

Tradename: AC ExoEternal

Code: 60200

CAS #: 7732-18-5 & 68333-16-4 (or) 92128-79-5 & 90082-61-4 (or) 68132-21-8 & 123465-35-0

Test Request Form #: 14437

Lot #: 9420611

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

Study Director: *Daniel Shill*

Principal Investigator: *Kayla Goodson*

Test Performed:

Cellular Renewal Study

Introduction

As the largest human organ, the skin's integrity is critical to properly function as a physical barrier and maintenance of a healthy appearance for aesthetics. The epidermis constantly undergoes major self-renewal as the superlayer of cells are lost by desquamation and replaced by cells in the basal layers. The constant replacement of cells mitigates the negative long-term effects of ultraviolet light damage and harmful agents (chemicals, pollutants, etc.) on the skin. Aiding in the processes of cellular renewal can improve the skin's physical appearance as well as function as a protective barrier.

Accordingly, a cellular renewal study was conducted to evaluate the ability of **AC ExoEternal** to accelerate skin cell replacement by assessing changes in pigmentation. Additionally, Retinol and a blend of **AC ExoEternal** and Retinol were tested to elucidate any synergistic effects and to demonstrate the superior nature of bioauthentic exosomes as a delivery system.

Study Principle

Dermal Dye Max™ (active ingredient: dihydroxyacetone (DHA) / glycerone) is applied to the skin and creates artificially high pigmented skin. The controls and test materials are applied to the artificially pigmented areas and pigmentation is measured overtime. The artificially pigmented areas provide a model to assess cellular renewal by measuring pigmentation with decreases in pigmentation representing cellular renewal.

Materials

- A. Equipment:** DermaLab Skin Combo (Skin Color Probe)
- B. Products:** Dermal Dye Max™ (Alpine Valley Naturals); Base Lotion (Cetaphil® Moisturizing Cream for All Skin Types); Retinol (Lot #: 25372061)
- C. Software:** Excel Analysis ToolPak (Microsoft)

Methods

Ten volunteers between the ages of 19 and 32, 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	

Six test sites were identified on the volar forearm of participants. The first test site was identified as the Comparative Skin Site, and no dye nor treatment were applied to this site to demonstrate normal pigmentation readings. Dermal Dye Max™ was applied to the remaining five test sites and allowed to develop for 24 hours prior to baseline readings. After dye development and prior to initial treatment application, baseline DermaLab pigmentation index readings were taken for all six identified sites. The skin test site conditions and treatments are described below (Table 2). The Untreated Dye Control received Dermal Dye Max™ but no treatment to demonstrate normal cellular renewal.

All lotion formulations and the Base Lotion 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. Approximately 0.2 g of each treatment was applied to four 2 cm x 2 cm respective locations on the volar forearm. Pigmentation readings were taken every 24 hours until the active test site returned to baseline. After each daily reading, treatment of each respective test site was performed following the same parameters listed above.

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

Skin Test Site	Condition	Dermal Dye Max™ Application?	Treatment / Test Material Application Description	Lotion pH
1	Comparative Skin Site	No	None	N/A
2	Untreated Dye Control	Yes	None	N/A
3	Base Lotion	Yes	Base Lotion	5.1
4	0.3% Retinol	Yes	0.3% Retinol in Base Lotion	5.3
5	2.0% AC ExoEternal	Yes	2.0% AC ExoEternal in Base Lotion	5.3
6	2.0% AC ExoEternal + 0.1% Retinol	Yes	2.0% AC ExoEternal + 0.1% Retinol in Base Lotion	5.3

The percent change of Pigmentation values was calculated for each test site at every timepoint relative to Baseline values, using the following equation:

$$\text{Percent Change (\%)} = \frac{\text{Pigmentation Index}_{\text{Day}} - \text{Pigmentation Index}_{\text{Baseline}}}{\text{Pigmentation Index}_{\text{Baseline}}} \times 100$$

Cumulative Cellular Renewal, represented by the Pigmentation Index values and relative to the Untreated Dye Control, was calculated as area under the curve (AUC) by using the following equation:

$$AUC = \frac{1}{2} \sum_{i=0}^{n-1} (t_{i+1} - t_i) (\text{Pigmentation Index}_i + \text{Pigmentation Index}_{i+1})$$

For pigmentation measurements $\text{Pigmentation Index}_1$ and $\text{Pigmentation Index}_2$ at times t_1 and t_2 , the AUC between those two time points is equivalent to the product of difference in time and the average of the two Pigmentation measurements. Provided Pigmentation Index values decreased over time AUC was calculated as an inverse and presented as a positive value to demonstrate the amount of cellular relative to the Untreated Dye Control.

Results

The data obtained from this study met criteria for a valid study as the Untreated Dye Control, Base Lotion, Retinol, and 2.0% AC ExoEternal performed as anticipated. Application of 2.0% AC ExoEternal + 0.1% Retinol accelerated cellular renewal as pigmentation values returned to the baseline levels of the Comparative Skin Site after three days.

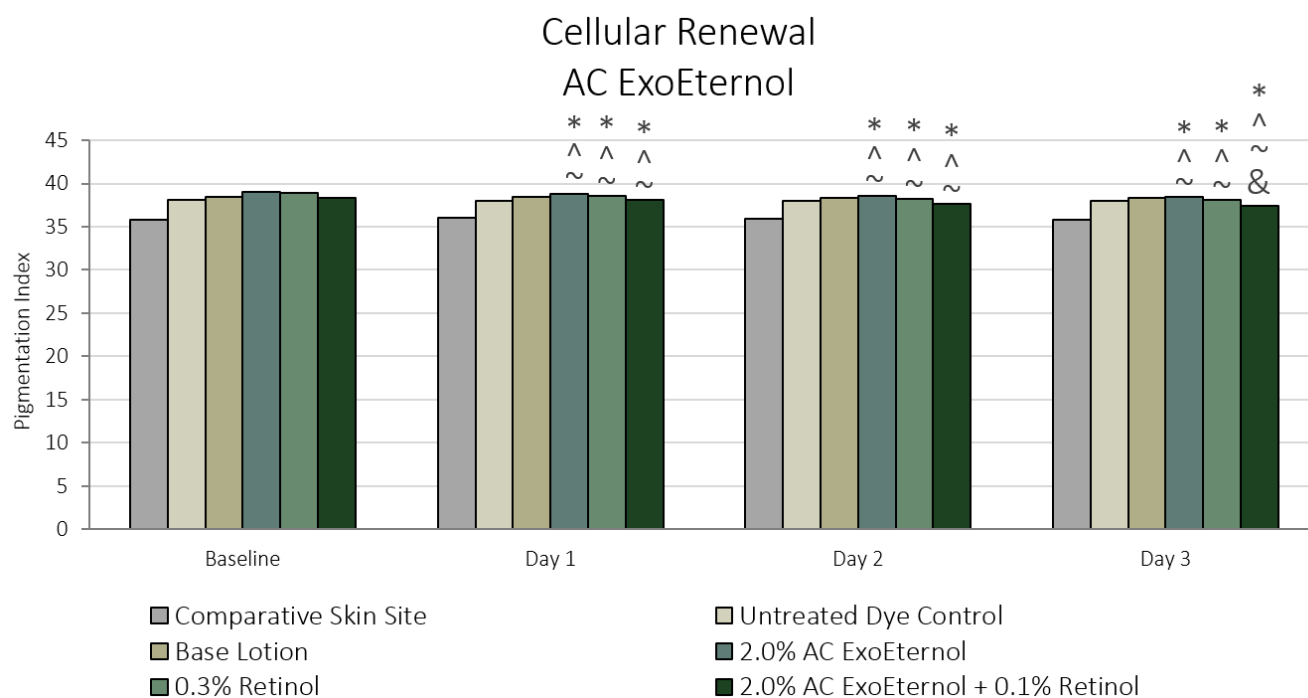


Figure 1. Cellular Renewal of Pigmentation Index Values Overtime. * indicates significance ($p \leq 0.05$) compared to Baseline within the same condition. ^ indicates significance ($p \leq 0.05$) compared to Untreated Dye Control within the same timepoint. ~ indicates significance ($p \leq 0.05$) compared to Base Lotion within the same timepoint. & indicates significance ($p \leq 0.05$) compared to Retinol within the same timepoint.

Table 3. Results from T-test Analyses of Pigmentation Index Values from Baseline to Day Three. * indicates significance ($p \leq 0.05$) compared to Baseline.

	Comparative Skin Site	Untreated Dye Control	Base Lotion	0.3% Retinol	2.0% AC ExoEternal	2.0% AC ExoEternal + 0.1% Retinol
P-value	0.123	0.115	0.102	0.014*	0.001*	< 0.001*

Table 4. Results from T-test Analyses of Pigmentation Index Values After Three Days of Application. ^ indicates significance ($p \leq 0.05$) between conditions.

	Untreated Dye Control vs Base Lotion	Untreated Dye Control vs 0.3% Retinol	Untreated Dye Control vs 2.0% AC ExoEternal	Untreated Dye Control vs 2.0% AC ExoEternal + 0.1% Retinol	Base Lotion vs 0.3% Retinol
P-value	0.142	0.001^	0.006^	0.001^	0.001^
	Base Lotion vs 2.0% AC ExoEternal	Base Lotion vs 2.0% AC ExoEternal + 0.1% Retinol	0.3% Retinol vs 2.0% AC ExoEternal	0.3% Retinol vs 2.0% AC ExoEternal + 0.1% Retinol	2.0% AC ExoEternal vs 2.0% AC ExoEternal + 0.1% Retinol
P-value	0.015^	0.010^	0.215	0.042^	0.287

Change in Pigmentation AC ExoEternal

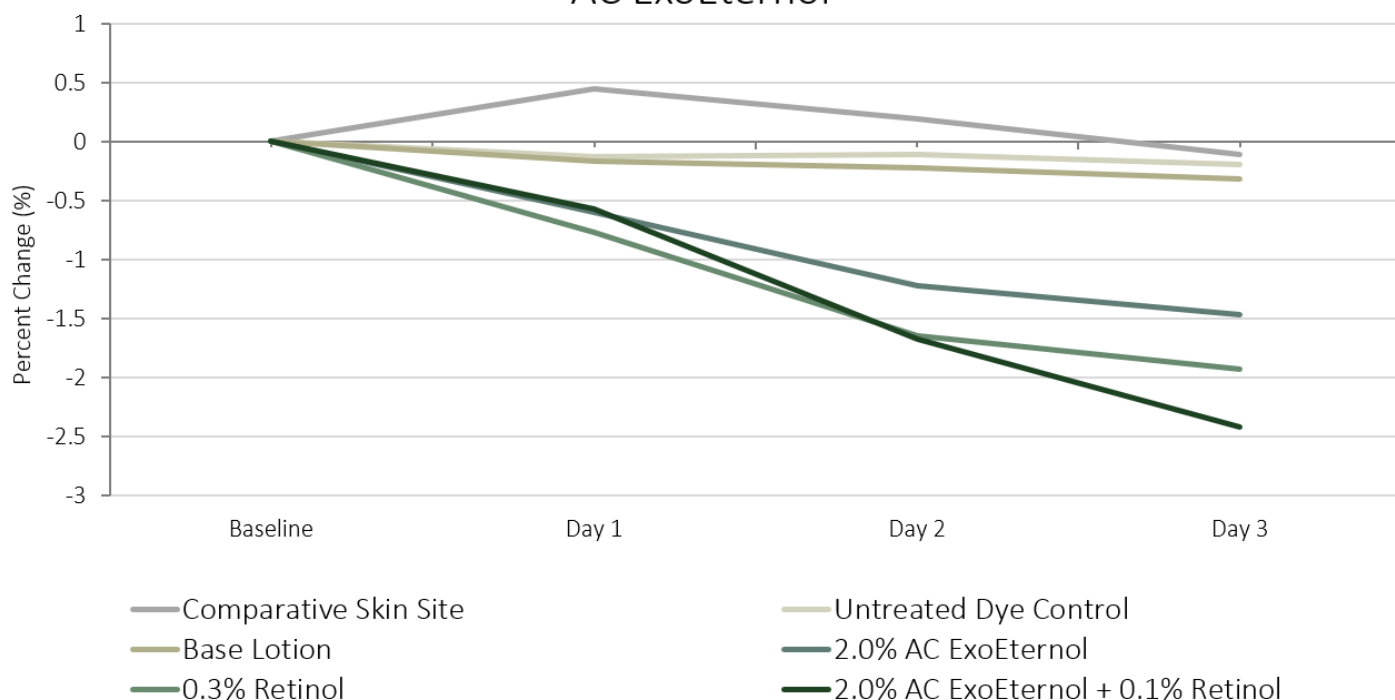


Figure 2. Percent Change in Pigmentation Relative to Baseline Readings

Cumulative Cellular Renewal AC ExoEternal

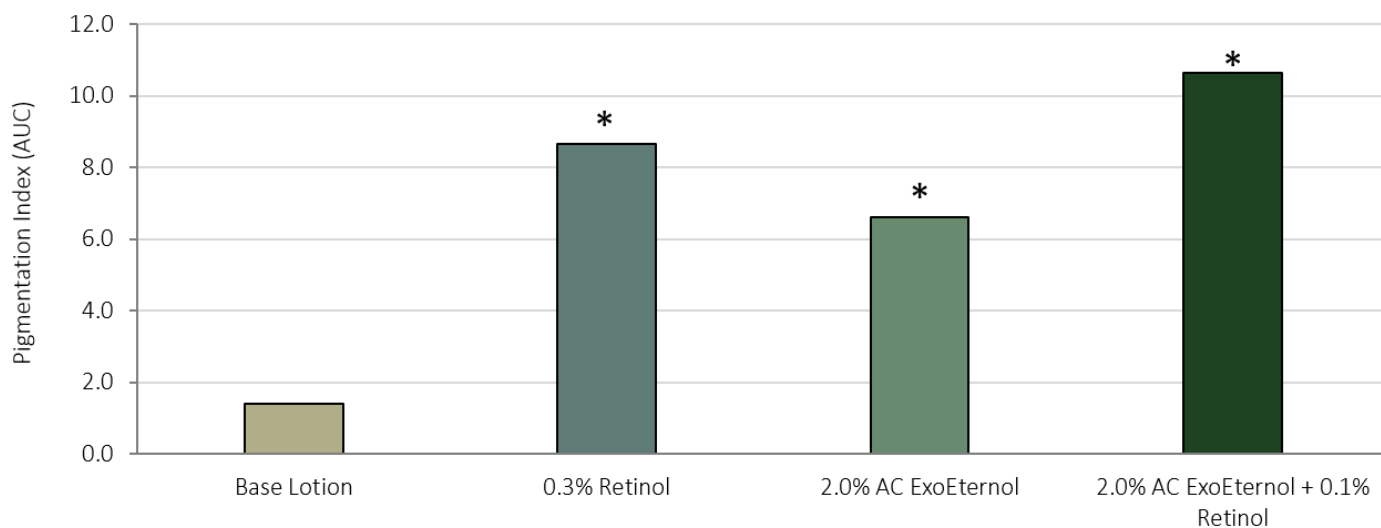


Figure 3. Cumulative Cellular Renewal in Pigmentation Relative to the Untreated Dye Control. * indicates significance ($p \leq 0.05$) between conditions.

Table 5. Results from One-way ANOVA Analysis of Cumulative Cellular Renewal. * indicates significance ($p \leq 0.05$) between conditions.

	Base Lotion vs 0.3% Retinol	Base Lotion vs 2.0% AC ExoEternal	Base Lotion vs 2.0% AC ExoEternal + 0.1% Retinol	0.3% Retinol vs 2.0% AC ExoEternal	0.3% Retinol vs 2.0% AC ExoEternal + 0.1% Retinol	2.0% AC ExoEternal vs 2.0% AC ExoEternal + 0.1% Retinol
P-value	0.029*	0.038*	0.011*	0.189	0.049*	0.042*

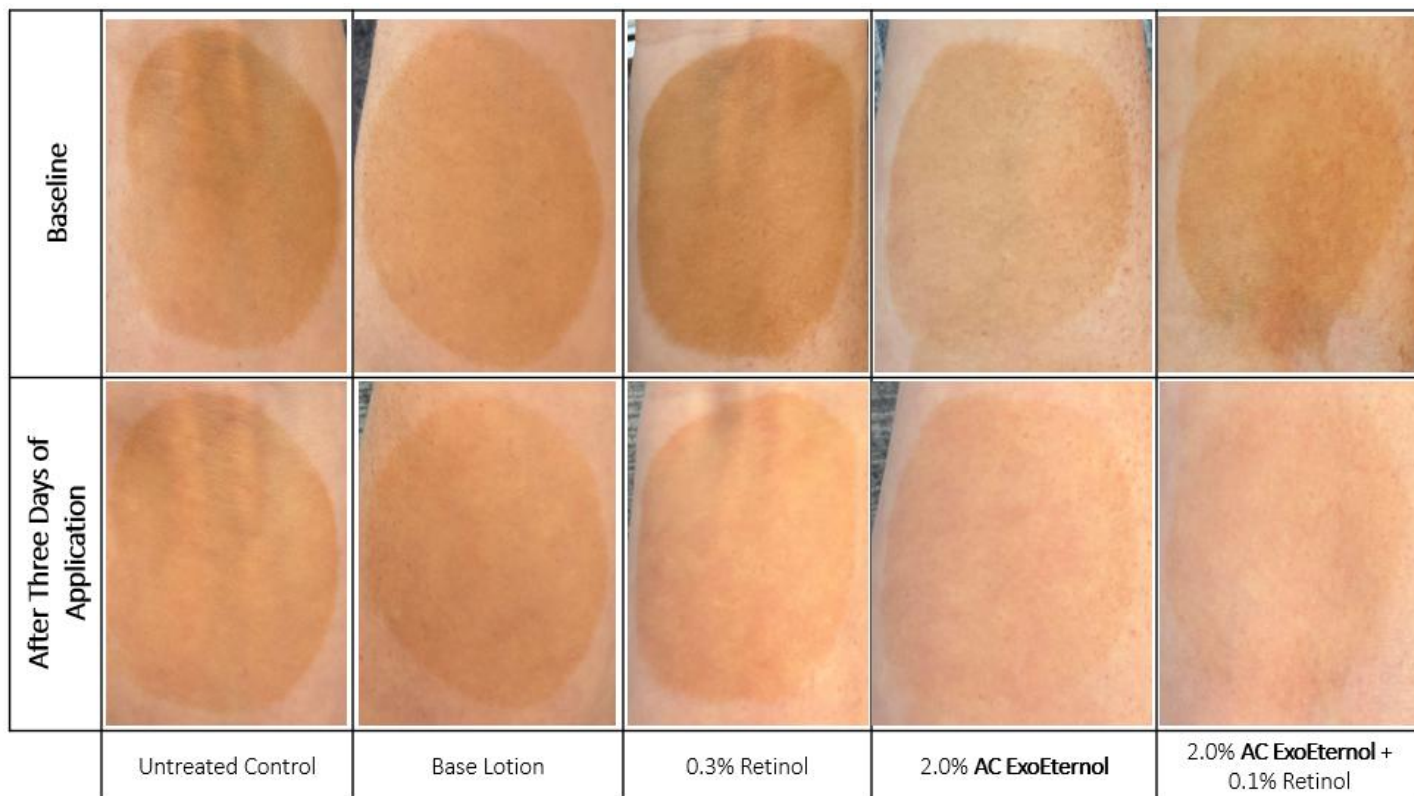


Image 1. Participant Images of Each Test Patch at Baseline (Top) and After Three Days (Bottom)

Discussion

The ability of **AC ExoEternal** to accelerate skin cellular renewal was assessed through changes in pigmentation. As shown in Figure 1 and 2, the Dermal Dye Max™ artificially augmented pigmentation values at baseline measurements. After three days, Untreated Dye Control demonstrated a 0.2% reduction in pigmentation indicating normal cellular renewal (Figures 1, 2; Tables 3, 4). Similarly, the Base Lotion exhibited a 0.3% decrease in pigmentation demonstrating a slight increase in normal cellular renewal (Figures 1, 2; Tables 3, 4). After three days of application, 0.3% Retinol induced a 1.9% decrease in pigmentation as expected (Figures 1, 2; Tables 3, 4). Similarly, 2.0% **AC ExoEternal** and 2.0% **ExoEternal** + 0.1% Retinol elicited a 1.5% and 2.4% reduction in pigmentation, respectively (Figures 1, 2; Tables 3, 4). These results demonstrate the synergistic effect of **AC ExoEternal** + 0.1% Retinol to accelerate cellular renewal.

Additionally, 2.0% **AC ExoEternal** induced a significant change in pigmentation after three days compared to the Untreated Control and Base Lotion (Figures 1, 2; Table 4). However, there was no significant difference in pigmentation between 0.3% Retinol and 2.0% **AC ExoEternal** after three days of application (Figures 1, 2; Table 4). Furthermore, 2.0% **AC ExoEternal** + 0.1% Retinol elicited a significant change in pigmentation after three days compared to 0.3% Retinol alone (Figures 1, 2; Table 4). These results indicate a synergistic effect between 2.0% **AC ExoEternal** + 0.1% Retinol in relation to cellular renewal.

Similar results are shown when the data is displayed to illustrate the collective effect of each treatment when the Untreated Dye Control pigmentation values are accounted for. As shown in Figure 3, 0.3% Retinol and 2.0% **AC ExoEternal** + 0.1% Retinol produced the largest change in cumulative cellular renewal compared to the Base Lotion (Table 5). Examining images of the skin test sites at Baseline and after three days of application, 0.3% Retinol, 2.0% **AC ExoEternal**, and 2.0% **AC ExoEternal** + 0.1% Retinol visually outperformed the Base Lotion in terms of reducing pigmentation (Image 1).

Taken together, these results indicate **AC ExoEternal** enhances cellular renewal when added to personal care applications at recommended use levels. Collectively, **AC ExoEternal** + 0.1% Retinol demonstrated significant synergistic effects by accelerating the processes of cellular renewal which can improve the skin's physical appearance with a healthier and more vibrant skin as well as function as a protective barrier to help reverse the signs of aging.

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/>