intensity white/yellow color while the dermis is a mixture of colors at varying intensities.

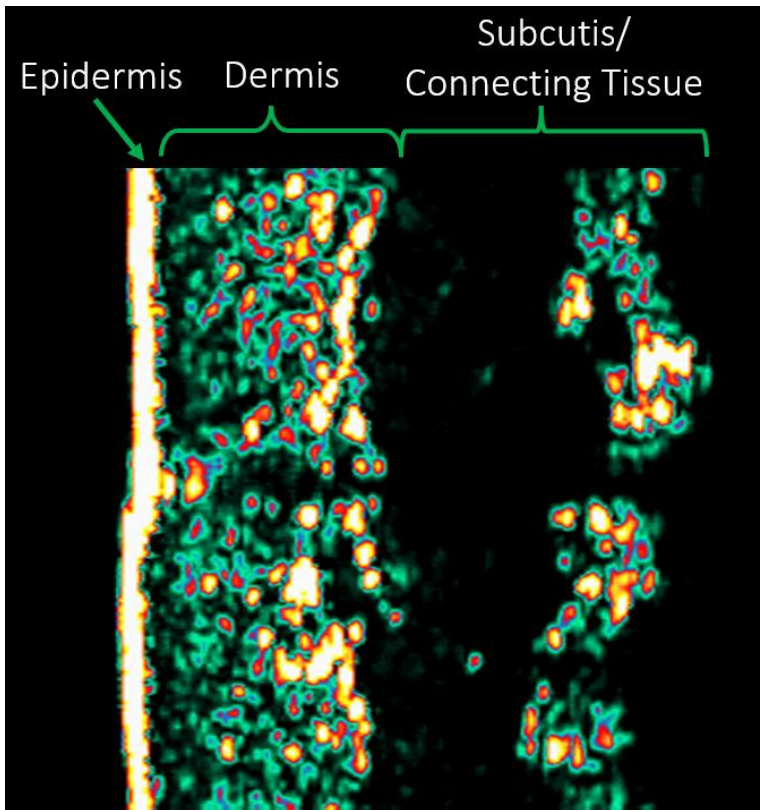


Figure 1. Representative Ultrasound Image with Measurement Areas

Materials

- A. **Equipment:** DermaLab Skin Combo (Ultrasound Probe)
- B. **Products:** Base Lotion (Cetaphil® Moisturizing Cream for All Skin Types)
- C. **Software:** Excel Analysis ToolPak (Microsoft)

Methods

10 volunteers between the ages of 23 and 45, who were known to be free of any skin pathologies with Fitzpatrick skin types I to III, participated in this study (Table 1).

Table 1. The Fitzpatrick Classification of Skin Types Chart¹

Fitzpatrick Skin Type Descriptions*	
Skin Type	Description
I	Always burns, never tans
II	Burns easily, tans minimally
III	Burns moderately, tans to light brown
IV	Burns minimally, tans to moderate brown
V	Rarely burns, tans to dark
VI	Never burns, least sensitive to changes

*Adapted from The Surgeon General's Call to Action to Prevent Skin Cancer

Three randomly assigned test sites were identified on the volar forearm of participants and baseline measurements were recorded. Following baseline measurements, participants applied 0.2 g of each test material on their volar forearms twice a day for four weeks. Measurements were recorded once a week for four weeks. The skin test site conditions and treatments are described below (Table 2). The Base Lotion utilized in this study was Cetaphil® Moisturizing Cream for All Skin Types.

Table 2. Descriptions of the Conditions and Treatments for each Skin Test Site

Skin Test Site	Condition	Treatment / Test Article Application Description
1	Untreated Control	None
2	Base Lotion	Base Lotion
3	2.0% AC CytoPure PF	2.0% AC CytoPure PF in Base Lotion

The Dermalab Skin Combo Ultrasound Probe Intensity. Intensity (Collagen Fiber Density) is an indicator of the amount of collagen within the dermis, with greater intensities correlating with higher levels of collagen.

An average of three consecutive measurements for Intensity (average) were recorded per condition at each time point. Data are displayed as averages from all volunteers and analyzed using t-tests with statistical significance accepted at $p \leq 0.05$. The percent change for Collagen Fiber Density (Intensity) measurements were calculated for each test site at every timepoint relative to baseline values, using the following equation:

$$\text{Percent Change (\%)} = \frac{\text{Measurement}_{\text{Week}} - \text{Measurement}_{\text{Baseline}}}{\text{Measurement}_{\text{Baseline}}} \times 100$$

Results

The data obtained met criteria for a valid study as the Untreated Control and Base Lotion performed as anticipated. Application of 2.0% AC CytoPure PF twice a day for four weeks demonstrated effective improvements in Intensity throughout the study duration compared to the Base Lotion.

Collagen Fiber Density AC CytoPure PF

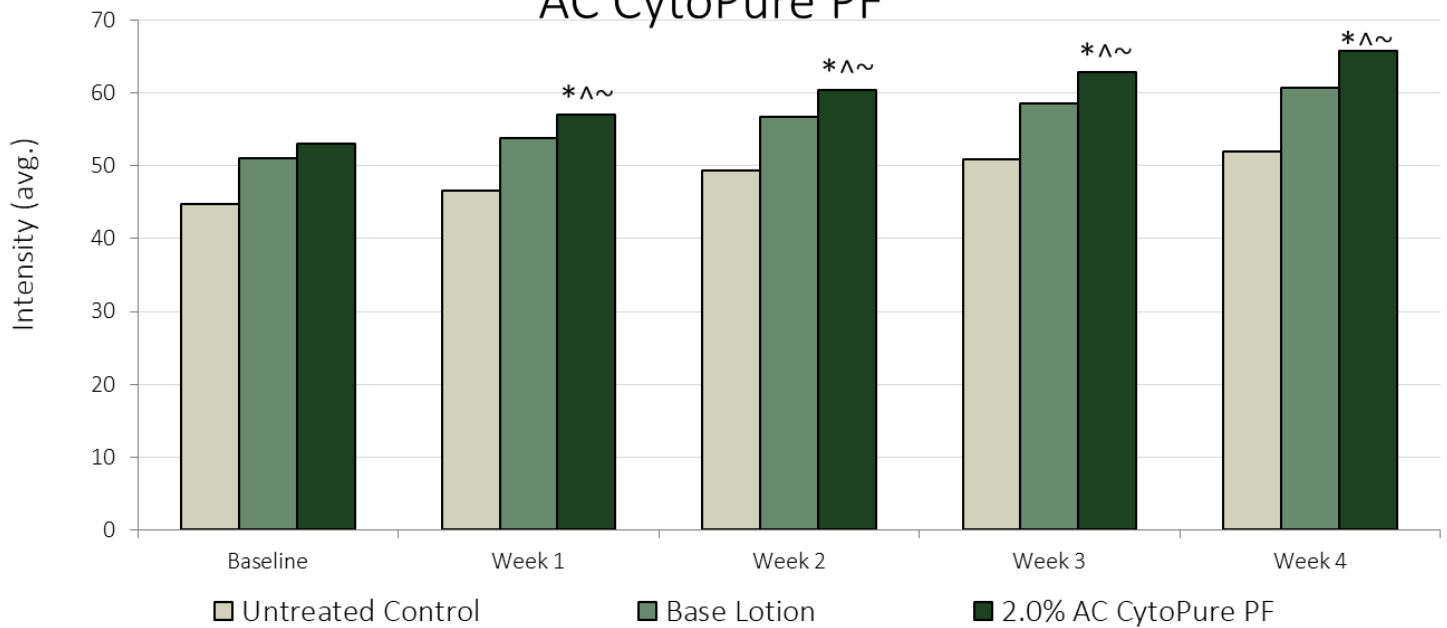


Figure 2. Collagen Fiber Density Overtime. * indicates significance ($p \leq 0.05$) compared to Baseline values within the same condition. ^ indicates significance ($p \leq 0.05$) compared to Untreated Control within the same timepoint. ~ indicates significance ($p \leq 0.05$) compared to Base Lotion within the same timepoint.

Table 3. T-test Analysis of Collagen Fiber Density from Baseline to After Four Weeks of Application. * indicates significance ($p \leq 0.05$) compared to Baseline values within the same condition.

	Untreated Control	Base Lotion	2.0% AC CytoPure PF
P-value	0.125	0.064	0.032*

Table 4. T-test Analysis of Collagen Fiber Density After Four Weeks of Application. ^ indicates significance ($p \leq 0.05$) compared to Untreated Control within the same timepoint. ~ indicates significance ($p \leq 0.05$) compared to Base Lotion within the same timepoint.

	Untreated Control vs Base Lotion	Untreated Control vs 2.0% AC CytoPure PF	Base Lotion vs 2.0% AC CytoPure PF
P-value	0.084	0.032 [^]	0.048 [~]

Change in Collagen Fiber Density AC CytoPure PF

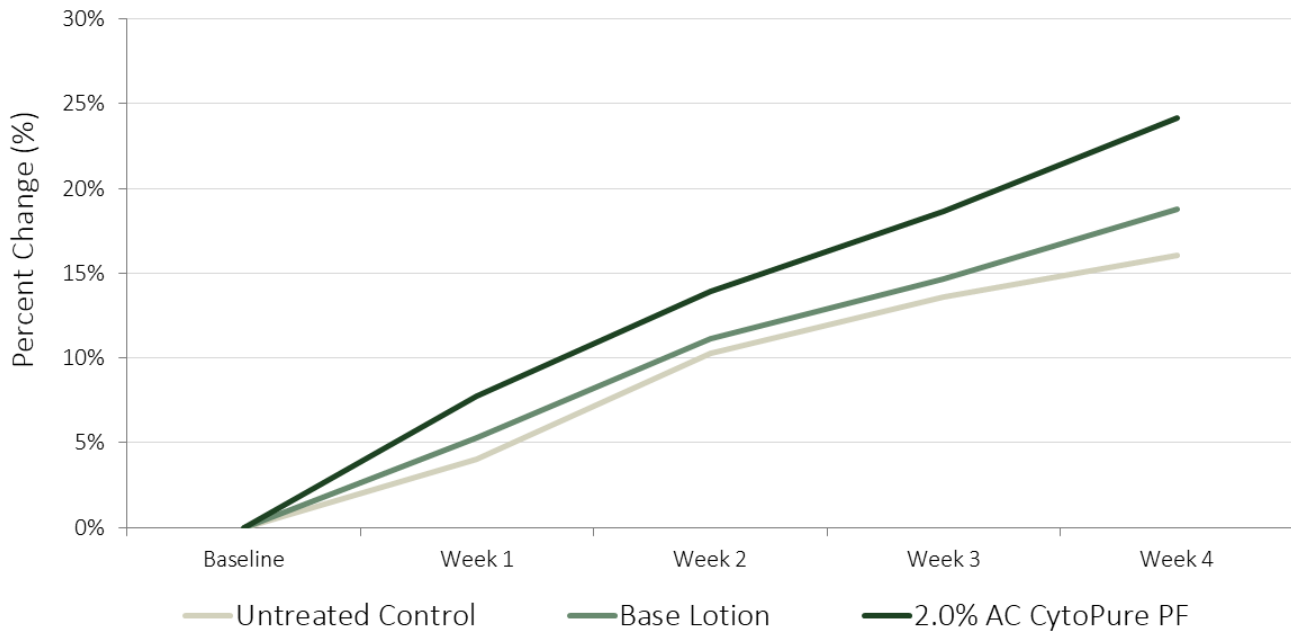


Figure 3. Percent Change in Collagen Fiber Density Relative to Baseline Values

Discussion

The ability of **AC CytoPure PF** to improve collagen fiber density was assessed via ultrasound measurements throughout four weeks of twice daily application.

Collagen Fiber Density was not significantly altered throughout the study with the Untreated and Base Lotion Test Sites, indicating consistent collagen density throughout the study (Figures 2, 3; Table 3). However, Collagen Fiber Density significantly increased by 24% after four weeks of 2.0% **AC CytoPure PF** application, respectively (Figures 2, 3; Table 3). After four weeks of application, there was no difference in Collagen Fiber Density between the Untreated Control and Base Lotion, indicating repeated applications of the Base Lotion did not alter Collagen Fiber Density (Figure 3; Table 4). Conversely, applying 2.0% **AC CytoPure PF** twice daily demonstrated a significant increase in Collagen Fiber Density compared to the Untreated Control and Base Lotion after four weeks (Figure 3; Table 4). These results indicate application of 2.0% **AC CytoPure PF** elicits an increase in collagen density, reducing the visual impacts of normal aging.

Taken together, these results indicate **AC CytoPure PF** improves collagen density when added to personal care applications at recommended use levels. Collectively, **AC CytoPure PF** improves normal skin aging by augmenting collagen density resulting in a healthier and more youthful skin appearance.

References

1. Sharma AN, Patel BC. Laser Fitzpatrick Skin Type Recommendations. [Updated 2022 Mar 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK557626/>