

INVESTIGATION INTO THE DELIVERY AND EVALUATION OF THE EFFECT OF AVENAPLEX ON SKIN BARRIER USING LC-MS/MS, RAMAN SPECTROSCOPY, IMMUNOSTAINING ANALYSIS AND SKIN EFFICACY.

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1 INTRODUCTION

The protective and regulatory barrier function of the skin is primarily achieved within the top layer of the epidermis, the stratum corneum. The stratum corneum consisting of 10 to 15 layers of corneocytes and highly organised intercellular lipids, forming water-resistant lamellae that prevent excessive water loss.¹ Close to 20% of the volume of the SC is made up of lipids. These lipids consist of 40% to 50% ceramides, 25% cholesterol and 10% to 15% free fatty acids.

Ceramides in the skin play a crucial role in maintaining the integrity of the stratum corneum, contributing to the skin barrier function and preventing moisture loss.² Plants contain phytoceramides, however the structure of these phytoceramides can differ significantly from those found in human skin. This structural disparity would indicate that plant-derived ceramides may not serve as direct replacements for those depleted in the skin.³

Several alterations in aged skin contribute to a defective skin barrier function. The reduction in production of epidermal lipids is evident in the aged epidermis. During ageing, ceramides become deficient. This reduction compromises the epidermal barrier, leading to a higher trans epidermal water loss, resulting in an increase of skin roughness and dryness.⁴

In this work, **AvenaPlex (Avena sativa (Oat) Kernel Extract)**, a powerful anti-ageing active extracted from oat using polar solvents and comprising 'skin similar' ratios of ceramide, sterol and fatty acids, was analysed. Objectives of this work were to:

1. Identify the oat ceramides in AvenaPlex and to determine its 'skin identical' ceramides.
2. Validate the delivery of AvenaPlex into the skin, to replenish skin ceramides.
3. Demonstrate the visual efficacy of AvenaPlex on the skin.

2 MATERIALS & METHODS

IDENTIFICATION OF OAT CERAMIDES IN AVENAPLEX AND DETERMINATION OF AVENAPLEX 'SKIN IDENTICAL' CERAMIDES

Ceramide Profiling
Total amounts of ceramides: Liquid Chromatography with tandem Mass Spectrometry (LC-MS/MS).
Ceramides classes profiling: Extraction of ceramides in the lower phase of isopropanol/hexane/water (55:20:25 v/v/v) at 60°C. Using LC-MS/MS, four gradients for the separation.

Skin Identical Lipids Increase (ex vivo)

Treatment	Skin explants
Single application of 100% AvenaPlex	Female, Caucasian 59 years old

Skin Identical Lipids Assessment:

The lipid conformation was measured using Raman Spectroscopy. Raman measurement were performed on 4 to 6 points of the stratum corneum, the epidermis and AvenaPlex in the 400-3800 cm⁻¹ range.

VALIDATION OF AVENAPLEX DELIVERY INTO THE SKIN TO REPLENISH SKIN CERAMIDES

Skin Ceramide Increase & Epidermal Barrier Integrity (in vivo/ex vivo)

Product Treatment
Application of 1% AvenaPlex in a serum on one volar forearm
Twice a day (AM/PM)
Over 2 months

Participant Panel
6 Female, Caucasian
43 to 59 years old

Non-invasive Sampling with Lipbarvis® (in vivo):

3 suction blisters of 7 mm per arm were raised thanks to a vacuum pump and removed.

Immunohistochemical Analysis (ex vivo):

Primary anti-Ceramides antibody was applied. Samples were analysed with a Leica fluorescence microscope and measurement of fluorescence intensities was carried out using ImageJ software.

Epidermal Barrier Integrity Analysis with Transmission Electron Microscopy (TEM) (ex vivo):

A homogeneous layer of corneocytes was chosen and prepared. In healthy skin as well as in dry skin, an intercellular space (ICS) is framed by two corneocytes. Within the chosen areas, an ICS and intercellular lipid lamellae (ICLL) were semi-automatically selected and marked. Samples were analysed using TEM.

DEMONSTRATION OF THE VISUAL EFFICACY OF AVENAPLEX ON THE SKIN

Skin Roughness and Wrinkles Reduction (in vivo)

Product Treatment
Application of 1% AvenaPlex in a serum or a placebo serum
Twice a day (AM/PM)
Over 3 months

2 Participant Panels
20 Female, Caucasian
40 to 59 years old

Measurement of Skin Roughness:

Average roughness was evaluated with PRIMOS-CR. Wrinkles (crow's feet area) were assessed by the parameter Ra (µm) which is defined as the average of absolute values of the profile heights in the roughness profile. The lower the value Ra is, the less rough the skin is.

Evaluation of Wrinkles Reduction:

Using PRIMOS-CR, high-resolution photographs were taken.

3 RESULTS & DISCUSSION

IDENTIFICATION OF OAT CERAMIDES IN AVENAPLEX AND DETERMINATION OF AVENAPLEX 'SKIN IDENTICAL' CERAMIDES

Ceramide Profiling

Ceramide Composition*	Percentage
Ceramides	1.4
Hydroxyceramides	
Glycosyl Inositol Phosphoryl Ceramides (Proceramides)	1.3
Glucosylceramide	1.3
Total Ceramides	4.0

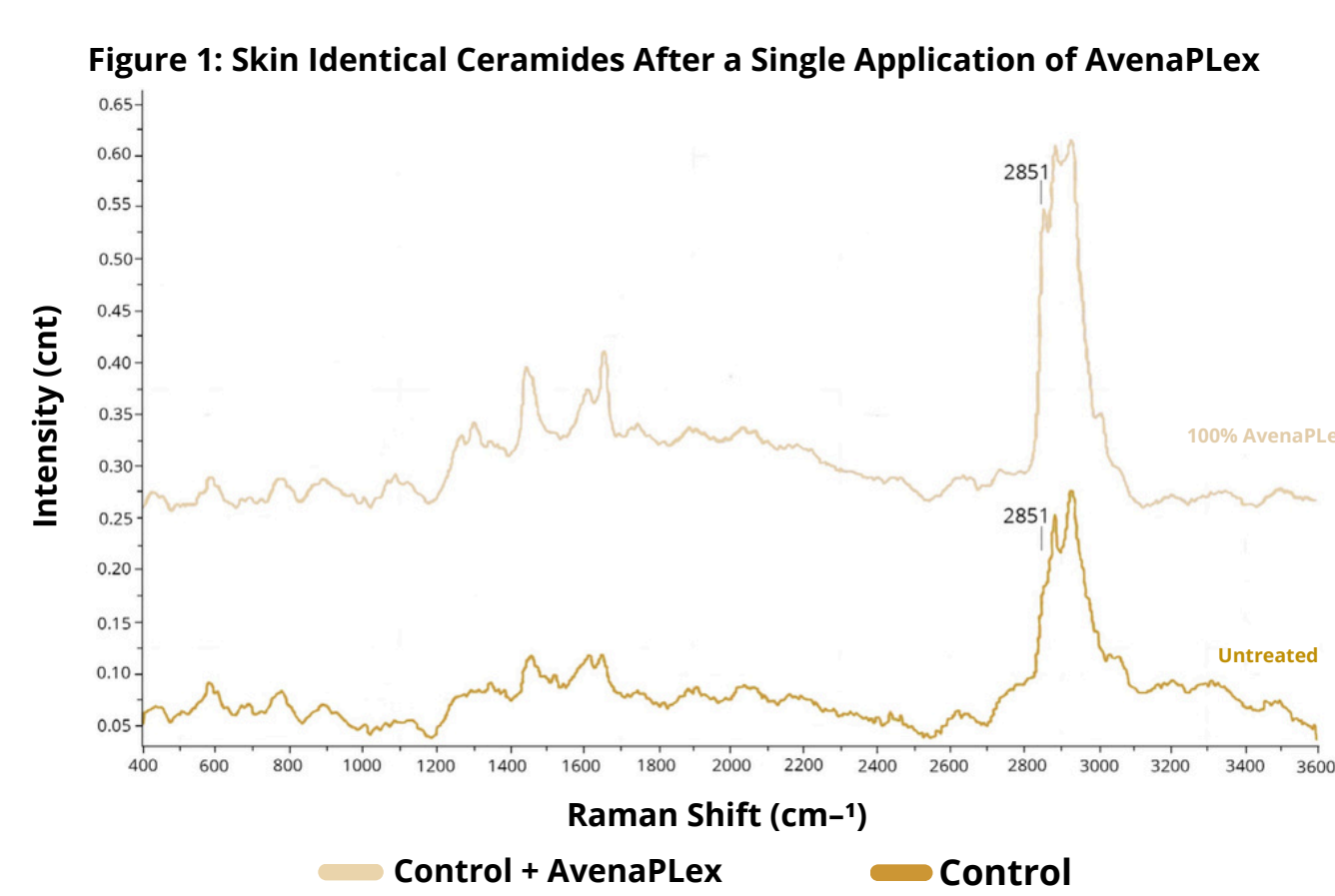
Table 1: Typical ceramide composition present in AvenaPlex.

Ceramide Class*	AvenaPlex Skin Identical (%)	AvenaPlex Total Incl. Isomers (%)
Ceramide 2: Non-hydroxy-sphingosine (NS)	0.07	0.52
Ceramide 3: Non-hydroxy-phytosphingosine (NP)	0.13	0.13
Ceramide 4: Omega-hydroxy-6-hydroxy-sphingosine (EOH)	0.47	0.47
Ceramide 5: Alpha hydroxy sphingosine (AS)	0.05	0.19
Ceramide 6: Alpha hydroxy-phytosphingosine (AP)	0.05	0.05
Total	0.77	1.36

Table 2: Ceramide classes present in AvenaPlex.

AvenaPlex has a typical concentration of 4 g.kg⁻¹ ceramide. 'Skin identical' is defined as compounds which have the same chemical, isotopic and stereochemical structure as those found in human skin. The percentage of each skin identical ceramide was calculated by the average of the line divided per the total of hydroxyceramides and ceramides detected. Then, this result was divided per the total of ceramides present in AvenaPlex. Ceramide class profiling of AvenaPlex revealed that of the ceramide classes identified, skin identical sphingosine and phytosphingosine bases were also present.

Skin Identical Ceramides Increase



Applying AvenaPlex to the skin increases the level of essential ceramides within the skin. The untreated control sample shows only a small peak at 2851 cm⁻¹ relating to ceramides and the explant AvenaPlex shows a significant increase in the ceramide peak at 2851 cm⁻¹.

VALIDATION OF AVENAPLEX DELIVERY INTO THE SKIN TO REPLENISH SKIN CERAMIDES

Skin Ceramide Increase

Figure 2: Ceramide Fluorescence Staining After 2 Months Without and With AvenaPlex

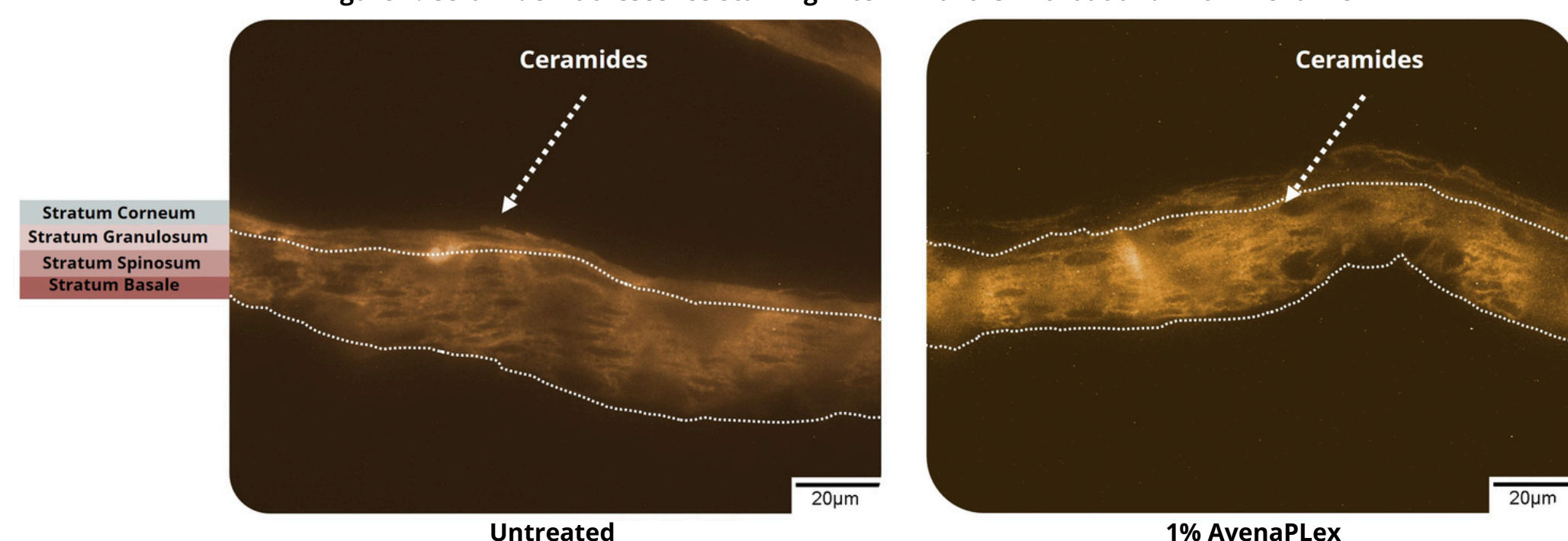


Figure 2 shows the immunofluorescence labelling of the ceramides (yellow). For both untreated and treated, the yellow fluorescence is distributed over the whole epidermis. The intensity of fluorescence with 1% AvenaPlex is significantly higher than the untreated. The ceramide content of the skin with 1% AvenaPlex is statistically significantly (p=0.010) 10% higher, compared to the untreated.

Epidermal Barrier Integrity

Figure 3a: Integrity of The Epidermal Barrier (TEM) Without and With AvenaPlex After 2 Months

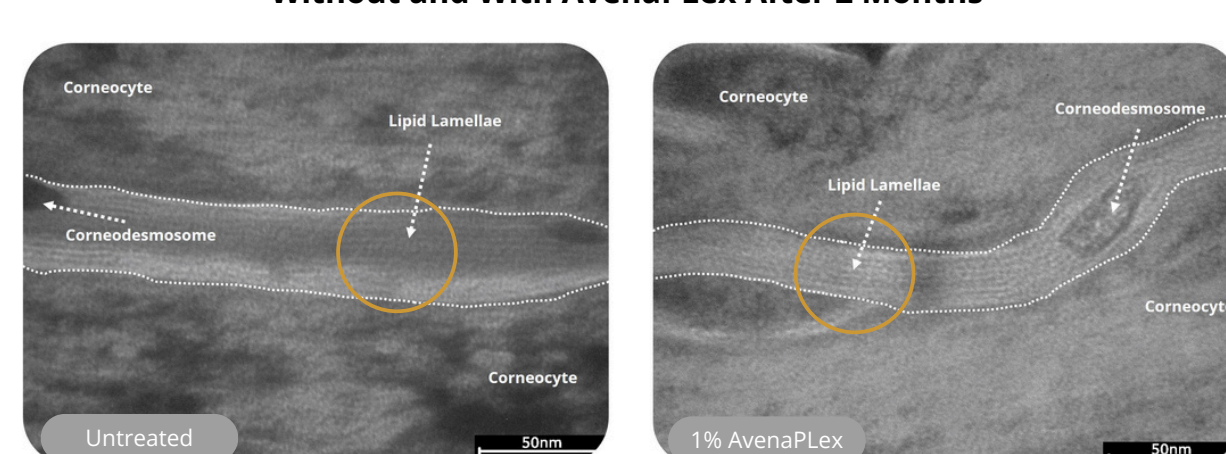


Figure 3b: Visual Interpretation of The Intercellular Spacing

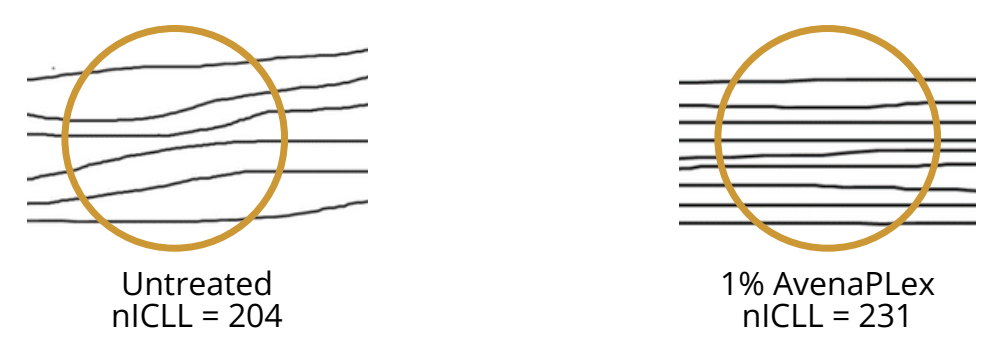
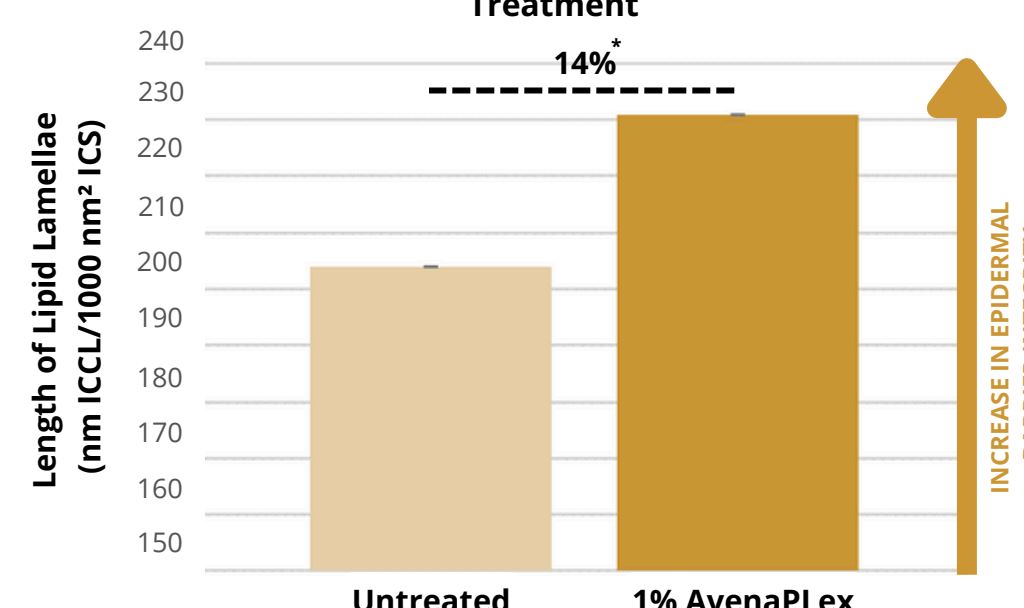


Figure 4: Epidermal Barrier Integrity After 2 Months of Treatment

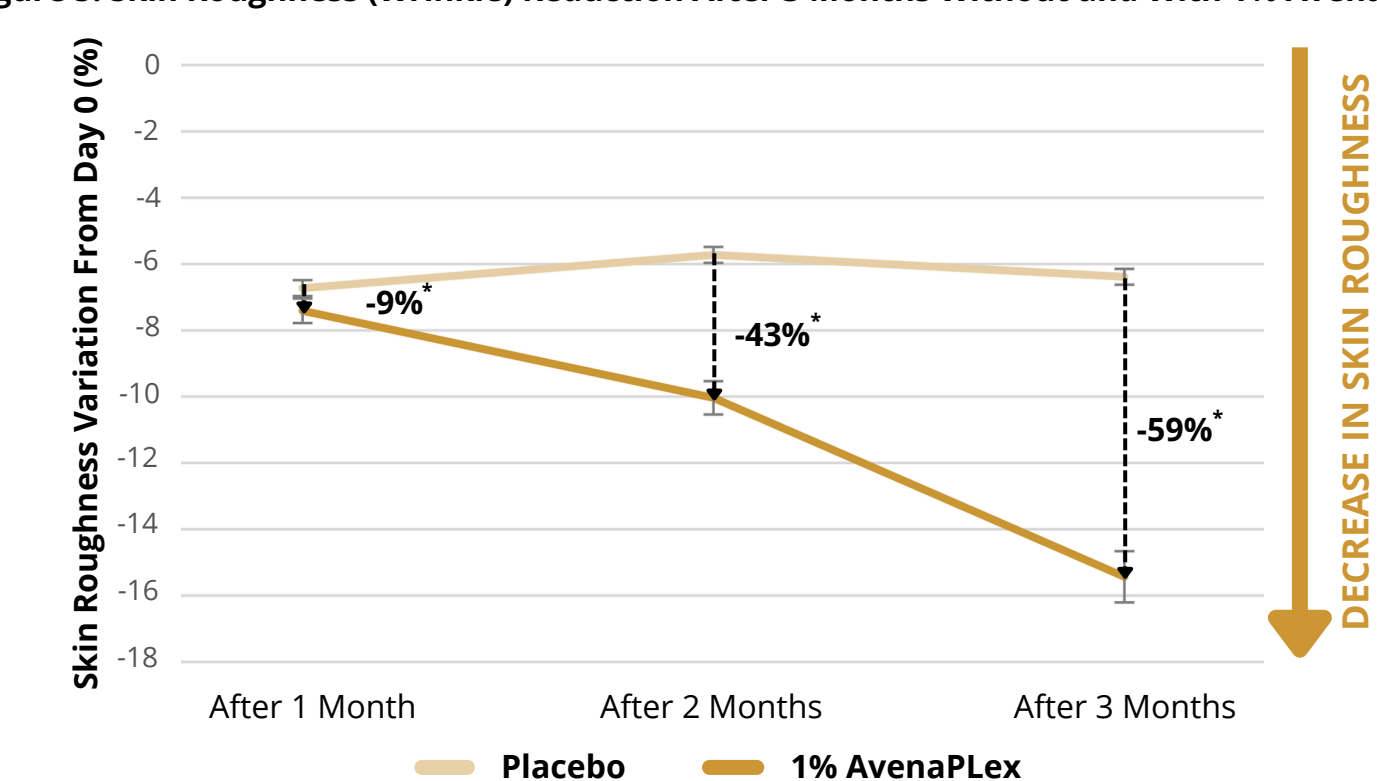


Two months of treatment with 1% AvenaPlex resulted in statistically significantly (p=0.028) higher values of intercellular lipid lamellae, indicating an improved skin barrier function in comparison to the untreated. Specifically, the amount of intercellular lipid lamellae is significantly 14% greater in the skin treated with 1% AvenaPlex compared to the untreated.

DEMONSTRATION OF THE VISUAL EFFICACY OF AVENAPLEX ON THE SKIN

Skin Roughness and Wrinkles Reduction

Figure 5: Skin Roughness (Wrinkle) Reduction After 3 Months Without and With 1% AvenaPlex



A statistically significant (p=0.050) reduction of skin roughness is observed after 3 months of treatment with 1% AvenaPlex, compared to placebo.

Figure 6: Visual Crow's Feet Wrinkles Reduction Without and With 1% AvenaPlex

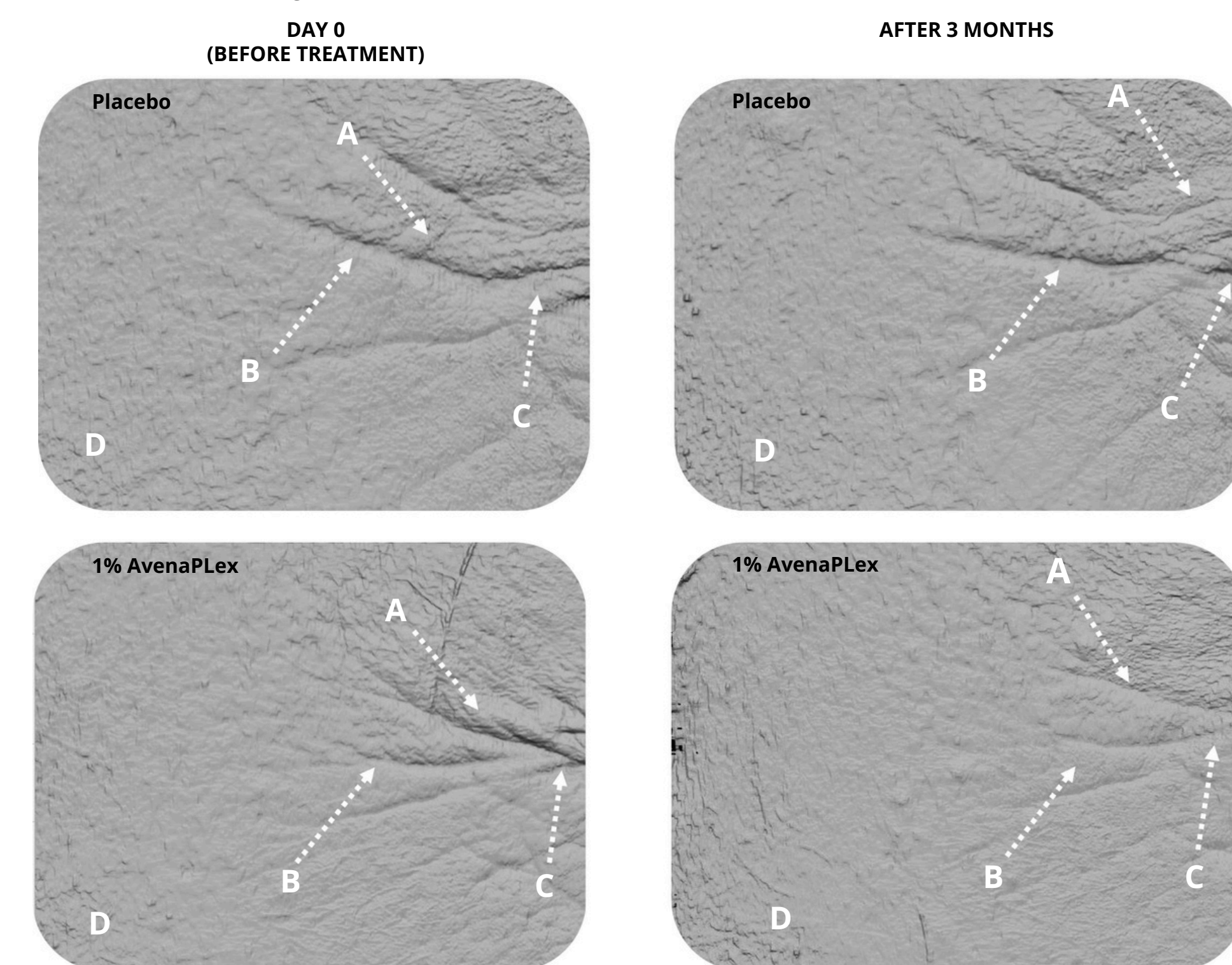


Figure 6 shows that the placebo serum did not reduce the appearance of crow's feet wrinkles, in comparison to day 0. Conversely, after 3 months of treatment with 1% AvenaPlex, the depth and length (A, B, C) of crow's feet wrinkles decreased and the surrounding skin (D) looks smoother.

4 CONCLUSIONS

The outermost layer of the skin, called the stratum corneum, consists of corneocytes that are bound together by mortar-like lipids (mainly ceramides). Ceramides are crucial molecules for the skin barrier function. During ageing, ceramides become deficient, this results in a disruption of skin barrier function. These studies have shown that AvenaPlex contains a significant proportion of 'skin identical' ceramides. AvenaPlex can be effectively delivered into the epidermis and are proven to be a good source of skin replenishing ceramides. The topical application of AvenaPlex results in an increase of the length of the lipid lamellae (in the intercellular space) resulting in an improved quality of the skin barrier. The anti-ageing potential of AvenaPlex was further demonstrated with the improvement in skin texture and wrinkles (crow's feet) reduction.

AvenaPlex is a powerful anti-ageing active, proven to balance the ceramides loss and restore the skin barrier.

Further analysis should be carried on in order to have a more detailed classification of the skin identical ceramides classes.

5 REFERENCES

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