e-ISSN 3050-8460
Journal of Dermatology Research

ATHENAEUM SCIENTIFIC PUBLISHERS

Case Report

Immunohistochemical Variations of Epidermal and Dermal Markers in Human Skin After Cosmetic Treatments: Some Cases Study

Lorenzo Alibardi[1*], Katia Masucci[2]

[1]Comparative Histolab, Padova, Italy

[2]Formamentis SRL, Padova, Italy

***Correspondence author:** Lorenzo Alibardi, Comparative Histolab, Padova, Italy; Email: lorenzo.alibardi@unibo.it

Citation: Alibardi L, et al. Immunohistochemical Variations of Epidermal and Dermal Markers in Human Skin After Cosmetic Treatments: Some Cases Study. J Dermatol Res. 2026;7(1):1-14.

https://doi.org/10.46889/JDR.2026.7106

Received Date: 31-12-2025

Accepted Date: 26-01-2026

Published Date: 02-02-2026

Abstract

Introduction: The present explorative study reports observations on variations in acidic keratin, filaggrin, loricrin, elastin and collagen in human skin after cosmetic treatments.

Objective: Comparison between individuals that customarily utilize cosmetic products ("conditioned") with individuals without cosmetic applications ("non-conditioned") was done. Materials and Methods: Treatments lasted four weeks and the skin from the left forearm was sampled two weeks later. After fixation, embedding in Bioacryl resin and immunofluorescence for the above-indicated markers, comparison between the five subjects was carried out.

Results: Conditioned subjects showed and intense and even distribution of acidic keratin and filaggrin in the granular layer that faded in the corneous layers where corneocytes were regularly desquamating. In contrast, the acidic keratin and filaggrin distribution was lower and discontinued in the two non-conditioned cases that showed a packed stratum corneum before the treatments. Qualitative observations indicated that desquamation, keratin and filaggrin distribution improved after treatment suggesting ameliorated epidermal turnover. No difference was observed for loricrin distribution. Numerous elastic fibrils were detected in conditioned vs non-conditioned cases. No change in the distribution of collagen fibers and number of dermal fibroblasts was observed but conditioned subjects apparently contained more elastic fibrils/fibroblasts that those non-conditioned.

Conclusion: Despite the limited number of subjects, some improvement was detected in the epidermis while the dermis was not affected in the short period of these cosmetic treatments. Longer treatments are likely more effective in producing positive changes in the skin.

Keywords: Human Skin; Histology; Immunofluorescence; Cosmetic Application

Introduction

The human skin is composed from the superficial epidermis of ectodermal origin and from a thicker dermis underneath, derived from the dermo-mesoderm [1-4]. When the human skin is observed superficially using a stereomicroscope, it features folds and lines of variable size and length that form irregular rectangular- or rombic- shaped patterns, indicated as the skin "texture". These lines increase during aging and in people with different lifestyles. Numerous scientific studies have shown that cosmetic treatments lasting 4-16 weeks, using a variety of cosmetic formulations, allow to improve the aspect and the morphological characteristics of the skin, in particular delaying its aging [2-5]. These diversified cosmetic applications are broadly utilized from women but also more and more frequently also from men. As a result of these cosmetic treatments, also the alteration in some key proteins of the epidermis and dermis has been detected.

A number of keratins are synthesized in the epidermis, including some in the upper spinosus and granular layers [6,7]. A recent study has detected a likely homologous K24 in the granular layer of human epidermis, resulting an interesting antigen marker for this epidermal layer [8]. Filaggrin is one of the main keratin-associated proteins of the granular and pre-corneous (transitional) layer and determines the clumping of keratin filaments inside corneocytes and later is degraded to produce a mix of amino acids with pyrrolidone carboxylic acid, lactate, urea, monosaccharides and ions, indicated as NMF (Natural Moisturizing Factor) [1,3,9,10]. NMF maintains soft, moisturized and shiny the epidermis to which it also confers a positive aesthetic aspect. A derived product, urocanoic acid, absorbs UV radiation protecting the skin from their damaging action. Loricrin, a major protein of the granular and corneous layers, is the principal protein of the cell corneous envelope, the 20 nm thick membrane of mature corneocytes that protects the skin from transpiration, chemicals and microbiological exposure [11,12].

The dermis is formed from various connective fibrils, mainly from collagens of different types containing largely predominant collagen type I, about 90% of collagens and from elastic fibrils [2-4]. Collagen determines the mechanical resistance of the skin to traction and compression while its degradation by fragmentation and dehydration during aging is the main cause of skin aging. Elastin is the main component of elastic fibrils that determine skin elasticity and keeps its smooth aspect after deforming or stretching. This protein opposes to the formation of wrinkles on the skin during mechanical stress and aging. Its amount and fibrils orientation, together fibrillin, is essential for keeping skin elasticity while its reduction and irregular distribution determines skin deterioration and accelerates skin aging.

From the above information, here we present an explorative study on the microscopic changes in the skin observed in some women after short cosmetic treatments using the procedure adopted from Formamentis SRL, a small Cosmetic Firm operating in the Venetian region of Italy. The microscopic study evaluated the general histology of the skin and the presence of some of the above antigen markers of the epidermis (keratin, filaggrin and loricrin) and of the dermis (elastin and collagen) before and after the cosmetic application.

Materials and Methods

Experimental Protocol

The study was conducted on five women, with variable ages, 31, 48, 48, 58 and 59 years old, here indicated as "subjects". The subjects voluntarily underwent to the treatments according to the Method Formamentis SRL and their skin was collected through biopsy sampling. Three subjects, indicated as "conditioned", age 48, 48 and 58 years, were customarily using the cosmetic applications while two subjects, here indicated as "un-conditioned", age 31 and 59 years, never used these or other cosmetic products. The three conditioned subjects mainly served as comparison with those un-conditioned before and after the cosmetic treatments. The skin from about midway in the left forearm was biopsied in the three conditioned subjects, while the skin of the left forearm of the two un-conditioned subjects was sampled before and after the treatment.

The treatment consisted in four weakly sessions in which the two un-conditioned subjects underwent to 70 minutes applications of a mixture product indicated as "01 Torba 70%" over the entire body while the face instead received a coating with the mixture product indicated as "01 Torba 100%" (code: UN01A, Producer Emmedue Cosmetics, Casale Monferrato, Alessandria, Italy). As indicated in the datasheet of the Producer, Torba contains heelmoor clay mud as the active principle and is generally utilized in hydrothermal cutaneous treatments. After the single "Torba treatment" of the week, the un-conditioned subjects were also using, almost daily, a series of cosmetic products on the face and arms. The latter consisted in a mixture of commercially available products (Emmedue Cosmetics, Casale Monferrato, Alessandria, Italy): 1) tonic dermobiota detergent (Unique, code UN-09, containing jojoba seed oil, argania spinosa kernel oil, aloe barbadensis juice, ficus-indica extract, saccharide isomerate, Lactococcus ferment lysate); 2) hydrating cream plus (Unique, code UN-13A, containing olive oil, phospholipids, sodium hyaluronate and ceramids, carnosin); 3) anti-oxidant cream (Unique, code UN-18NEW, containing borago seed officinalis oil, propoli extract, ribes fruit extract, copper and zinc gluconate); 4) nutrient cream (Unique, code UN-14A, containing apricoat kernel oil, phospholipids, ceramides and phytosphingosine). At the end of the four weeks of treatments, the two un-conditioned subjects interrupted the treatments and biopsies were collected 2 weeks later, for a total of six weeks of experimentation.

Sampling and Tissue Preparation

From the chosen skin area of the forearm, a small biopsy of 3 by 3 by 2 mm, was collected in all the five subjects. In the two un-conditioned subjects after 6 weeks from the initial sampling another biopsy was collected in the forearm, at a distance of about

3 cm from the initial biopsy. The distance of the second from the first biopsy was decided in order to avoid the use of wounding skin derived from the initial sampling.

The sampled tissues were immediately fix in 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4 for about 8 hours at 4°C, dehydrated in ethanol and embedded in the metacrylate Resin Bioacryl, adapted for histology and immunohistochemistry [13]. After sectioning using an ultramicrotome with special glass blades, sections of 1-5μm in thickness were collected on gelatin-chromoalume coated microscope slides and dried for 2-3 hour on a hot plate at 40-45°C.

Microscopic Methods

Some sections served for the histological study after staining with 0.5% toluidine blue or with Giemsa stain. Other sections instead underwent immunohistochemical detection of epidermal or dermal markers. The following proteins (antigen) were detected using antibodies produced in rabbits: an acidic keratin previously studied in the granular layer using the AK2 antibody [8], human filaggrin using the antibody 8959 [9], human loricrin using the SAB2108557 antibody (Sigma-Merck). For the dermis, a mouse antibody against elastin (E4013, Sigma-Merck) was utilized. For the detection of collagen, aside the staining and fibers aspect using toluidine blue or Giemsa, the use of the blue fluorophore DAPI was utilized for a panoramic view of the dermis. The latter die produces a brilliant nuclear blue fluorescence but also stains with minor intensity connective fibrils, in particular of collage (see later description).

Briefly, after an overnight application of the primary antibodies at 4°C with a dilution 1:70 of the primary antibodies against the above antigens (omitting primary abs in controls), sections were rinsed in phosphate buffer and incubated for 70 minutes with the secondary anti-rabbit (for keratin, filaggrin and loricrin detection) or anti-mouse (for elastin detection) IgG antibodies conjugated with TRIT (red fluorescence, dilution 1:150 v:v). Nuclei were counterstained with DAPI (blue fluorescence, 1:1000 dilution v:v) for 10 minutes in the dark. The sections were studied using a epifluorescence microscope equipped with filters for TRITC and DAPI detection and images were acquired by a digital camera and they were input in a computer. Selected figure plates were composed in plates by using Photoshop 8.0 program.

Results

Histology

Both non-conditioned subjects, after the treatment reported to feel their skin smoother, silkier, more elastic and shiner than before the treatment. The epidermis of conditioned subjects, 48, 48 and 58 years of age, showed a 30-40μm thick epidermis with numerous epidermal papillae (Fig. 1). Collagen fibers in the dermis appeared thicker (0.5-1,5μm), long and less undulated in comparison to the smaller and more undulated elastic fibrils of 0.1-0.5μm (Fig. 1). Collagen fibrils however remained relatively thin and separated with some anastomosis so that individual fibrils were often not distinguishable one from another. The epidermis was composed of 3-4 suprabasal spinosus layers, 2-3 granular layers made of thin spindle-shaped cells and very thin corneocytes were distinguishable, stratified to form a 4-8μm thick stratum corneum with superficial desquamation.

In comparison, the non-conditioned subjects before the treatment featured a compact and pale stratum corneum with corneocytes often not distinguishable (Fig. 2). In the younger subject (31 years old), large collagen fibers of 3-4μm in diameter and frequently anastomosed were present in the lower dermal layer (arrows in Fig. 2). After the treatment, the epidermis of this subject showed areas of the corneous layer with distinct corneocytes, also flacking off the surface while the dermis remained in general similar to the pre-treatment aspect (Fig. 2). In the older, non-conditioned subject (59 years old) before the treatment, the corneous layer was also pale and compact with poor distinction of the corneocytes (Fig. 2). Generally thinner collagen fibers were seen in the dermis and few fibers were thicker than 1μm and apparently less numerous than in the younger subject (arrows in Fig. 2). After the treatment, under histology this subject evidenced a stratum corneum with visible corneocytes along many areas of the epidermis and also desquamating on surface (Fig. 2). Furthermore, in numerous dermal regions more fibrils and in particular thicker collagen fibers were present, sometimes even over 2μm in diameter (Fig. 2).

Immunofluorescence for the Epidermis

The epidermis of the three conditioned subjects showed a continuous and intensely immunofluorescent, 1-3 stratified granular layer for the acidic granular keratin, AK2 positive (Fig. 3). A very weak staining for keratin was seen in upper spinosus layers, while negative controls were unlabeled (Fig. 3). The epidermis of both un-conditioned subjects before the treatment showed an

un-even and weaker positive staining in the granular layer in comparison to conditioned subjects (Fig. 3). Also, the granular layer appeared discontinuous while the corneous and upper spinosus layers were very weakly immunolabeled or completely unlabeled. After the treatment, in both subjects the granular layer appeared continuous over most of the surface and also the stratum corneous showed some immunofluorescence (Fig. 3).

Immunofluorescence for filaggrin was sharp and intense in a continuous granular layer, like for the acidic keratin in conditioned subjects (Fig. 4). In the two non-conditioned subjects before the treatment, the immunolabeling was instead generally discontinuous and weaker than in the conditioned subjects (Fig. 4). After the treatment both younger and older subjects evidenced a more continuous and intense immunofluorescent granular layer (Fig. 4). Control sections did not show any labeling (Fig. 4).

The immunofluorescence for loricrin instead appeared similar in the conditioned and non-conditioned individuals and it was prevalently located in the stratum granular, pre-corneum and corneum, despite the different ages (Fig. 5). In the conditioned subjects, the corneous layer varied its thickness depending from the plane of sectioning and was more intensely labeled than the granular layer (Fig. 5). In the two non-conditioned subjects, before and after treatments, loricrin immunolabeling was observed in both variably thick corneous and granular layer (depending from the plane of sectioning) and was often more intense in some regions of the granular layer (arrows in Fig. 5). Control sections resulted immunonegatives (Fig. 5).

Immunofluorescence for the Dermis

In random planes of sectioning, numerous elastin-positive short fibrils were seen in the softer superficial dermis of the three conditioned subjects after immunostaining with the E4013 antibody (Fig. 6). Also, in the two non-conditioned subjects before the treatment numerous elastic fibrils were immunodetected (Fig. 6). The qualitative observations indicated that generally shorter and less numerous elastic fibrils were seen in some areas of non-conditioned subjects in pre-treatment in comparison to the conditioned subjects, but in other areas of the dermis this was not evident. In the non-conditioned subjects, after the treatment, numerous and longer fibrils were, however, detected in some areas (Fig. 7), suggesting increase in comparison to the pre-treatment, but in other areas this was not evident. Control sections were not immunolabeled (Fig. 7).

The large dermal fibers were evident after DAPI staining, generally beginning from the boundary between the loose and deep dermal layers, at 30-50μm from the epidermis, depending also from the level of sectioning aside local differences (Fig. 8,9). As previously indicated, collagen fibers varied in thickness (0.5-3μm) increasing their size in the deep dermis, waving and were often apparently anastomosed in histological sections, preventing their counting as separate elements. In the three conditioned subjects, collagen fibers were numerous and often crossed, forming an irregular meshwork (Fig. 8). In contrast, the fiber pattern varied between the two non-conditioned subjects. In the younger, as previously noticed under histology (Fig. 2), in pre-treatment the fibers were numerous, long and thick (1-3μm) and generally anastomosed (Fig. 8). No true difference was evident also in post-treatment in this subject (Fig. 8).

In the oldest subject, collagen fibers were less numerous and thinner (0.5-1.5μm, infrequently thicker even in the deep dermis) as observed under histology in pre-treatment (Fig. 2, 8, 9). After the treatment, in various regions of the dermis, the fibers appeared increased in number, thickness in the deep dermis (even thicker than 2.5μm) and were also more regularly oriented, in parallel with respect the surface (Fig. 9). However, the conclusive general impression was for no significative change occurred in the dermis of the two subjects after treatment.

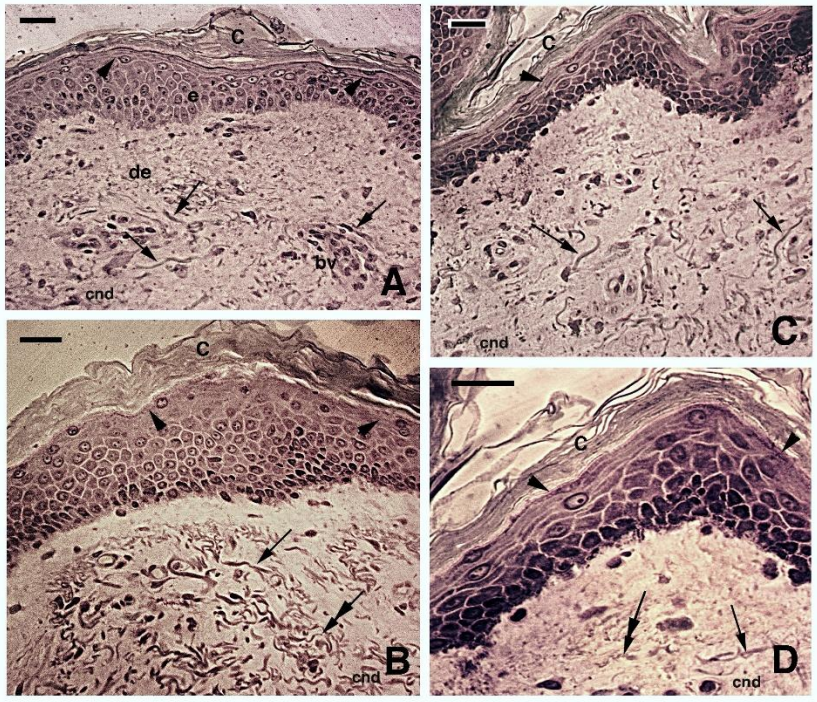

Figure 1: Histology of the skin of conditioned subject (toluidine blue stain). A, thin connective fibrils (arrows) are seen in the loose dermis of 48 years old subject. A thin granular layer (arrowhead) is present underneath the narrow stratum corneum mad of stratified corneocytes. Bar, 10μm. B, numerous connective fibers, thicker of collagen (arrow) and thinner of elastin (double arrow) are seen in this section from a 58 years old individual. Also, a thin granular layer (arrowhead) is present underneath the corneous layer. Bar, 10μm. C, section from another 48 years old subject, showing sparse collagen fibers (arrows), thicker granular layer (arrowhead) and stratified corneoum layer. Bar, 10μm. D, detail of previous image showing 2-3 layers of granular cells. The arrow indicates a collagen fiber while the double arrow points an elastic fibril. Bar, 20μm. Legends: bv, blood vessel; c, corneum layer; cnd, conditioned subject; d, dermis; e, epidermis.

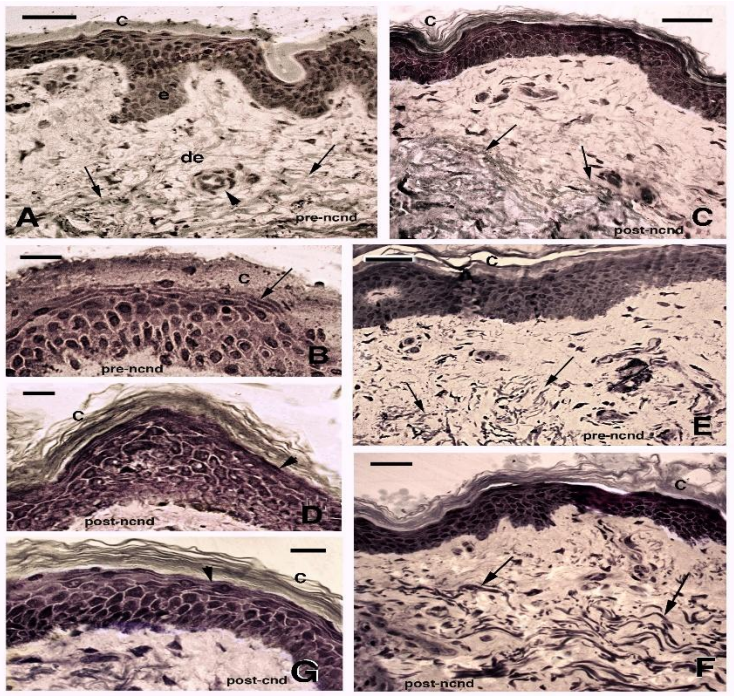

Figure 2: Histology of non-conditioned subjects before (A, B, E) and after (C,D, F, G) treatments (toluidine blue stain). A, in the 31 years old subject before treatment, the papillated epidermis feature a compacted corneous layer and numerous thick collagen fibers (arrows) are present in the dermis. The arrowhead indicates a gland duct. Bar, 25μm. B, detail of the compact

corneous layer with 2-3 layers of granular cells in the epidermis of the young subject. Bar, 10μm. C, after treatments the epidermis of the young subject shows numerous collagen fibrils and a flaking corneous layer with distinct corneocytes. Bar, 30μm. D, young subject after the treatment, featuring a corneous layer with separated corneocytes in some regions. The arrowhead indicates the granular layer. Bar, 10μm. E, older subject (59 years old) in pre-treatment that shows a compact pale corneous layer (artifactually split) with thin connective fibrils (arrows). Bar, 25μm. F, treated older subject featuring visible corneocytes in some areas of the corneous layer and large collagen fibrils (arrows) in the dermis. Bar, 30μm. G, older subject after the treatment, with 2-3 layers of granular cells (arrowhead) and evident corneocytes in the corneous layer. Bar, 10μm. Legends: c, corneous layer; de, dermis; e, epidermis; pre-ncnd, post-ncnd, post-conditioned; pre-conditioned.

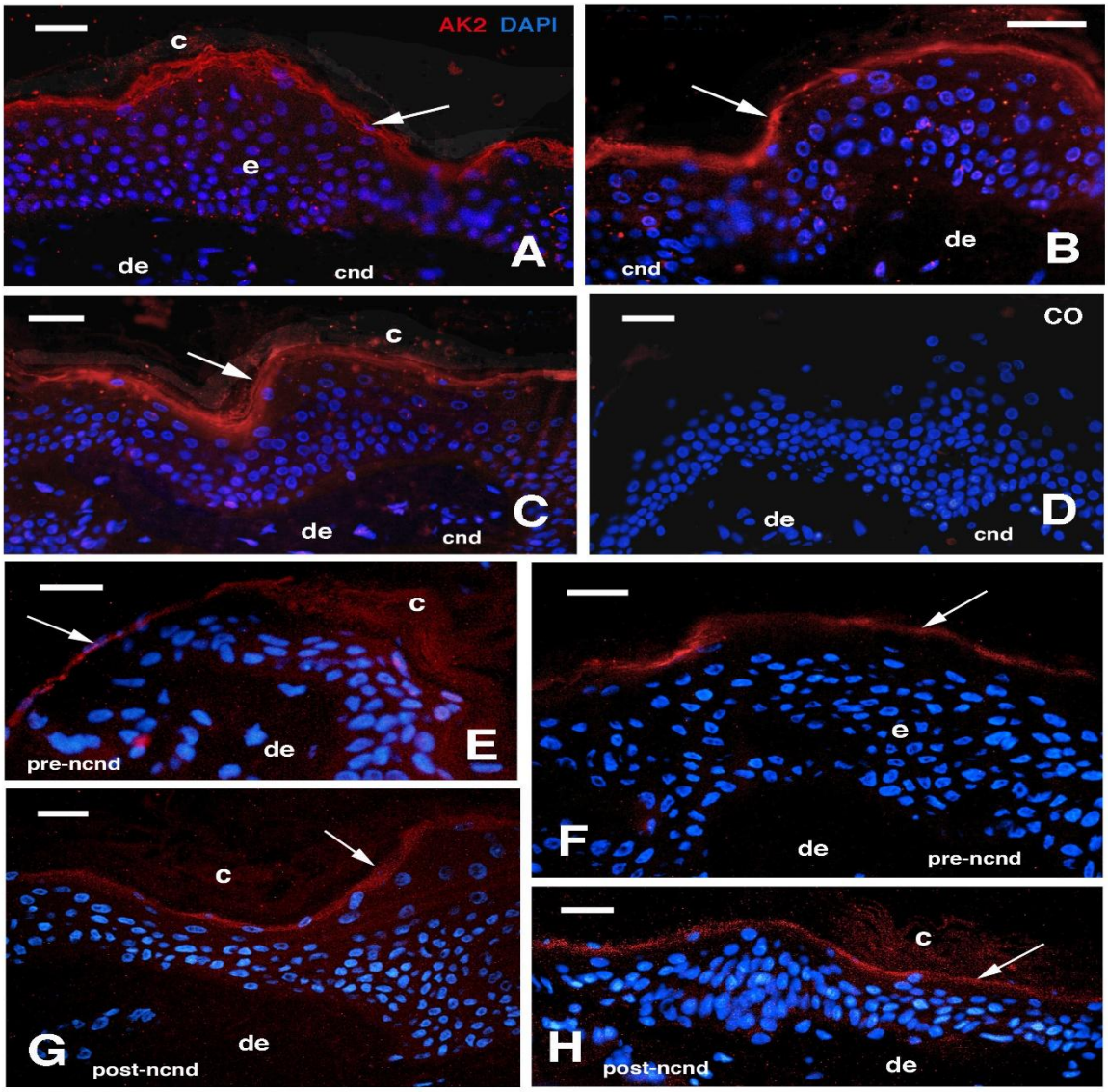

Figure 3: Immunofluorescence for acidic keratin (AK2 positive). In the epidermis of conditioned (A-D) and non-conditioned (E-H) subjects. A, 48 years old subject with continuous immunolabeled granular layer (arrow). Bar, 15μm. B, other 48 years old subject with labeled granular layer (arrow). Bar, 15μm. C, 58 years old subject with a continuous immunofluorescent granular layer (arrow). Bar, 15.μm. D, immunonegative control section. Bar, 15μm. E, younger subject before treatment showing a weak immunofluorescence in the granular (arrow) and corneous layer. Bar, 15μm. F, older subject before treatment with interrupted labeling in the stratum granulosum (arrow). Bar, 15μm. G, young subject that evidences a well labeled and continuous granular layer and weak immunofluorescent corneous layer (desquamating). Bar, 15μm. H, continuous immunolabeled granular layer (arrow) in older subject after treatment. Also the corneous layer appear weakly labeled. Bar, 15μm. Legends: c, corneous layer; cnd, conditioned; CO, control; de, dermis; e, epidermis; post-ncnd, post-treatment non-conditioned; pre-ncnd, pre-treatment non-conditioned.

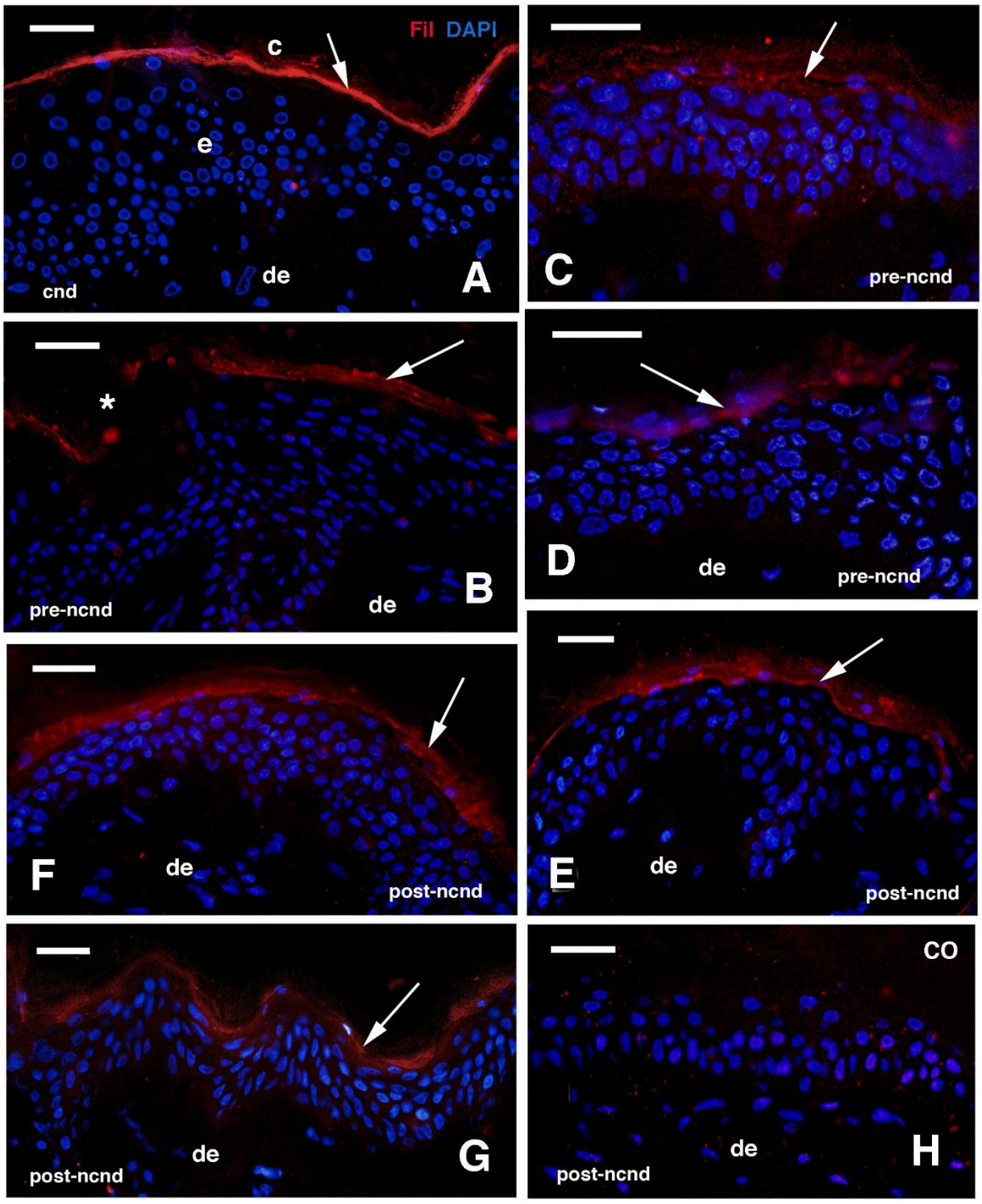

Figure 4: Immunolocalization of filaggrin in conditioned epidermis (A) and non-conditioned (B-H) epidermis. A, epidermis of a 48 years old subject with intensely labeled granular layer (arrow). Bar, 15µm. B, non-conditioned oldest subject before treatment, showing a discontinuous (asterisk) immunolabeled granular layer (arrow). Bar, 15µm. C, young non-conditioned subject before treatment with weakly immunofluorescent granular layer (arrow). Bar, 15µm. D, other area of the epidermis of young non-conditioned subject in pre-treatment evidencing low and discontinuous labeling in the granular layer (arrow). Bar, 15µm. E, non-conditioned young subject after treatment featuring an almost continuous and more intensely labeled granular and transitional layers (arrow). Bar, 15µm. F, oldest non-conditioned subject after treatment with well labeled granular-transitional layer (arrow). Bar, 15µm. G, old non-conditioned subject after treatment with continuous immunofluorescent granular layer (arrow). Bar, 15µm. H, immunonegative control section. Bar, 15µm. Legends: c, corneous layer; CO, control; de, dermis; e, epidermis; post-ncnd, post-treatment not-conditioned; pre-ncnd, pre-treatment nonconditioned.

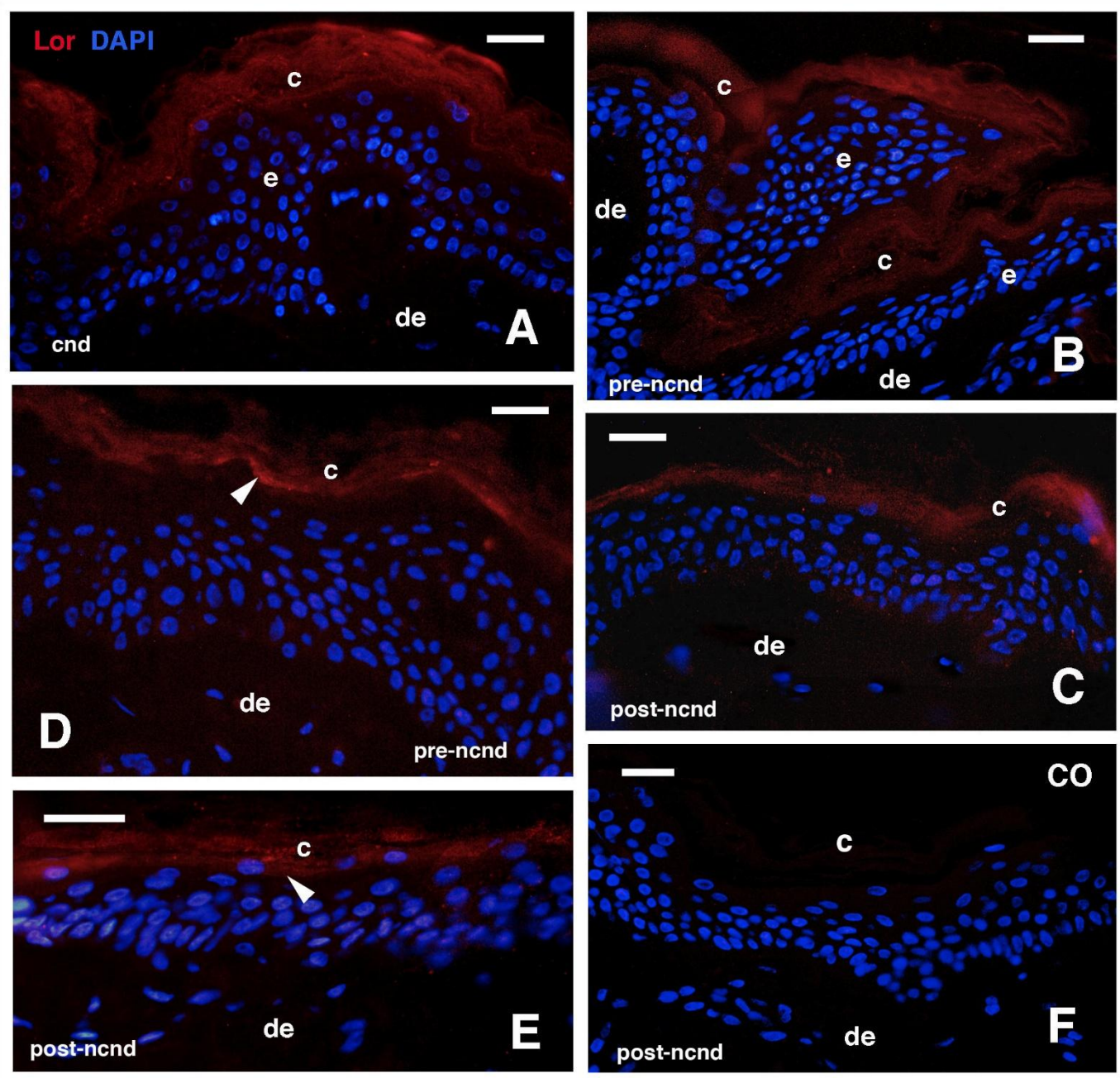

Figure 5: Immunolocalization of loricrin in conditioned (A) and non-conditioned (B-F) subjects. A, labeled thick corneous layer of 58 years old subject. Bar, 15µm. B, folded skin with immunofluorescent corneous layer in younger non-conditioned subjects before treatment. Bar, 15µm. C, other skin region of younger non-conditioned subject before treatment. Bar, 15µm. D, skin of oldest non-conditioned subject before treatment (the arrowhead indicates the granular layer). Bar, 15µm. E, other section from the oldest subjects with labeled granular layer (arrowhead). Bar, 15µm. F, immunonegative control section. Bar, 15µm. Legends: c, corneous layer; cnd, conditioned; CO, control; de, dermis; e, epidermis; pre-ncnd, pre-non conditioned subject (before treatment); post-ncnd, post-non conditioned subject (after treatment).

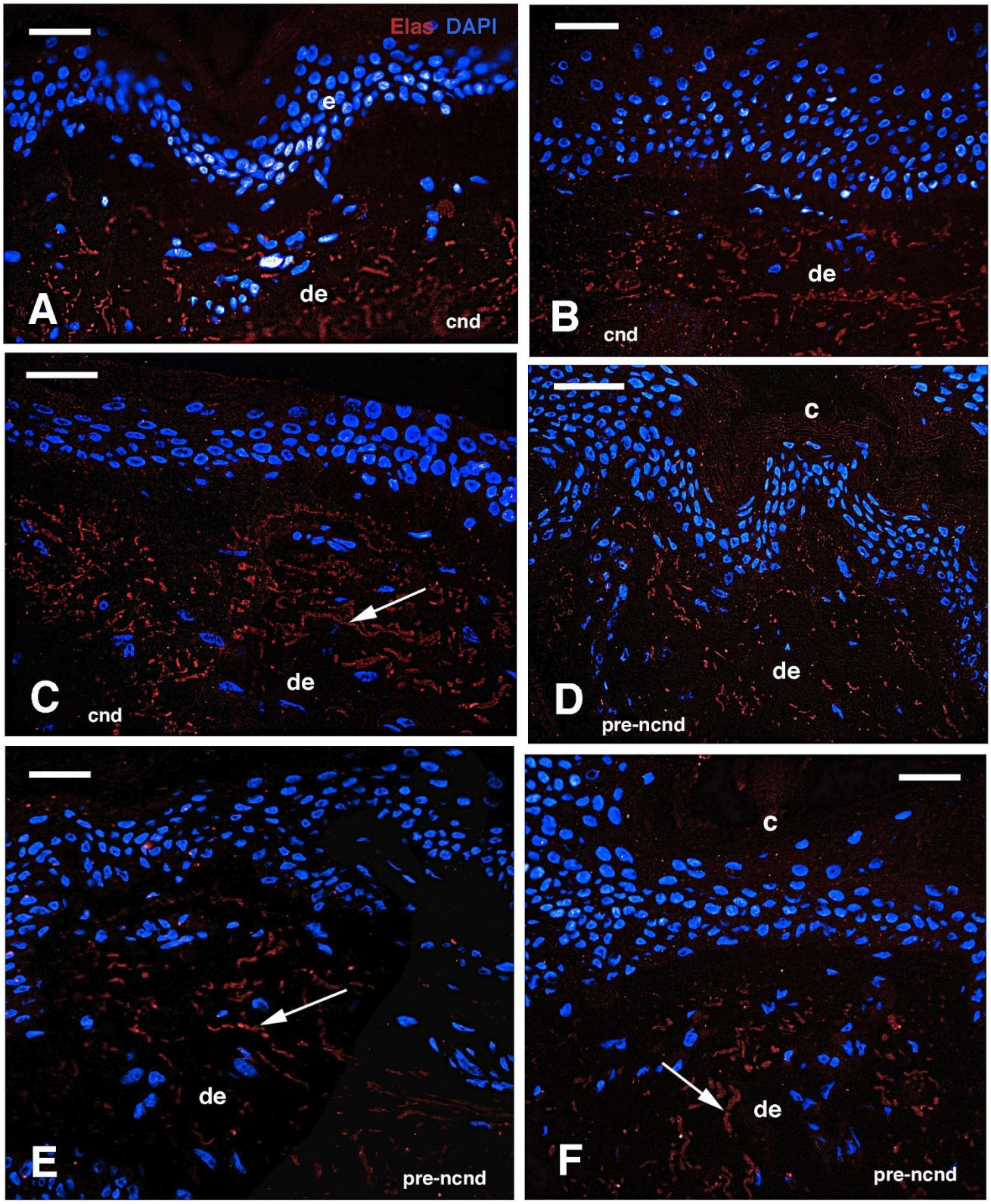

Figure 6: Immunofluorescence for elastin in the dermis of conditioned (A-C) and non-conditioned (D-F) subjects. A, numerous elastic fibrils are observed in the dermis of the 58 years old subject. Bar, 25µm. B, a 48 years old subject shows numerous dermal elastic fibrils. Bar, 25µm. C, another 48 years old subject featuring numerous elastic fibrils, some also long (arrow). Bar, 25µm. D, sparse elastic fibrils in the dermis of the non-conditioned young subject before treatment. Bar, 25µm. E, oldest non-conditioned subject before treatments, evidencing few elastic fibrils (arrow) in this area of the dermis. Bar, 25µm. F, another area of the dermis in the oldest subject before treatment with sparse elastic fibrils (arrow). Bar, 25µm. Legends: c, corneous layer; cnd, conditioned; de, dermis; e, epidermis; pre-ncnd, pre-conditioned.

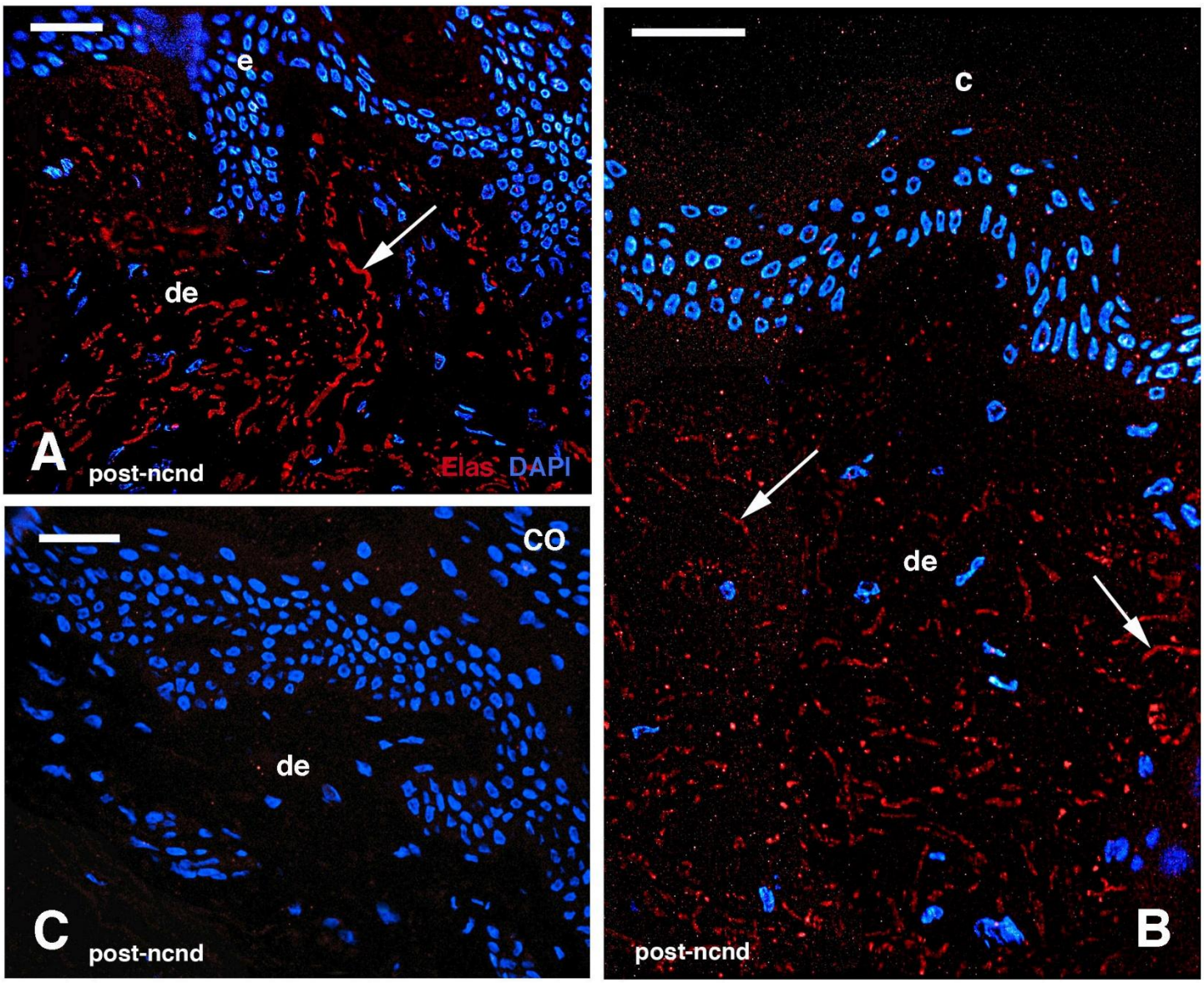

Figure 7: Immunofluorescence for elastin in non-conditioned subjects after treatments. A, younger subject showing numerous elastic fibrils (arrow) in the dermis. Bar, 25µm. B, numerous elastic fibrils, some long ones (arrows), are seen in the dermis. Bar, 30µm. C, immunonegative control section. Bar, 25µm. Legends: c, corneous layer; CO, control; de, dermis; e, epidermis; post-ncnd, post-conditioned.

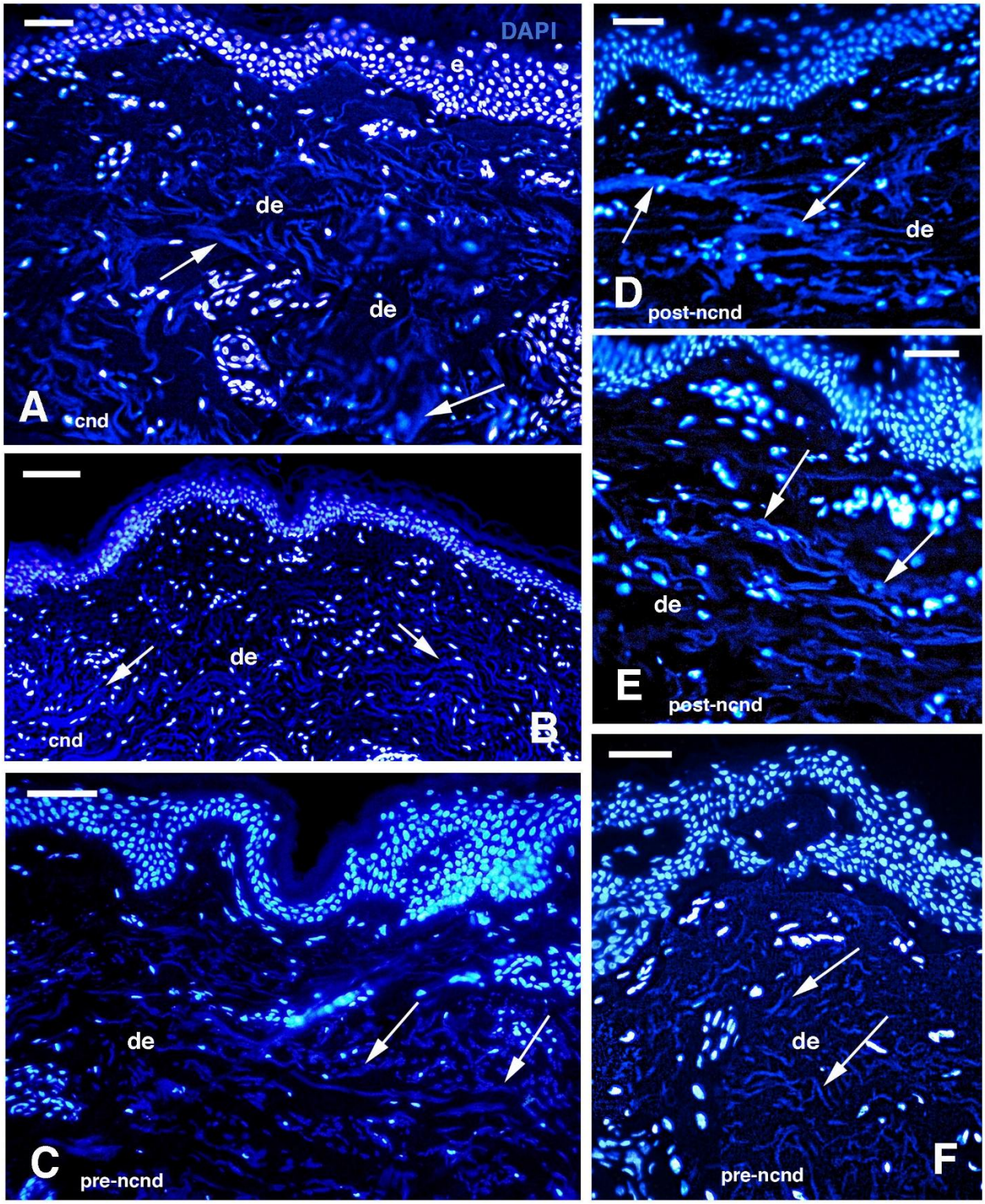

Figure 8: Blue DAPI fluorescence that illustrates the distribution, shape and sizes of dermal collagen fibers in conditioned (A, B) and non-conditioned (C-F) subjects. A, numerous large fibers (arrows) are seen in the dermis of at 48 years old subject. Bar, 25μm. B, numerous large fibers (arrows) are present in the 58 years old subject. Bar, 30μm. C, numerous large fibers (arrows) are visible in the younger subject (31 years old) before treatment. Bar, 30μm. D, large and anastomosed fibers (arrows) are present in the dermis of the younger subject after treatment. Bar, 25μm. E, another dermal region in the younger subjects after treatment with large fibers(arrows). Bar, 25μm. F, thin fibers (arrows) are observed in the dermis of the non-conditioned older subject before treatment. Bar, 30μm. Legends: c, corneous layer; de, dermis; e, epidermis; pre-ncnd, post-ncnd, post-conditioned; pre-conditioned.

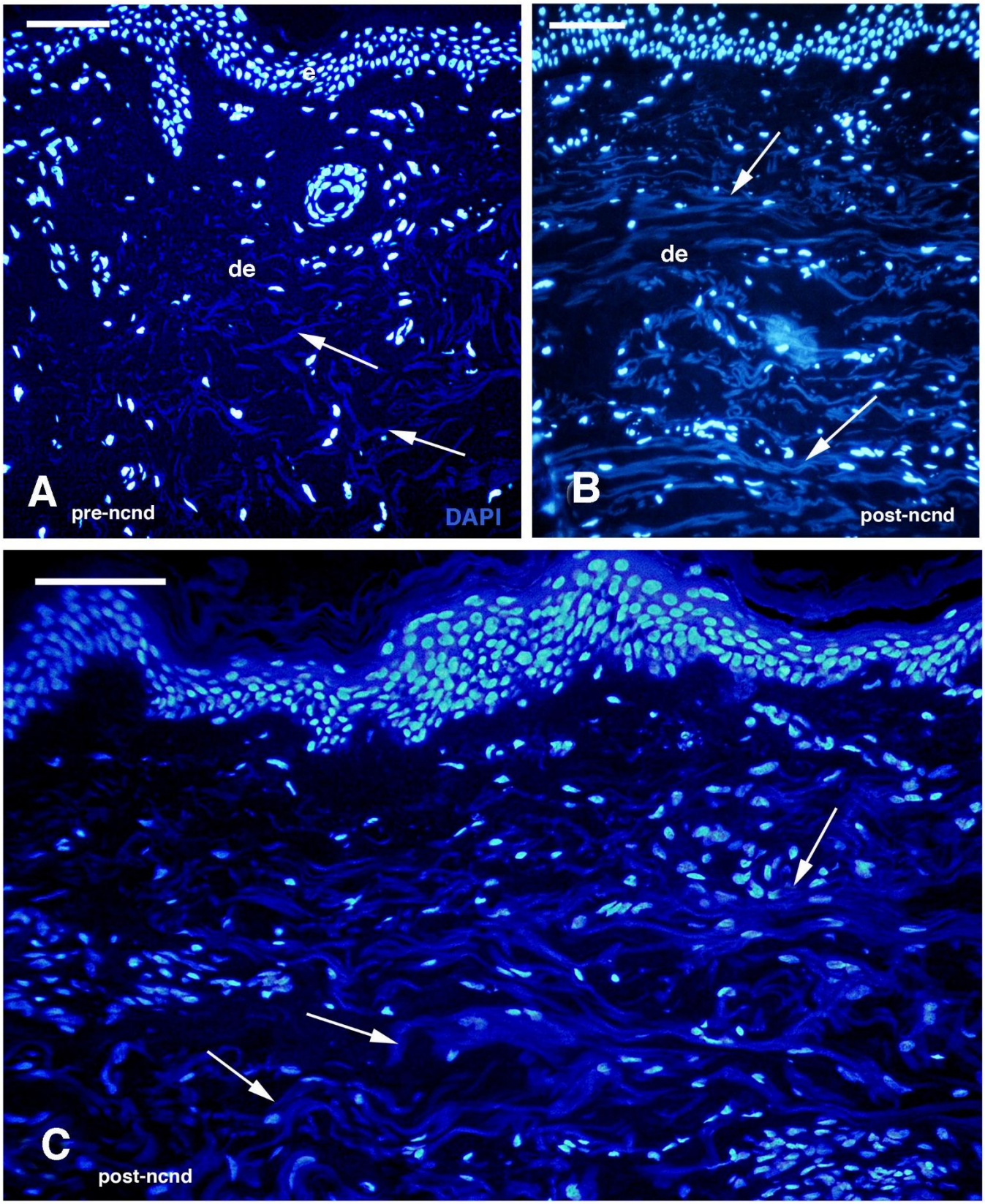

Figure 9: Blue DAPI fluorescence showing large, irregular and often anastomosed collagen fibrils in the oldest non-conditioned subject before (A) and after (B, C) treatment. A, the dermis in pre-treatment shows sparse fibrils and some with large dimension (arrows). Bar, 30μm. B, after treatment various large collagen fibers are seen (arrows) in the superficial and deep dermis. Bar, 30μm. C, other area of the dermis where large collagen fibers are present (arrows). Bar, 30μm. Legends: c, corneous layer; de, dermis; e, epidermis; pre-ncnd, pre-non conditioned; pre-ncnd, pre-non conditioned.

Discussion

In the three conditioned subjects, the normal pattern of distribution of AK2-positive keratin, filaggrin and loricrin in the stratum corneous with stratified corneocytes and superficial desquamation, suggest an optimal epidermal turnover that keeps the epidermis thin, soft, sensitive and shiny [14]. In fact, while a thin skin is more versatile and responsive to environmental changes and higher sensitivity, a thicker skin is designed to withstand constant or long periods of pressure and mechanical friction and loads, as these are present on the palms of the hands and soles of the feet, derived from walking or handling objects. Also, the thickness of the epidermis and the dermal fibroblasts and fibers decrease during aging, influencing the comparison among subjects of different ages (31-48-58-59 years) [14-16].

On that ground, the present qualitative and preliminary study, albeit carried out only for a brief period on few subjects, 4 weeks treatments plus 2 weeks post-treatment, has shown that some positive changes have occurred in the epidermis of the non-conditioned subjects. The changes in particular were seen in the barrier layer formed in the granular and corneous layers of the epidermis, as indicated from the continuous acidic keratin and filaggrin immunolabeling [8]. This barrier was clearly identified in conditioned subjects, but was less integral in the non-conditioned subjects before the treatments. The epidermal barrier appears to improve after the cosmetic treatment in only 4 weeks, suggesting that during these brief treatments, the epidermis was affected. The utilized formulation, as indicated in their coded compositions (see Materials and Methods), include different vegetable extracts and oils, probiotics, phospholipids, ceramides and phytosphyngosins, chemicals that allow trans-epidermal penetration [5,17]. Among the various formulations utilized in the present study, the “hydrating cream plus” also contains sodium hyaluronate and carnosine, molecules that also previous studies have shown to rapidly penetrate, within 1-2 hours, across the epidermis and reaching the dermis to stimulate keratinocytes and fibroblasts proliferation [5,17-19]. This suggests that the present treatments were also effective on the establishment and improvement of a continuous epidermal barrier.

In contrast, loricrin appears not affected from the treatment, although the stratum corneous is composed from more clearly separated corneocytes that also desquamates in the treated subjects, as observed in the conditioned individuals. From these qualitative observations, the epidermis appears influenced from the treatments, confirming previous and longer cosmetic trials [5]. The dermis on both subjects, located deeper in the skin, instead resulted no or insensibly affected from the short cosmetic treatment here performed. In fact, elastic and collagen fibrils do not appear significantly changed after this brief treatment. It is likely that, after longer treatments, e.g. 6-12 weeks, some more significant results also in the dermis could have been obtained, as indicated in other studies [5,18,19]. These studies have shown that cosmetic formulation containing growth factors, short peptides and matrikines, can stimulate fibroblasts to proliferate and also to produce collagen and hyaluronate after 8-16 weeks or longer treatments.

Conclusion

The present, preliminary study has indicated a positive effect on epidermal markers for barrier integrity but no significative changes in the dermis after the short period of protocol treatments. Further experiments after longer treatments are needed to determine whether the penetration of cosmetic applications can also influence fibroblasts in their proliferation and synthesis of collagen and elastin.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Funding Statement

The study was jointly supported from Formamentis SRL Headquarters, via del Lavoro 18c, 35020, Ponte San Nicolò, Padova, Italy (KM) and Comparative Histolab, Padova, Italy (LA). We thank Dr. Bruno Rizzuto (Dr. Day Surgery, Padova, Italy) for sampling the biopsies.

Acknowledgement

None.

Data Availability Statement

Not applicable.

Ethical Statement

The project did not meet the definition of human subject research under the purview of the IRB according to federal regulations and therefore, was exempt.

Informed Consent Statement

Informed consent was taken for this study.

Authors' Contributions

LA planned, performed lab analyses and wrote the MS. KM, performed the cosmetic treatments. Both authors red and approved the MS.

References

1. Wickett R, Visscher MO. Structure and function of the epidermal barrier. Am J Infect Control. 2006;34:S98-110.
2. Bonta M, Daina L, Mutiu G. The process of ageing reflected by histological changes in the skin. Rom J Morphol Embryol. 2013;54:797-804.
3. Eckhart L, Lippens S, Tschachler E, Declercq W. Cell death by cornification. Biochim Biophys Acta. 2013;1833:3471-80.
4. Shin JW, Kwon SH, Choi JY. Molecular mechanisms of dermal aging and antiaging approaches. Int J Mol Sci. 2019;20:2126.
5. Aldag C, Teixeira DN, Leventhal PS. Skin rejuvenation using cosmetic products containing growth factors, cytokines and matrikines: A review of the literature. Clin Cosmet Investig Dermatol. 2016;9:411-9.
6. Moll R, Divo M, Langbein L. The human keratins: Biology and pathology. Histochem Cell Biol. 2008;129:705-33.
7. Salas PJ, Forteza R, Mashukova A. Multiple roles for keratin intermediate filaments in the regulation of epithelial barrier function and apico-basal polarity. Tissue Barriers. 2016;4:e1178368.
8. Alibardi L. Immunolabeling for filaggrin and acidic keratins in the granular layer of mammalian epidermis indicates that an acidic-basic interaction is involved in cornification. Tissue Cell. 2024;88:102397.
9. Resing KA, Dale BA. Proteins of keratohyalin. In: Goldsmith LA, editor. Physiology, biochemistry and molecular biology of the skin. New York: Oxford University Press. 1991;1:148-67.
10. Rawlings AV, Scott IR, Harding CR, Bowser PA. Stratum corneum moisturization at the molecular level. J Invest Dermatol. 1994;103:731-40.
11. Kalinin AE, Kajava AV, Steinert PM. Epithelial barrier function: Assembly and structural features of the cornified cell envelope. BioEssays. 2002;24:789-800.
12. Elias PM, Choi EH. Interactions among stratum corneum defensive functions. Exp Dermatol. 2005;14:719-26.
13. Scala C, Cenacchi G, Ferrari C, Pasquinelli G, Preda P, Manara G. A new acrylic resin formulation: A useful tool for histological, ultrastructural and immunocytochemical investigations. J Histochem Cytochem. 1992;40:1799-804.
14. Tagami H. Location-related differences in structure and function of the stratum corneum with special emphasis on those of the facial skin. Int J Cosmet Sci. 2008;30:413-34.
15. Wada M. Changes of the thickness of epidermal tissue with aging. Okajimas Folia Anat Jpn. 1978;54:341-60.
16. Walker M. Human skin through the ages. Int J Pharm. 2022;622:121850.
17. Ng KW, Lau WM. Skin deep: the basics of human skin structure and drug penetration. In: Dragicevic N, Maibach HI, editors. Percutaneous penetration enhancers: Chemical methods in penetration enhancement. Berlin: Springer. 2015;3-11.
18. Brown TJ, Alcom D, Fraser RE. Absorption of hyaluronan applied to the surface of intact skin. J Invest Dermatol. 1999;113:740-6.
19. Yang JA, Kim ES, Kwon JH, Kim H, Shin JH, Yun SH, et al. Transdermal delivery of hyaluronic acid-human growth hormone conjugate. Biomaterials. 2012;33:5947-54.