

Tradename: AC WonderShroom

Code: 21029

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Test Request Form #: 11686

Lot #: N240509A

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

Study Director: *Daniel Shill*

Principal Investigator: *Kayla Goodson*

Test Performed:

In vivo DermaLab Analysis: Neck Wrinkles

Introduction

Wrinkles are furrows, folds, or creases in the skin associated with a decrease in skin elasticity and overexposure to the sun. As skin ages, cell production slows resulting in the dermis becoming thinner and allowing depressions to form on the skin's surface. Aging skin is often slower to retain moisture, less elastic, and prone to damage which can exacerbate the formation of unappealing skin wrinkles. Furthermore, horizontal creasing on the neck, most often from repeated movement, leads to reductions in collagen and elastin. Horizontal neck folds, also known as "Tech Neck", can appear more prudent from repeatedly looking down at technological devices. The lines from "Tech Neck" are similar to those formed on a repetitively folded piece of paper, the creases remain even when unfolded. It is important to monitor the visual manifestations of skin damage, such as fine lines and wrinkles, because limiting skin damage provides a healthier and more youthful appearance.

Accordingly, an *in vivo* study was conducted over a period of four weeks to evaluate the ability of **AC WonderShroom** to improve the appearance of horizontal wrinkles on the neck as well as Collagen Fiber Density, Dermal Collagen Thickness, and Skin Elasticity.

Study Principle

Participants applied specific products to their neck twice a day for four weeks. Ultrasound and Elasticity measurements were collected once a week during the four-week use period. Photographs of participant necks were obtained using a camera at baseline and after four weeks of product application. Ultrasound skin imaging is based on measuring reflections of an emitted acoustic pulse transmitted into the skin. After processing the reflected signals, a cross-sectional image is generated based on the intensity of the reflected signals (Diagram 1). The Elasticity Probe is based on a suction cup method, which measures the elevation of the skin, as a function of a predefined negative pressure setting. Photographs of neck wrinkles were utilized for qualitative and quantitative purposes.

Materials

- A. **Equipment:** DermaLab Skin Combo (High Resolution Skin Imaging Probe and Elasticity Probe); Canon EOZ Rebel T3 Digital Camera
- B. **Products:** Cetaphil Moisturizing Cream for All Skin Types
- C. **Software:** ImageJ (National Institutes of Health (NIH)); Excel Analysis ToolPak (Microsoft)

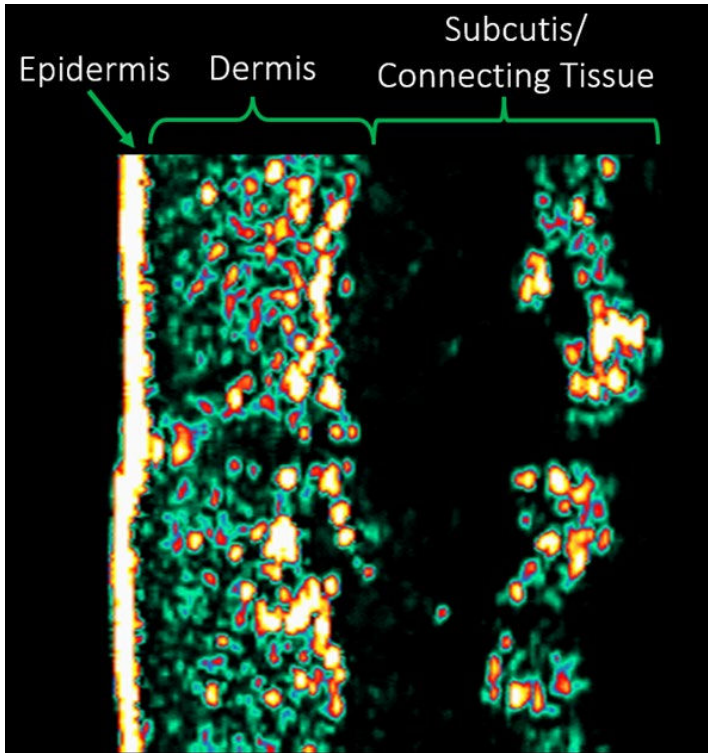


Diagram 1. Representative Ultrasound Image with Measurement Areas

Methods

Ten volunteers between the ages of 23 and 70, 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

Two randomly assigned test sites were identified on each neck of participants and Baseline images, Ultrasound, and Elasticity measurements were recorded prior to any product application. Following Baseline measurements, participants were instructed to apply 0.2 g of each treatment to each half of their neck twice a day for a four-week period. Participants were instructed to continue their everyday skin care routine and to apply the lotion once their routine was finished. Participants were instructed not to wear makeup or SPF products on their neck for each weekly measurement session. The skin test site conditions and treatments are described below (Table 2). All lotion formulations were adjusted to a direct pH of 5.0 – 5.5 (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 Neck Test Site

| Neck Test Site | Condition | Treatment / Test Article Application Description | Lotion PH |
|----------------|----------------------|--|-----------|
| 1 | Base Lotion | Base Lotion | 5.2 |
| 2 | 2.0% AC WonderShroom | 2.0% AC WonderShroom in Base Lotion | 5.4 |

Neck Wrinkle Length was assessed for each condition from pictures using ImageJ software and is expressed in centimeters by dividing the wrinkle length (exported in pixels) by a conversion factor of a given number of pixels per centimeter. The data are displayed as averages and t-test analyses were performed with statistical significance accepted at $p \leq 0.05$. Percent change is expressed relative to Baseline values and calculated by the following equation:

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

Results

The data obtained met criteria for a valid study as the Base Lotion performed as anticipated. Application of 2.0% AC WonderShroom twice a day for four weeks demonstrated an increase in neck Collagen Fiber Density, Collagen Thickness, Skin Elasticity, and reduced Neck Wrinkle Length throughout the four-week treatment period.

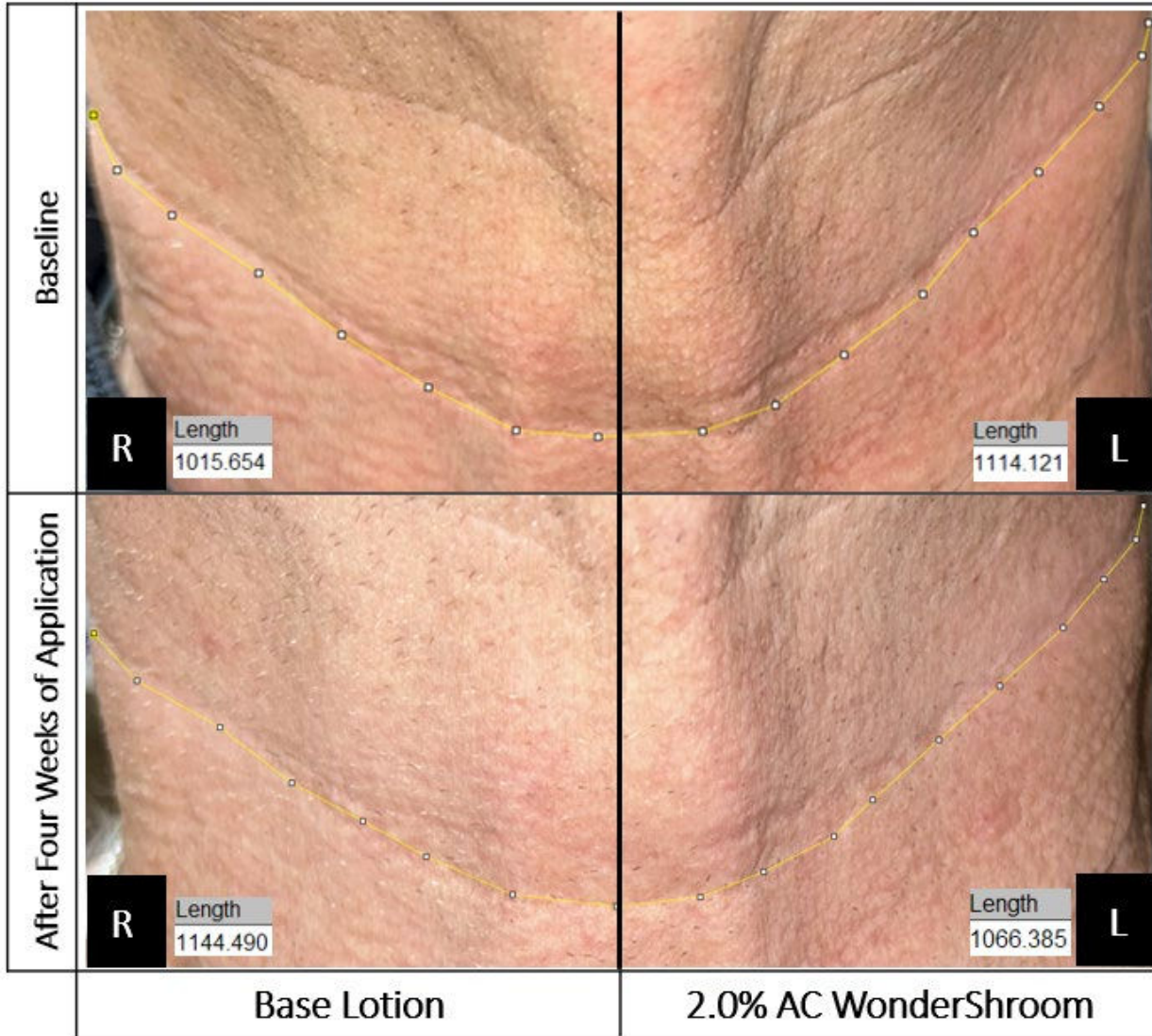


Image 1. Images of Participant 3's Neck Wrinkle Length Over Time Measured in Pixels (Right Side (R)= Base Lotion, Left Side (L)= 2.0% AC WonderShroom). Only the bottom horizontal neck wrinkle for Participant 3 was measured as the top horizontal neck wrinkle is not continuous and does not meet proper ImageJ analysis threshold.

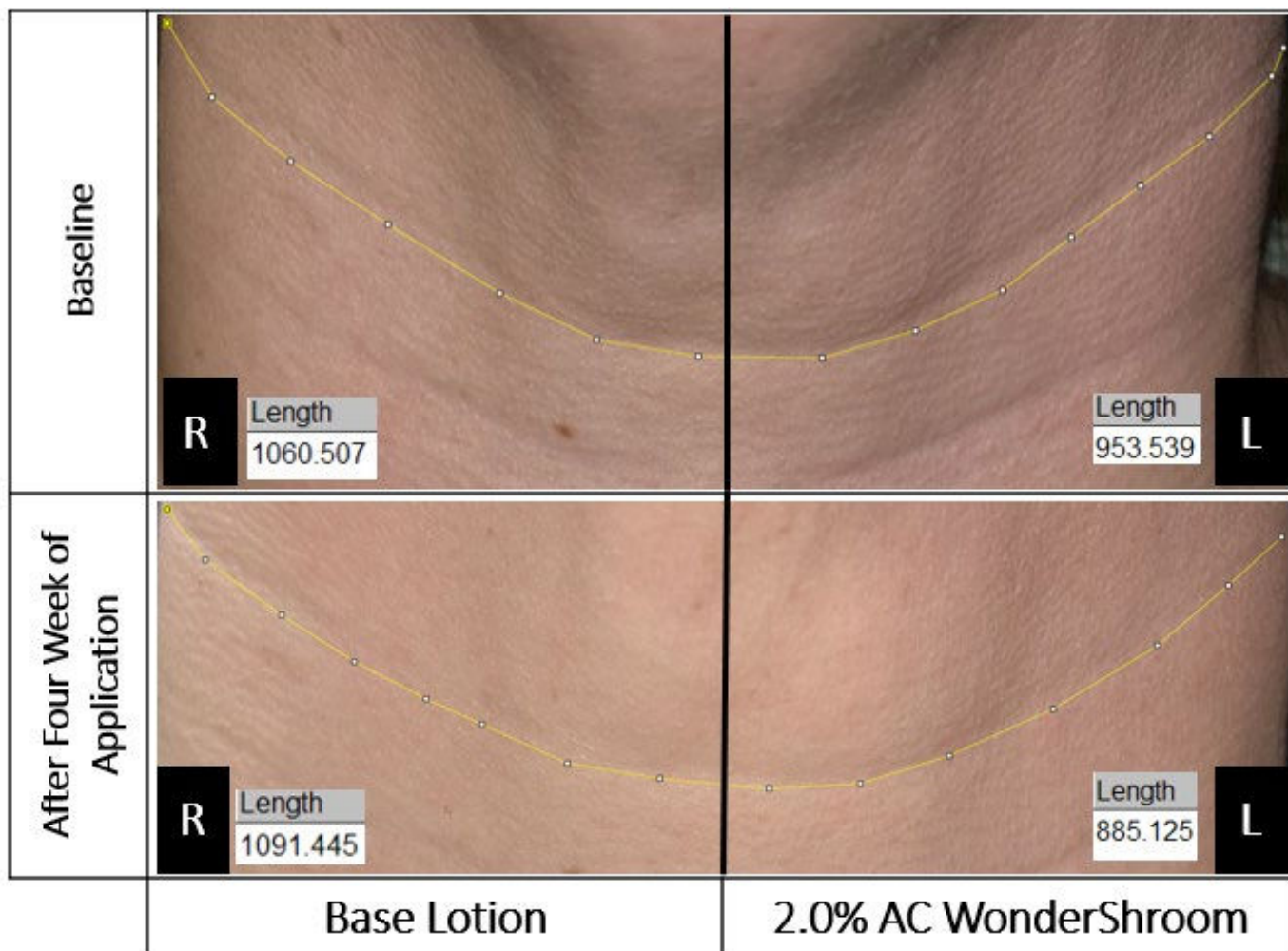


Image 2. Images of Participant 9's Top Neck Wrinkle Length Over Time Measured in Pixels (Right Side (R)= Base Lotion, Left Side (L)= 2.0% AC WonderShroom). Both horizontal neck wrinkles (top and bottom) were measured for Participant 9 as they were continuous and met the proper ImageJ analysis threshold.

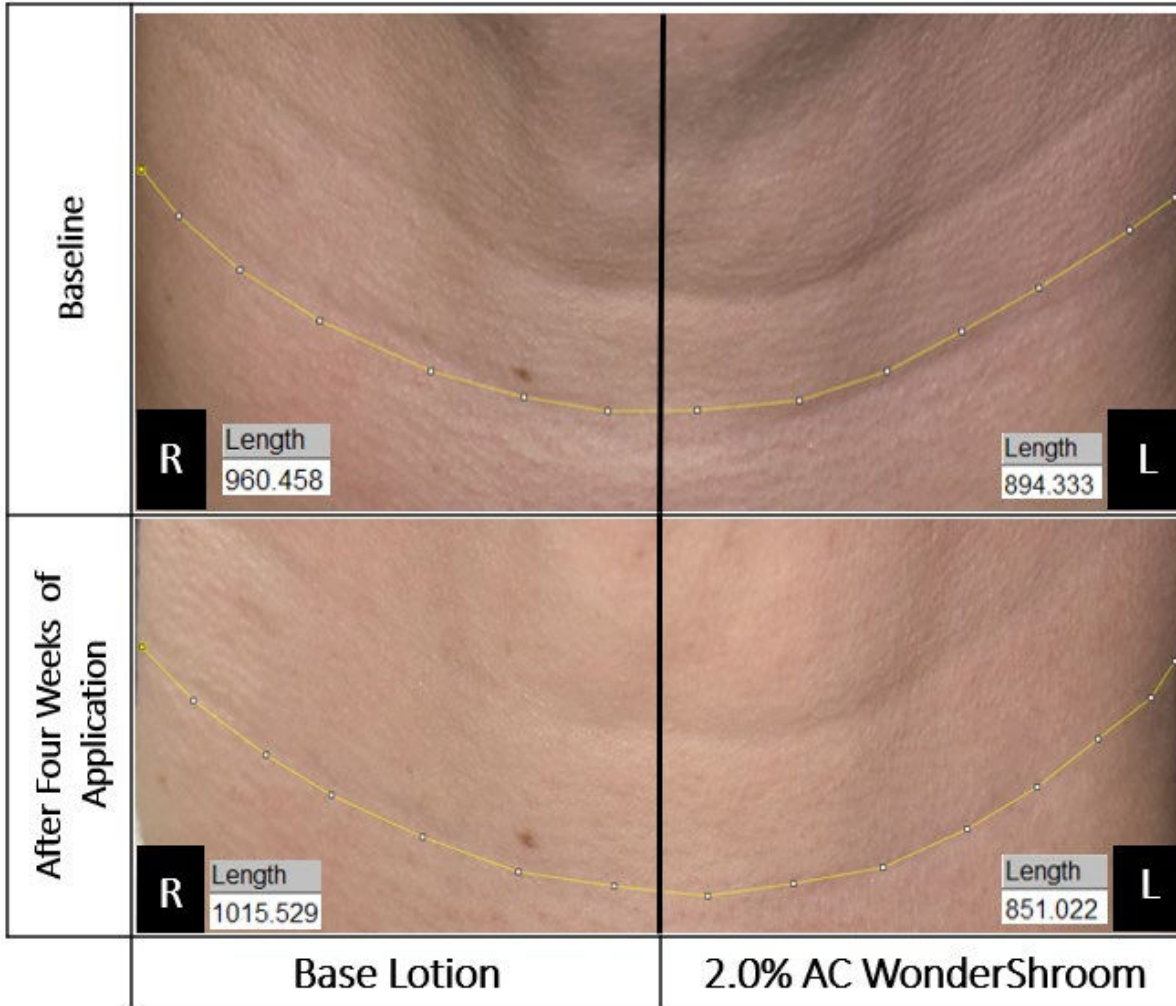


Image 3. Images of Participant 9's Bottom Neck Wrinkle Length Over Time Measured in Pixels (Right Side (R)= Base Lotion, Left Side (L)= 2.0% AC WonderShroom). Both horizontal neck wrinkles (top and bottom) were measured for Participant 9 as they were continuous and met the proper ImageJ analysis threshold.

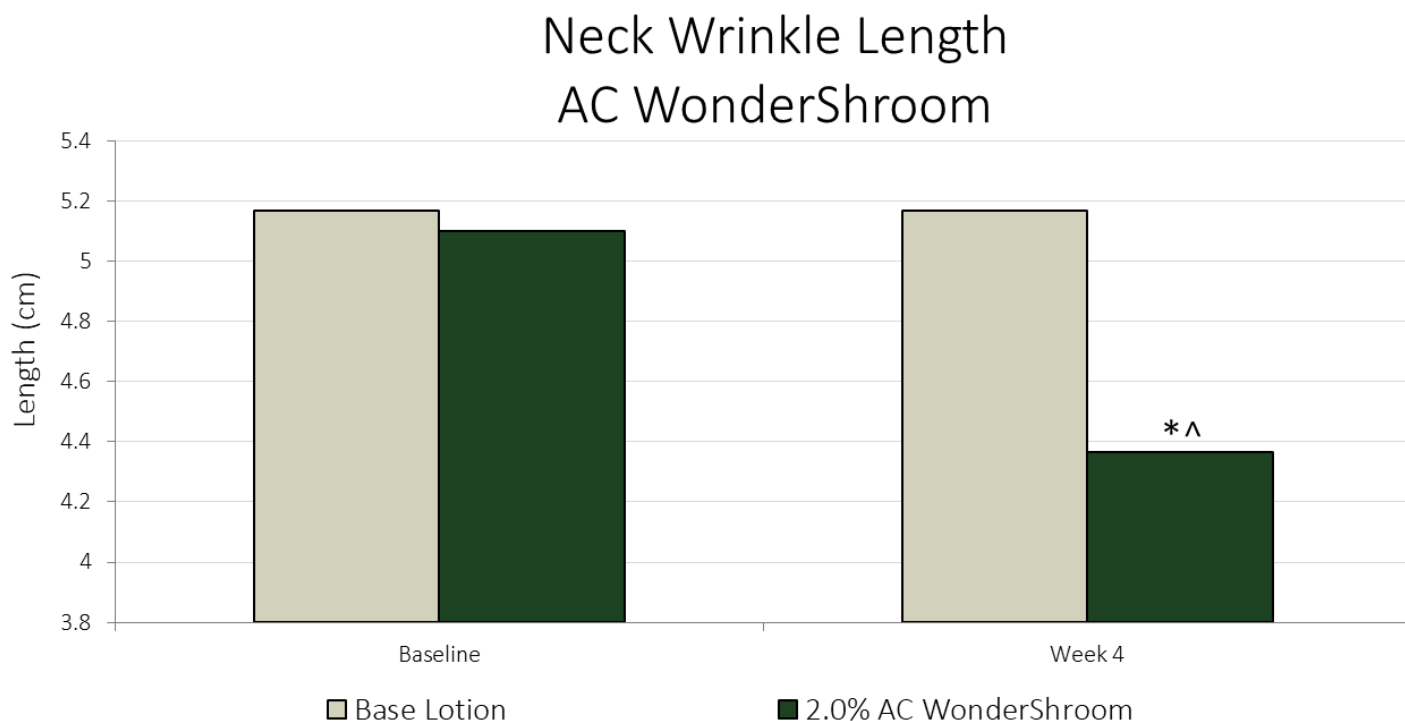


Figure 1. Neck Wrinkle Length Overtime. * indicates significance ($p \leq 0.05$) compared to Baseline values within the same condition. ^ indicates significance ($p \leq 0.05$) compared to Base Lotion within the same timepoint.

Table 3. T-test Analysis of Neck Wrinkle Length from Baseline to After Four Weeks of Application. * indicates significance ($p \leq 0.05$) compared to Baseline.

| | Base Lotion | 2.0% AC WonderShroom |
|----------------|-------------|----------------------|
| P-value | 0.851 | 0.040* |

Table 4. T-test Analysis of Neck Wrinkle Length After Four Weeks of Application. ^ indicates significance ($p \leq 0.05$) compared to Base Lotion within the same timepoint.

| | Base Lotion vs 2.0% AC WonderShroom |
|----------------|-------------------------------------|
| P-value | 0.015^ |

Collagen Fiber Density AC WonderShroom

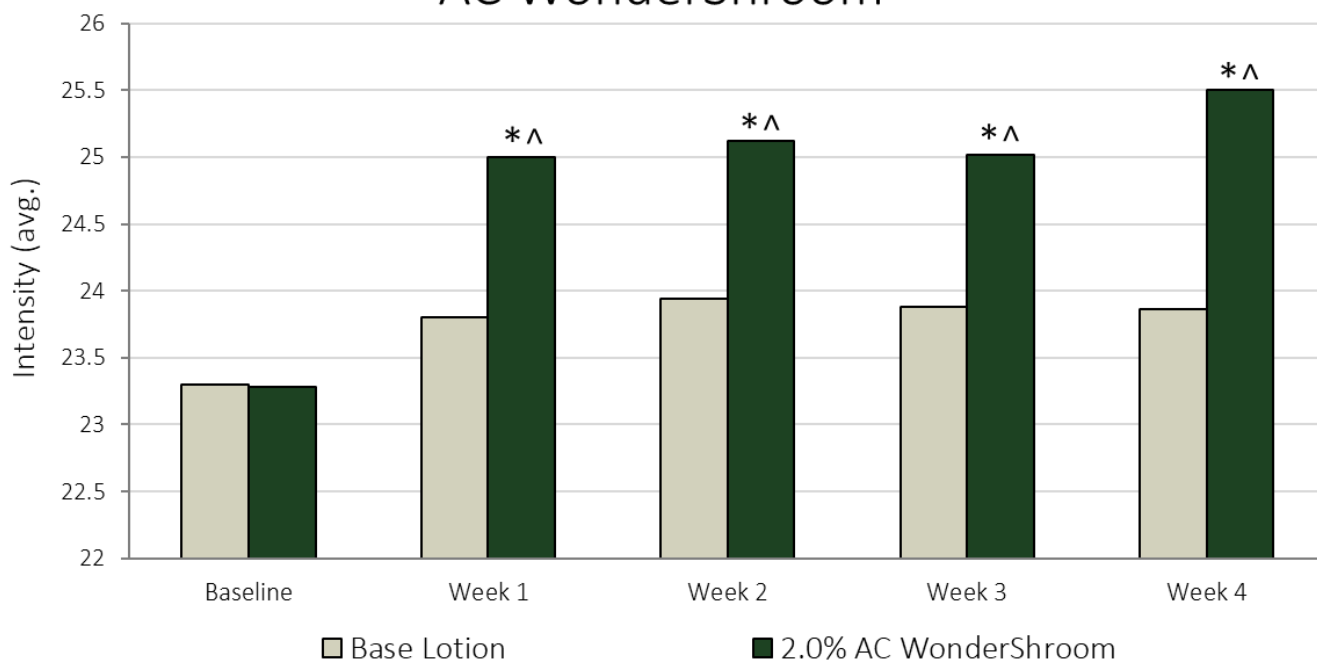


Figure 2. Collagen Fiber Density Overtime. * indicates significance ($p \leq 0.05$) compared to Baseline values within the same condition. ^ indicates significance ($p \leq 0.05$) compared to Base Lotion within the same timepoint.

Table 5. T-test Analysis of Collagen Fiber Density from Baseline to After Four Weeks of Application. * indicates significance ($p \leq 0.05$) compared to Baseline.

| | Base Lotion | 2.0% AC WonderShroom |
|---------|-------------|----------------------|
| P-value | 0.437 | 0.003* |

Table 6. T-test Analysis of Collagen Fiber Density After Four Weeks of Application. ^ indicates significance ($p \leq 0.05$) compared to Base Lotion within the same timepoint.

| | Base Lotion vs 2.0% AC WonderShroom |
|---------|-------------------------------------|
| P-value | 0.018^ |

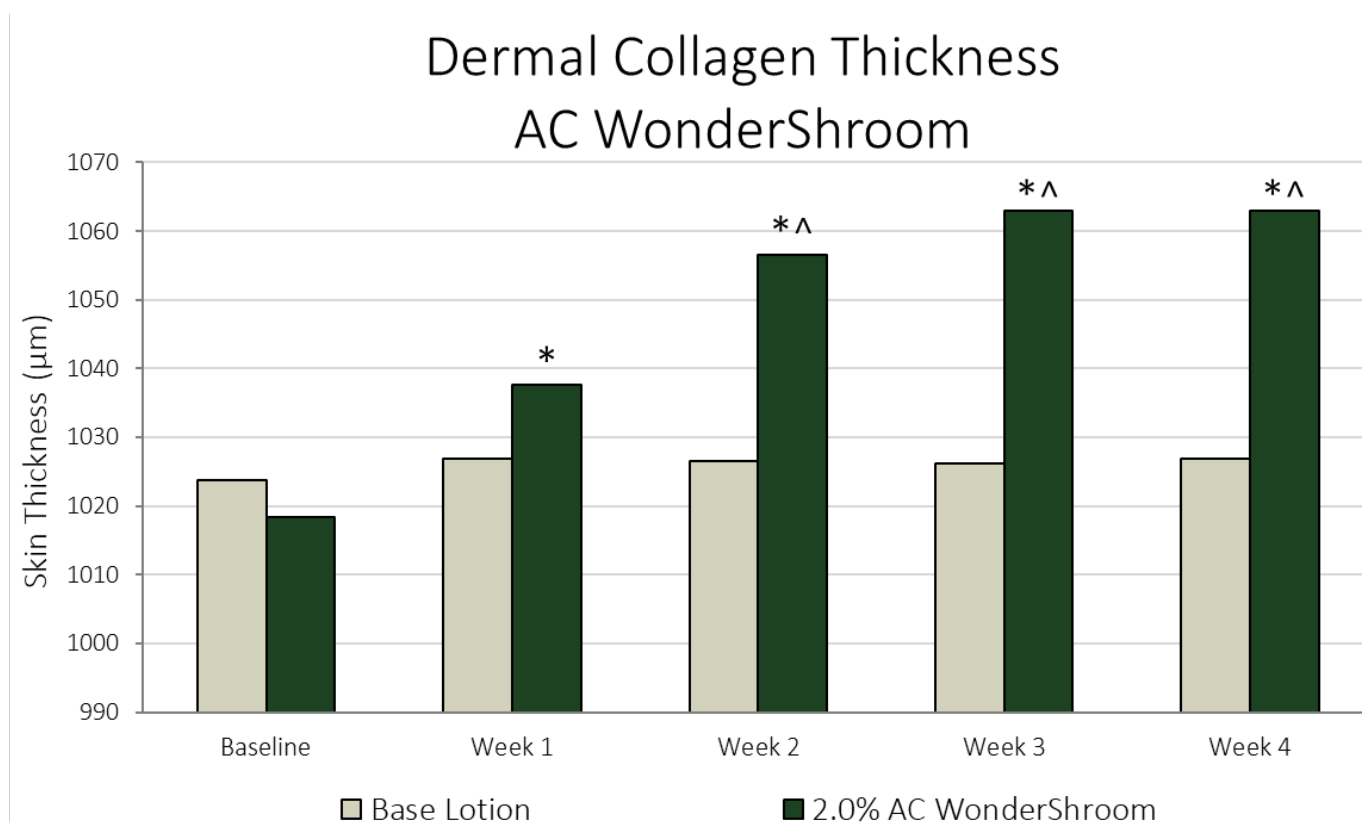


Figure 3. Dermal Collagen Thickness Overtime. * indicates significance ($p \leq 0.05$) compared to Baseline values within the same condition. ^ indicates significance ($p \leq 0.05$) compared to Base Lotion within the same timepoint.

Table 7. T-test Analysis of Dermal Collagen Thickness from Baseline to After Four Weeks of Application. * indicates significance ($p \leq 0.05$) compared to Baseline.

| | Base Lotion | 2.0% AC WonderShroom |
|---------|-------------|----------------------|
| P-value | 0.876 | < 0.001* |

Table 8. T-test Analysis of Dermal Collagen Thickness After Four Weeks of Application. ^ indicates significance ($p \leq 0.05$) compared to Base Lotion within the same timepoint.

| | Base Lotion vs 2.0% AC WonderShroom |
|---------|-------------------------------------|
| P-value | 0.023^ |

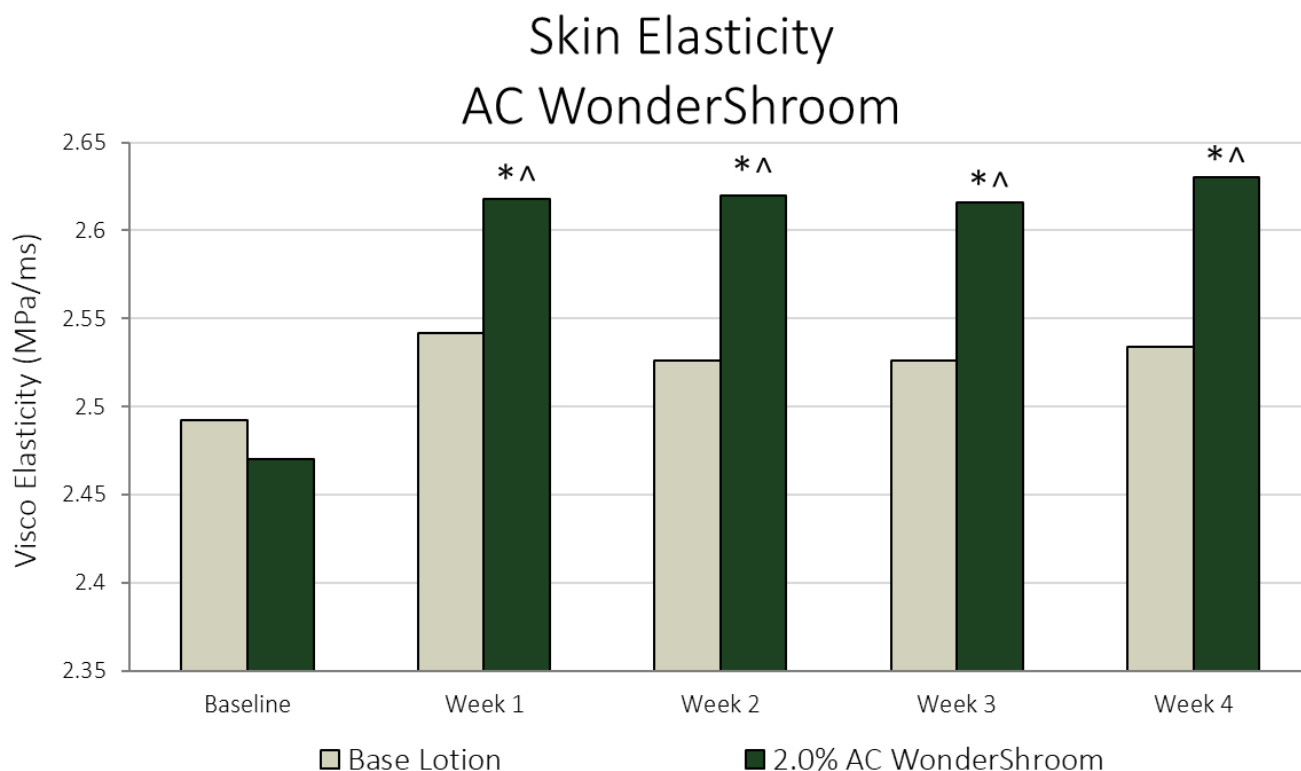


Figure 4. Skin Elasticity Overtime. * indicates significance ($p \leq 0.05$) compared to Baseline values within the same condition. ^ indicates significance ($p \leq 0.05$) compared to Base Lotion within the same timepoint.

Table 9. T-test Analysis of Skin Elasticity from Baseline to After Four Weeks of Application. * indicates significance ($p \leq 0.05$) compared to Baseline.

| | Base Lotion | 2.0% AC WonderShroom |
|----------------|-------------|----------------------|
| P-value | 0.825 | 0.008* |

Table 10. T-test Analysis of Skin Elasticity After Four Weeks of Application. ^ indicates significance ($p \leq 0.05$) compared to Base Lotion within the same timepoint.

| | Base Lotion vs 2.0% AC WonderShroom |
|----------------|-------------------------------------|
| P-value | 0.008^ |

Table 11. Percent Change in Collagen Fiber Density, Dermal Collagen Thickness, and Skin Elasticity Overtime Relative to Baseline

| Outcome | Treatment | Weeks of Application | | | |
|---------------------------|-----------------------------|----------------------|--------|--------|--------|
| | | Week 1 | Week 2 | Week 3 | Week 4 |
| Collagen Fiber Density | Base Lotion | 2.2% | 2.8% | 2.5% | 2.4% |
| | 2.0% AC WonderShroom | 7.4% | 7.9% | 7.5% | 9.5% |
| Dermal Collagen Thickness | Base Lotion | 0.3% | 0.3% | 0.2% | 0.3% |
| | 2.0% AC WonderShroom | 1.9% | 3.8% | 4.4% | 4.4% |
| Skin Elasticity | Base Lotion | 2.0% | 1.4% | 1.4% | 1.7% |
| | 2.0% AC WonderShroom | 6.0% | 6.1% | 5.9% | 6.5% |

Discussion

The ability of **AC WonderShroom** to improve the appearance of horizontal wrinkles on the neck as well as Collagen Fiber Density, Dermal Collagen Thickness, and Skin Elasticity was assessed via software measurements, ultrasound, and elasticity measurements throughout four weeks of twice daily application.

Visually, participants experienced an improvement in horizontal neck wrinkles after four weeks of 2.0% **AC WonderShroom** application compared to the Base Lotion alone (Images 1, 2, 3, 4, 5). Moreover, Base Lotion application alone had no effect on horizontal Neck Wrinkle Length after four weeks of application, compared to baseline (Figure 1; Tables 3, 4). Conversely, twice daily application of 2.0% **AC WonderShroom** resulted in a significant 14% reduction in horizontal Neck Wrinkle Length after four weeks of application ($p < 0.05$; Figure 1; Tables 3, 4). These results indicate **AC WonderShroom** improves the visibility and length of horizontal neck wrinkles.

As shown in Figure 2, Collagen Fiber Density was not significantly altered over four weeks with Base Lotion, indicating consistent collagen density throughout the study (Tables 5, 11). However, Collagen Fiber Density significantly increased by 9%, relative to baseline, after four weeks of 2.0% **AC WonderShroom** application (Figure 2; Tables 5, 11). Compared to Base Lotion after four weeks of application, 2.0% **AC WonderShroom** demonstrated a significant increase in Collagen Fiber Density (Figure 2; Table 6). These results indicate application of 2.0% **AC WonderShroom** elicits an increase in collagen density, reducing the visual impacts of normal aging on the neck.

Similarly, Dermal Collagen Thickness did not significantly change throughout the four-week protocol with Base Lotion application, indicating consistent skin thickness throughout the study (Figure 3; Tables 7, 11). Conversely, applying 2.0% **AC WonderShroom** twice daily significantly augmented Dermal Collagen Thickness by 4%, relative to baseline, after four weeks of application (Figure 3; Tables 7, 11). Cumulatively, applying 2.0% **AC WonderShroom** twice daily demonstrated a significant increase in Dermal Collagen Thickness compared to the Base Lotion after four weeks (Figure 3; Table 8). These results indicate **AC WonderShroom** augments dermal collagen production resulting in a more youthful neck appearance.

With respect to Skin Elasticity, Base Lotion did not elicit any significant alterations, indicating consistent skin elasticity throughout the four-week study (Figure 4; Tables 9, 11). However, Skin Elasticity significantly increased by 6% after four weeks of 2.0% **AC WonderShroom** application, relative to baseline (Figure 4; Tables 9, 11). Compared to Base Lotion after four weeks of twice daily application, 2.0% **AC WonderShroom** demonstrated a significant increase in Skin Elasticity (Figure 4; Table 10). These results indicate **AC WonderShroom** improves skin elasticity, improving the visual impacts of normal aging on the neck.

Taken together, these results indicate **AC WonderShroom** improves the appearance of horizontal wrinkles on the neck in addition to neck Collagen Fiber Density, Dermal Collagen Thickness, and Skin Elasticity when added to personal care applications at recommended use levels. Collectively, **AC WonderShroom** improves visual neck aging by augmenting collagen production, collagen density, and elasticity resulting in a healthier and more youthful neck 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/>