Antiaging Treatment of the Facial Skin by Fat Graft and Adipose-Derived Stem Cells

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Background: The regenerative property of fat grafting has been described. However, it is not clear whether the clinical results are attributable to the stem cells or are linked to other components of the adipose tissue. This work is aimed at analysis of the histologic and ultrastructural changes of aged facial skin after injection of fat graft in addition to its stromal vascular fraction, obtained by centrifugation, and to compare the results with those obtained by the injection of expanded adipose-derived mesenchymal stem cells.
Methods: This study was performed in six consecutive patients who were candidates for face lift and whose ages ranged between 45 and 65 years. The patients underwent sampling of fat by liposuction from the abdominal region. The injection of fat and its stromal vascular fraction or expanded mesenchymal stem cells was performed in the preauricular areas. Fragments of skin were removed before and 3 months after each treatment and analyzed by optical and electron microscopy.
Results: After treatment with the autologous lipidic component and stromal vascular fraction, the skin showed a decrease in elastic fiber network (elastosis) and the appearance of new oxytalan elastic fibers in papillary dermis. The ultrastructural examination showed a modified tridimensional architecture of the reticular dermis and the presence of a richer microvascular bed. Similar results following treatment with expanded mesenchymal stem cells were observed.
Conclusion: This study demonstrates that treatment with either fat and stromal vascular fraction or expanded mesenchymal stem cells modifies the pattern of the dermis, representing a skin rejuvenation effect. (*Plast. Reconstr. Surg.* 135: 999, 2015.)

In recent years, there has been an increased interest in use of the regenerative properties of adipose tissue grafts.[1–3] In this regard, some studies have suggested the rejuvenating properties of fat grafting in the skin.[4,5] By this approach, studies in animal models have described an improvement in the texture of the skin and, in particular, the increased elasticity, normalization of excess in the pigmentation, and aesthetic improvement of all scars, regardless of the provoking cause.[6]

For this reason, fat grafting is now widely used in plastic surgery, mostly in the treatment of the aging face and hands.[7,8] In the absence of standardized procedures, multiple surgical procedures and techniques are used in fat grafting, considering that adipose tissue can be purified by gravity or by different means of centrifugation.[9,10] Fat has also been used in combination with autologous adipose-derived mesenchymal stem cells or with enriched autologous plasma (platelet-rich plasma). To date, it is widely known that some surgeons are grafting only the stromal vascular fraction of the adipose tissue, without the mature adipocytic component.[11–13] Other approaches are based exclusively on the injection of adipose-derived mesenchymal stem cells after in vitro expansion.[14]

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A Video Discussion by Michael T. Longaker, M.D., M.B.A., accompanies this article. Go to PRSJournal.com and click on "Video Discussions" in the "Videos" tab to watch.

However, despite the wide availability of surgical techniques and the remarkable results obtained in some experiments,[15] the biological mechanism responsible for the rejuvenating and regenerative effect obtained with the grafted lipoaspirate and/or its components is not yet clearly understood, and there is no consensus concerning the structural and ultrastructural changes that occur in the skin and subcutaneous tissue that justify the long-lasting improvement clinically visible after fat grafting.[15–17]

The purpose of this article is to analyze the histologic and ultrastructural changes of aged facial skin after the injection of expanded mesenchymal stem cells and fat with its stromal vascular fraction, to assess whether the subcutaneous connective tissue may constitute a good clinical model with which to study the related effects of antiaging mechanisms.

In addition, we have evaluated whether a difference exists in the therapeutic results comparing specimens after treatment with two different protocols. The first was based on the injection of expanded mesenchymal stem cells, whereas in the second, we used centrifuged fat and its stromal vascular fraction. This last phase of the work was aimed at evaluating the best approach that could be used in the regenerative therapy of the face.

PATIENTS AND METHODS

The study involved six consecutive patients (mixed races), five women and one man, who were candidates for facial rejuvenation surgery (face lift). Age ranged between 45 and 65 years, and none of them had a history of chronic viral, metabolic, ischemic, autoimmune, or other systemic disease. Also, patients who reported a smoking habit were excluded from the study. The patients consented under approved guidelines set forth for human clinical trials according to the Brazilian investigation ethical committee board (protocol no. 28063) and the Brazilian Clinical Trials Registry (Universal Trial Number, U1111-1145–3081).

Under local anesthesia, the patients underwent removal of 30 cc of fat by liposuction from the abdominal region. The removed fat was processed according to two different protocols.

Part of the aspirated fat was harvested from the inferior abdomen using a 10-ml syringe and cannula (3 mm in diameter) and processed in a centrifuge (IEC Medilite Microcentrifuge; Thermo Electron Corp., Byron Medical, Waltham, Mass.) for mechanical isolation of the stromal vascular fraction. One syringe was spun at 3000 rpm for 3 minutes, equivalent to 1286 g. In the final centrifugation process, a layer at the bottom of the centrifuged sample, called a pellet, was revealed and separated by mechanical dissociation (centrifugation) without enzymatic processing.[10] The pellet, containing the stromal vascular fraction and rich in regenerative cells, was collected. This pellet was mixed with another layer of intact adipocytes (1 ml), to create an enriched fat graft that was injected into the subdermal layer, 2 cm distally from the tragus, in the right preauricular region. It was performed as retrograde injection in a fan-wise maneuver, in an area of 1 cm^2, using a 1.5-mm blunt-tipped cannula connected to a 3-ml syringe. Another pellet, from a second syringe, was collected and sent for quantitative analysis of the mesenchymal cell fraction in pellet samples by flow cytometry (CD105$^+$/CD90$^+$/CD73$^+$/CD146$^+$/CD14/CD45$^-$/CD34$^-$), representing the total number of mesenchymal cells, and showed an average of 16,204 ± 5516 mesenchymal stem cells.

The other part of the aspirated fat was sent for expansion of its stem cells. After expansion, the expanded stem cells were diluted in 0.9% saline and packaged in syringes of 1-ml solution containing $2 × 10^6$ mesenchymal cells diluted in a volume of 0.4 ml. We carried out the subdermal application of mesenchymal stem cells with a 1-ml syringe (volume, 0.4 ml) coupled to a 30-gauge needle, in the left preauricular area (1-cm^2 area), 2 cm distal from the tragus.

In terms of experimental design, we decided to use expanded adipose-derived mesenchymal stem cells in vitro rather then just using fresh isolated adipose-derived stem cells for two main reasons: (1) to verify whether the number of stem cells could influence the quality of the result; and (2) because, if so, the method could be applied and repeated also in patients with insufficient fat deposits.

Before injection, a fragment of skin and subcutaneous tissue was removed from the preauricular areas and sent to the Section of Human Anatomy and Histology of Verona (Verona, Italy) and the Federal University of Rio de Janeiro (Rio de Janeiro, Brazil) for morphologic analysis by optical and electron microscopy. Histologic analysis of skin biopsy specimens was performed by hematoxylin and eosin, picrosirius red (for visualization of collagen), and orcein (for visualization of elastic fibers) staining.

The morphologic examination was repeated after 3 months, which is time for the action of the lipidic components,[18] taking a piece of skin and subcutaneous tissue in the same area where the expanded stem cells and fat with stromal vascular fraction were injected. The regenerative antiaging activity was evaluated by comparing the

morphology of the skin and subcutaneous areas before and after the two types of treatment.

The biopsy specimens, consisting of fragments of skin measuring 0.5 × 1 cm, were limited inside of the 1-cm^2 preauricular area (pretragal) and split into two equal parts of 0.5 × 0.5 cm. One half was sent in a plastic bottle immersed in 4% formaldehyde to the Institute of Biophysics Carlos Chagas Filho, Laboratory of Immunology, Federal University of Rio de Janeiro (Rio de Janeiro, Brazil) for histologic and immunohistochemical study. The other half (0.5 × 0.5 cm) was sent in a plastic bottle with a solution of buffered 2% glutaraldehyde to the Section of Human Anatomy and Histology of Verona (Verona, Italy) to be subjected to histomorphometric analysis with electron microscopy.

The first skin biopsies (untreated skin biopsy) of both preauricular regions were performed at the same time of the fat harvesting, measuring 0.5 × 1 cm, under local anesthesia with lidocaine 0.5% and 1:500,000 epinephrine, on an outpatient basis. At the same time, fat with its stromal vascular fraction was injected in the subdermal plane in an pretragal area measuring 1 cm^2, from far 2 cm of the right tragus. After 5 weeks, the expanded mesenchymal stem cells are injected into the other preauricular region, in an area measuring 1 cm^2, also in the subdermal plane.

Three months after treatment with fat graft and its stromal vascular fraction or with expanded mesenchymal stem cells, the second biopsy was performed. These biopsies of treated skin were performed at different times, because of the necessity of performing the expansion of mesenchymal stem cells during 5 weeks. Thus, the right preauricular biopsy (fat and its stromal vascular fraction protocol) was performed 5 weeks before the face-lifting operation. The left preauricular biopsy (expanded mesenchymal stem cells) was carried out concurrent with face lifting, to guarantee the same time to the treatment effect of both protocols.

All biopsy specimens, consisting of fragments of skin, were split into two equal parts (0.5 × 0.5 cm). One half was sent in a plastic bottle immersed in 4% formaldehyde to the Institute of Biophysics Carlos Chagas Filho (Laboratory of Immunology, Federal University of Rio de Janeiro) for histologic and immunohistochemical study. The other part (0.5 × 0.5 cm) was sent in a plastic bottle with a solution of buffered 2% glutaraldehyde to the Section of Human Anatomy and Histology of Verona, to be subjected to histomorphometric analysis with electron microscopy. During the period between the two biopsies, patients were instructed to not use any cosmetic product and/or peeling treatments on the face.

Preparation and Expansion of Mesenchymal Stem Cells from Adipose Tissue

To obtain mesenchymal stem cells from adipose tissue, the lipoaspirate underwent enzymatic dissociation. Briefly, a volume of 10 ml of lipoaspirate was processed in cabin class 100 (International Organization for Standardization 5) biosecurity, located in a clean room class 10,000 (International Organization for Standardization 7) and, after washing in phosphate-buffered saline (pH 7.4, sterile), was weighed dissociated with collagenase IA (Sigma-Aldrich, St. Louis, Mo.), 200 units/mg tissue, and incubated at a temperature of 37°C under constant agitation for 1 hour. The material was then centrifuged and the sediment was filtered through 70-μm nylon mesh. The cells were resuspended in culture medium supplemented with 10% fetal bovine serum. Cells were quantified using trypan blue dye and plated in low-glucose Dulbecco's Modified Eagle Medium (LGC) supplemented with 20% fetal bovine serum and antibiotics (penicillin and streptomycin sodium). Cultures were maintained at 37°C in 5% carbon dioxide; the following day, nonadherent cells were removed and adherent cells were expanded in culture bottles. Two days before application, the cells were washed with physiologic saline and incubated with culture medium supplemented with autologous plasma at a temperature of 37°C in 5% carbon dioxide. One day before application, 1-ml Luer-Lok (Becton Dickinson AG, Basel, Switzerland) syringes were prepared containing 2 million cells diluted in 0.4 ml of physiologic saline.

Characterization of the Adipose-Derived Mesenchymal Stromal Cell Surfaces with Surface Markers (CDs) and Flow Cytometry (Fluorescence-Activated Cell Sorting)

For characterization of adipose-derived mesenchymal stromal cells, surface markers were used. One milliliter of phosphate-buffered saline buffer, pH 7.4, was added to test tubes containing 1 × 10^6 cells and centrifuged (400 g) for 5 minutes at 4°C. The supernatant was discarded, and 50 μl of phosphate-buffered saline and 50 μl of human AB plus serum was added to each reaction tube, maintained in incubation for 5 minutes at room temperature. The fluorochrome-conjugated monoclonal antibodies were added to the tubes and incubated for 20 minutes at 4°C. The readings were performed using a FACSCalibur flow cytometer (Becton Dickinson) using the CellQuest Pro

program (Becton Dickinson). The phenotype of the stromal cells was defined using combinations of surface markers of the mesenchymal cells, pericytes, and fibroblasts: CD90, CD44, CD45RO, CD105, and CD1066.

In Vitro Cell Differentiation of Adipose-Derived Mesenchymal Stromal Cells

The cultured cells were induced to differentiate in vitro to the osteogenic and adipogenic lineages according to Zuk et al.[1] For adipogenic differentiation, the cells were cultured in 24-well plates containing 1 ml of Dulbecco's Modified Eagle Medium (LGC; HyClone Laboratories, Logan, Utah) supplemented with 10% fetal bovine serum and penicillin/streptomycin at a concentration of 100 units/ml and 100 mg/ml, respectively, 1 mM dexamethasone (Sigma, catalogue no. D4902), 0.5 mM 3-isobutyl-1-methylxanthine (Sigma, catalogue no. I7018), 10 mM human insulin (Humulin N; Eli Lilly and Co., Indianapolis, Ind.), and 0.2 mM indomethacin (Sigma, catalogue no. I7378). The induction medium was changed two times per week. At the end of culture, cells were fixed with 4% paraformaldehyde, washed with phosphate-buffered saline, and stained with a 0.5% solution of oil red O (Sigma, catalogue no. O0625) for reading differentiation. For osteogenic differentiation, the cells were cultured in six-well plates containing 2 ml of low-glucose Dulbecco's Modified Eagle Medium (LGC) supplemented with 10% fetal bovine serum, and penicillin and streptomycin (at a concentration of 100 U/ml and 100 mg/ml, respectively), 10 nM dexamethasone (Sigma, catalogue no. D4902), 10 mM β-glycerophosphate (Calbiochem-EMD Millipore, Darmstadt, Germany; catalogue no. 35675), and 50 mM L-ascorbic acid 2-phosphate (Sigma, catalogue no. A8960). The induction medium was changed two times per week. At the end of culture, cells were fixed with 4% paraformaldehyde, washed with phosphate-buffered saline, and stained with 1% alizarin red solution S (Sigma, catalogue no. A5533), pH 4.2.

Transmission Electron Microscopy

Different samples were fixed with glutaraldehyde 2% in Sorensen buffer, pH 7.4, for 2 hours; postfixed in 1% osmium tetroxide in aqueous solution for 2 hours; and dehydrated in graded concentrations of acetone. At the end of the dehydrating process, samples were positioned in a multiwell grid for electron microscopy and observed using an FEI Morgagni 268D transmission electron microscope (FEI, Hillsboro, Ore.).

Scanning Electron Microscopy

Specimens were fixed with 2% glutaraldehyde in 0.1 M phosphate buffer, dehydrated in graded ethanol, critical point dried (CPD 030; Oerlikon Balzers, Vaduz, Liechtenstein), fixed to stubs with carbon-based adhesive, sputtered with carbon or gold by a MED 010 coater (Oerlikon Balzers), and examined with an XL30 environmental scanning electron microscope (FEI).

RESULTS

The study was based on the following assumptions: a portion of the facial skin was taken adjacent to the area that would later be treated with fat grafting plus stromal vascular fraction or with expanded adipose-derived mesenchymal stem cells. These portions of skin represent the control models that were compared with the specimens obtained after
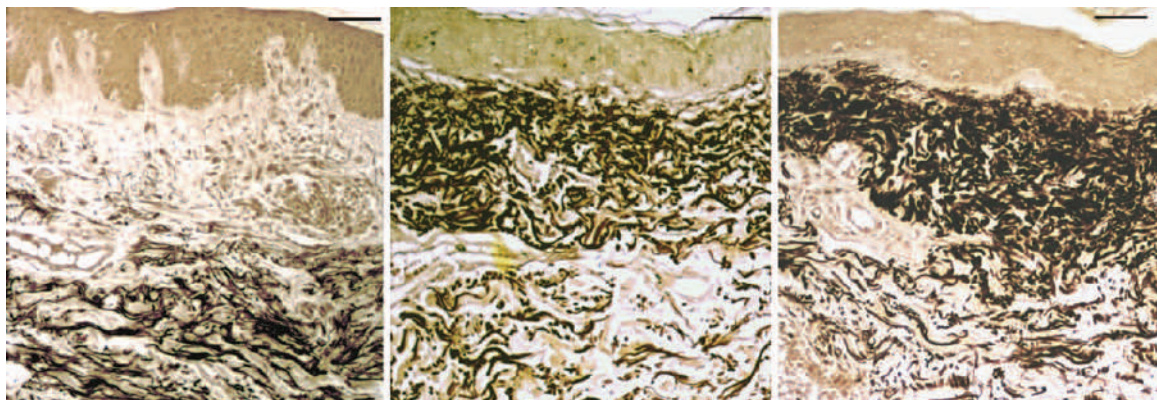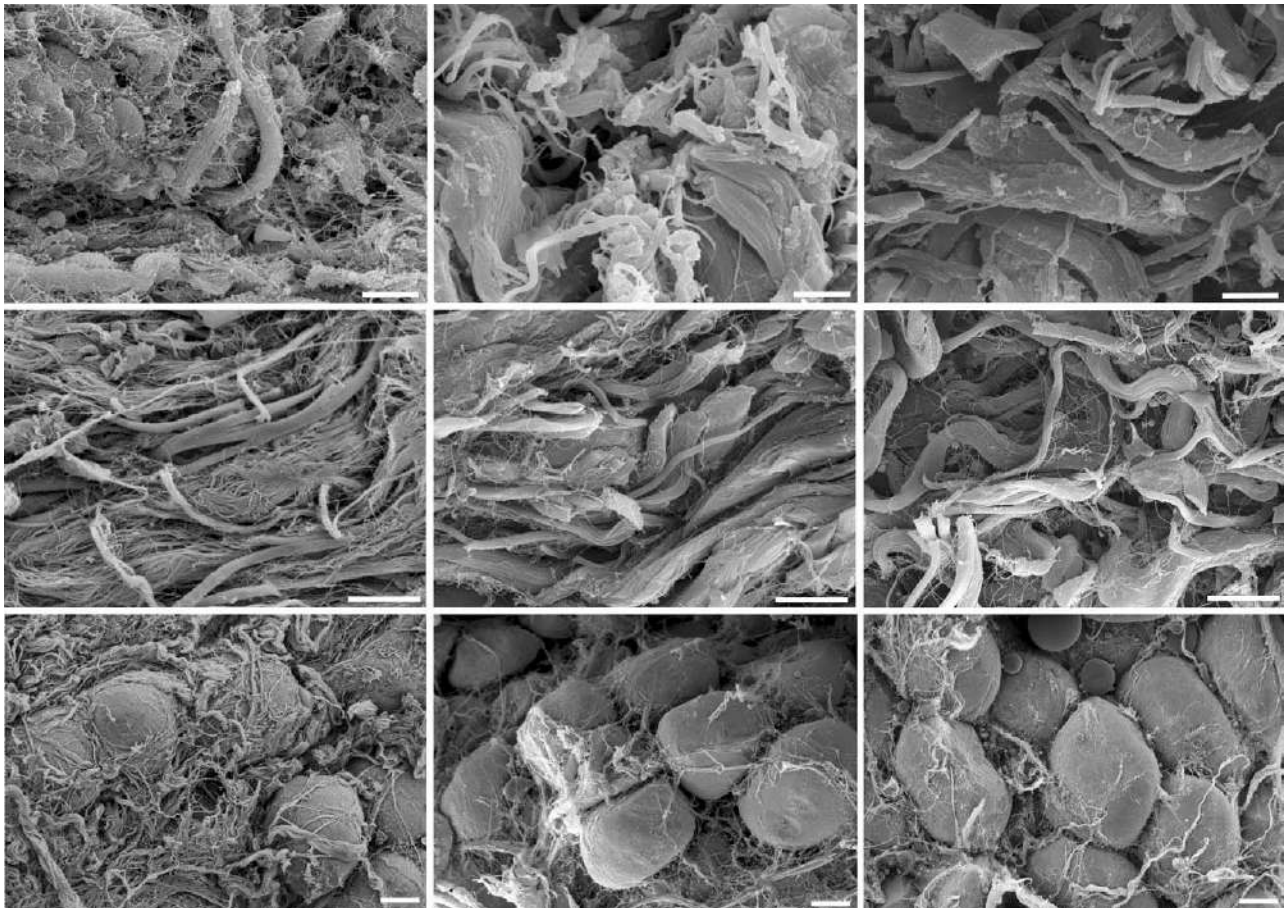

Fig. 1. Orcein-stained skin (*left*) before treatment, (*center*) after treatment with expanded mesenchymal stem cells, and (*right*) after treatment with fat and stromal vascular fraction. After both treatments, the increment of elastic fibers, stained in black and localized in the superficial layer of dermis, is detectable. In contrast, the elastic fibers are scarce in the deep layer of dermis. *Scale bars* = 50 μm.
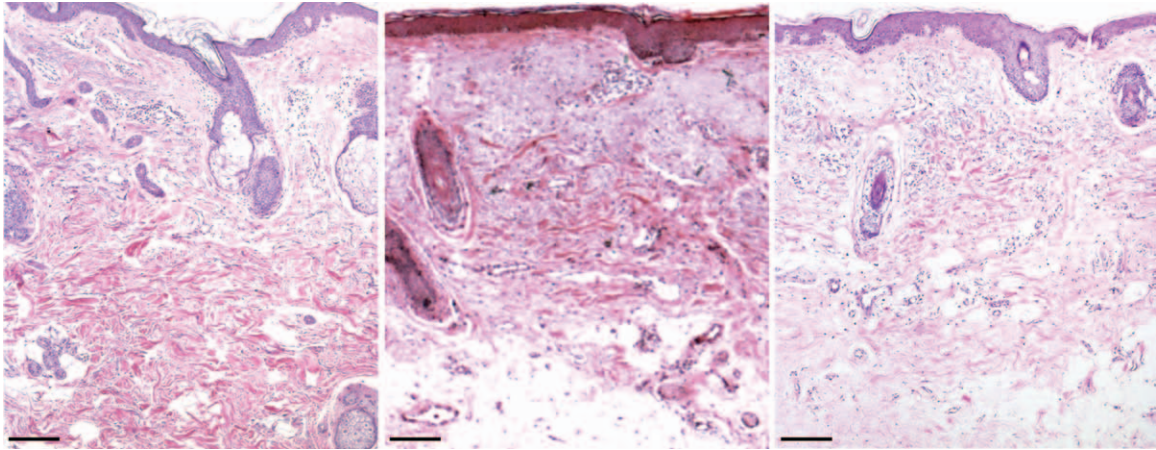
Fig. 2. Hematoxylin and eosin–stained skin (*left*) before treatment, (*center*) after treatment with expanded mesenchymal stem cells, and (*right*) after treatment with fat and stromal vascular fraction. After both treatments, the presence of vessels is noted in the area of the dermal-hypodermic junction. *Scale bars* = 150 μm.

Fig. 3. Scanning electron microscopy of skin. (*Above*) Superficial layer of dermis (*scale bars* = 5, 2.5, and 2.5 μm); (*center*) deep layer of dermis (*scale bars* = 10, 10, and 5 μm); and (*below*) adipose cells localized at the dermis-hypodermis junction (*scale bars* = 25 μm). (*Left*) Dermis portions before treatment. (*Center*) Dermis after treatment with expanded mesenchymal stem cells. (*Right*) Dermis after treatment with fat and stromal vascular fraction. In dermis, both treatments (with expanded mesenchymal stem cells and fat with stromal vascular fraction) determined the modification of collagen and reticular fiber networks. The role of collagen fibers in forming a finer pattern in the papillary dermis rather than in the reticular dermis is clear, where they are arranged to form thick bundles. The adipocyte component shows a reduction of collagen fibers, which envelop the mature cells.

treatment with fat grafting plus stromal vascular fraction or with the expanded adipose-derived stem cells.

Aged Facial Skin

Histologic examination of the facial skin before treatment showed a lesion consistent with a mild degree of actinic elastosis. On hematoxylin and eosin staining, the skin showed a mild mononuclear inflammatory infiltrate in the dermis and elastic fiber degeneration or elastosis. On orcein staining, the skin showed disorganized elastic fibers in the region of the papillary and reticular dermis, associated with a decrease in oxytalan fibers or "new elastic fibers." In the papillary dermis, oxytalan fibers were generally not visible. No significant alteration was visible on picrosirius red staining in the papillary or reticular dermis. In some areas, an increase in the network of elastic fibers, sometimes forming compact blocks stained by orcein, was present in the reticular dermis. The presence of a mass of elastic fibers reactive to orcein was also found, appearing either amorphous or irregular without a fibrillar aspect to varying degrees. On hematoxylin and eosin–stained sections, these areas showed focal mononuclear cell infiltration. On ultrastructural examination, the dermis showed irregularly dispersed elastic fibers generally of large diameter enmeshed in a network of collagen fibers. In some areas, this appeared rather sparse; in others, it was composed of dense bundles. The adipocytes located at the boundary of the dermis with the subcutaneous tissue were covered by a dense bundle of collagen strictly adherent to the plasma membrane.

Posttreatment Biopsies of the Skin: Fat and Stromal Vascular Fraction

After treatment with fat graft and its stromal vascular fraction, the skin showed a decrease in the elastic fiber network (elastosis) and an increase of the oxytalan elastic network in the papillary dermis. On hematoxylin and eosin–stained sections, in the perivascular areas, a mild mononuclear cell infiltration and edema were visible. An increased density of new oxytalan fibers disposed perpendicular to the dermis-epidermis junction was visible on orcein staining. Significant changes with respect to the pretreatment biopsy specimens were not visible by picrosirius red staining. In the reticular dermis, on hematoxylin and eosin staining, the dermis showed blood capillaries surrounded by edematous areas and mononuclear infiltrates. The most relevant finding was a decrease in the number of elastic fibers. In general, the skin appeared more hydrated, with an increase of the oxytalan network in the papillary dermis and a decrease in the area of elastosis in the reticular dermis (Figs. 1 and 2).

Ultrastructural examination showed a modified tridimensional architecture of the reticular dermis, in which the elastic fibers, with respect to the pretreatment specimens of the same patients, appeared with a reduced diameter and with a smoother surface because of a less developed fibrillar component at their periphery (Fig. 3). Another relevant finding was the relative scarcity of the network of collagen reticular fibers that wrapped the elastic fibers in the reticular dermis. In specimens removed after treatment, the elastic fibers appeared more dissociated, being reduced in the collagenic net characterizing the pretreatment biopsy specimens, in particular, in the deepest portion of the reticular dermis. At the junction between the reticular dermis and the subcutaneous tissue, the treatment was associated with the presence of a richer microvascular bed. The collagenic net wrapping the adipocytes appeared composed of loose collagen fibers (Figs. 1 through 5).

**Posttreatment Biopsies of the Skin: Expanded Mesenchymal Stem Cells**

The second protocol of treatment with expanded mesenchymal stem cells resulted in modifications of the skin, which were not significantly different from those generated by the first protocol (i.e., lipidic component and stromal vascular fraction of the autologous adipose tissue) (Figs. 1 through 5). The only detectable difference was a larger amount of adipose tissue visible at the interface between the reticular dermal end of the subcutaneous layer when the fat graft was performed. However, this aspect was difficult to quantify with precision because of the incomplete evaluation of the subcutaneous adipose tissue across its entire thickness.

Direct Comparison between Light and Electron Microscopy

The relationship between collagen and elastic fibers was also studied by an innovative method that we have developed in previous studies. This approach allows a direct comparison of hematoxylin and eosin–stained histologic slices and scanning electron microscopy that is performed on the same fibers. The method showed the three-dimensional morphology of the elastic fibers after treatment. The curl-like fibers appeared generally of small diameter and were disposed in nests surrounded by a thin shell of collagen (Fig. 6).

After treatment, immunocytochemistry examination showed the appearance of small-diameter
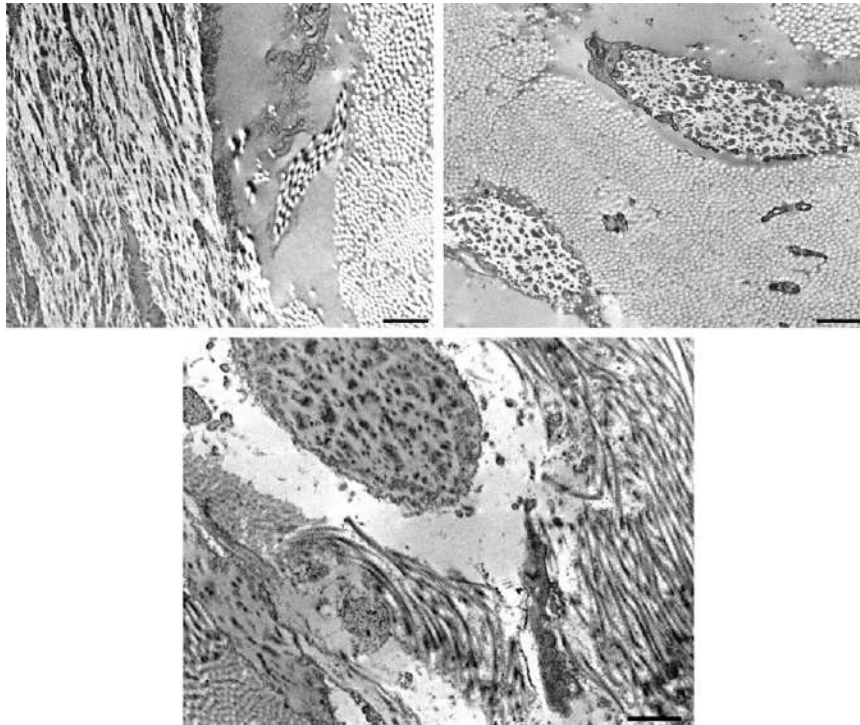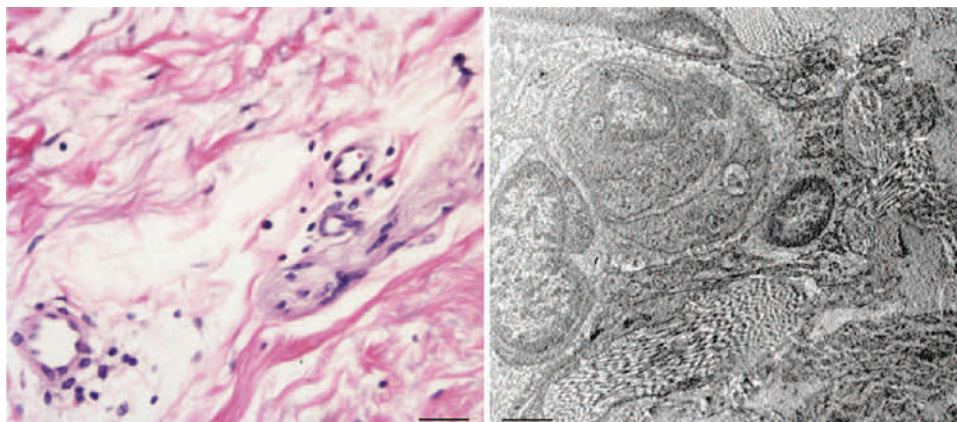
Fig. 4. Transmission electron microscopy of skin. (*Above, left*) Before treatment. (*Above, right*) After treatment with expanded mesenchymal stem cells. (*Below*) After treatment with fat and stromal vascular fraction. Before treatment, elastic fibers with a large diameter are visible with the characteristic patchy "leopard spots." Also, collagen in reticular form and in bands (*right*) is visible. After treatment, the areas of extracellular matrix are expanded. *Scale bars* = 1 μm.

nerve fibers positive for fibrillin in the papillary dermis. On this occasion, there is also a moderate increase in positivity for tropoelastin (Fig. 7).

Patients in whom the increase of the fibrils in the papillary dermis was higher also presented in the reticular dermis a more marked reduction of the large elastic fibers. In these patients, a proliferation of small blood vessels in the reticular dermis and the subcutaneous fat layer was detectable in some areas. In patients with small perivascular lymphocytic infiltrates, a lesser effect of the treatment was recorded (Table 1).

Fig. 5. Hematoxylin and eosin–stained skin (*left*) and transmission electron microscopy of skin (*right*). The morphology of a vessel representative of the dermis-hypodermis junction is noted, characterized by perivascular elements with a high nucleus-to-cytoplasm ratio that resemble stem cells. *Scale bars* = 30 μm (*left*) and 2 μm (*right*).
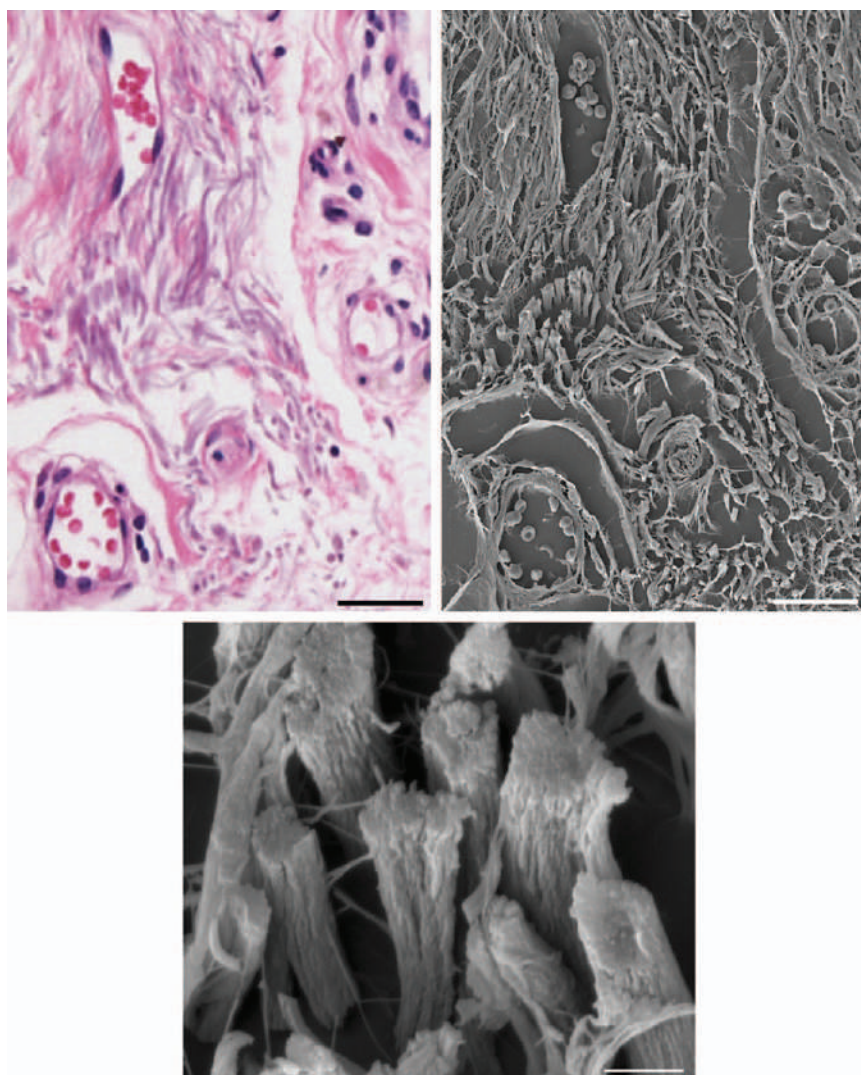
Fig. 6. Skin after treatment. Direct comparison between light and electron microscopy. The same area of the section is visualized by optical microscopy (*above*, *left*) (hematoxylin and eosin staining) and scanning electron microscopy (*above*, *right*). The *square* marks an area with thick elastic fibers that appears enlarged (*below*). Elastic fibers of small diameter are visible, arranged in an irregular manner with the individual vermiform curved fibers. *Scale bars* = 25 μm (*above*) and 2.5 μm (*below*).

DISCUSSION

Aging Process of Facial Skin

During aging, degenerative processes affecting the skin and deep structures of the face are well known to occur.[19,20] This aging process is the result of the action of intrinsic skin events associated with extrinsic agents, in particular, exposure to ultraviolet radiation.[21–24] Considering the histologic aspect of skin aging, several lesions have been described, such as degradation of extracellular matrix fibers (including oxytalanic elastin fibrils and collagen types I, III, and IV) and loss of the oligosaccharidic fraction. These lesions seem to impair the ability of the dermis to retain water. In addition, in aged skin, a reduction in the number of fibroblasts has been described. In particular, the reticular dermis features copious amounts of elastic fibers in a disorganized distribution pattern, leading to loss of elasticity and tonus. Within the deep layer, tissue atrophy is observed, causing volumetric loss of contour and angularity in the face.[19,25]

Adipose-Derived Mesenchymal Stem Cells

Some data suggest that the above-reported lesions could be treated by injection of adipose-derived mesenchymal stem cells.[24] Mesenchymal cells found in the stromal vascular fraction of adipose tissue are multipotent elements that can be extracted easily.[10,26] They have high potential for self-renewal and ex vivo expansion, with the capacity to differentiate into multiple cell lineages, giving them an important role
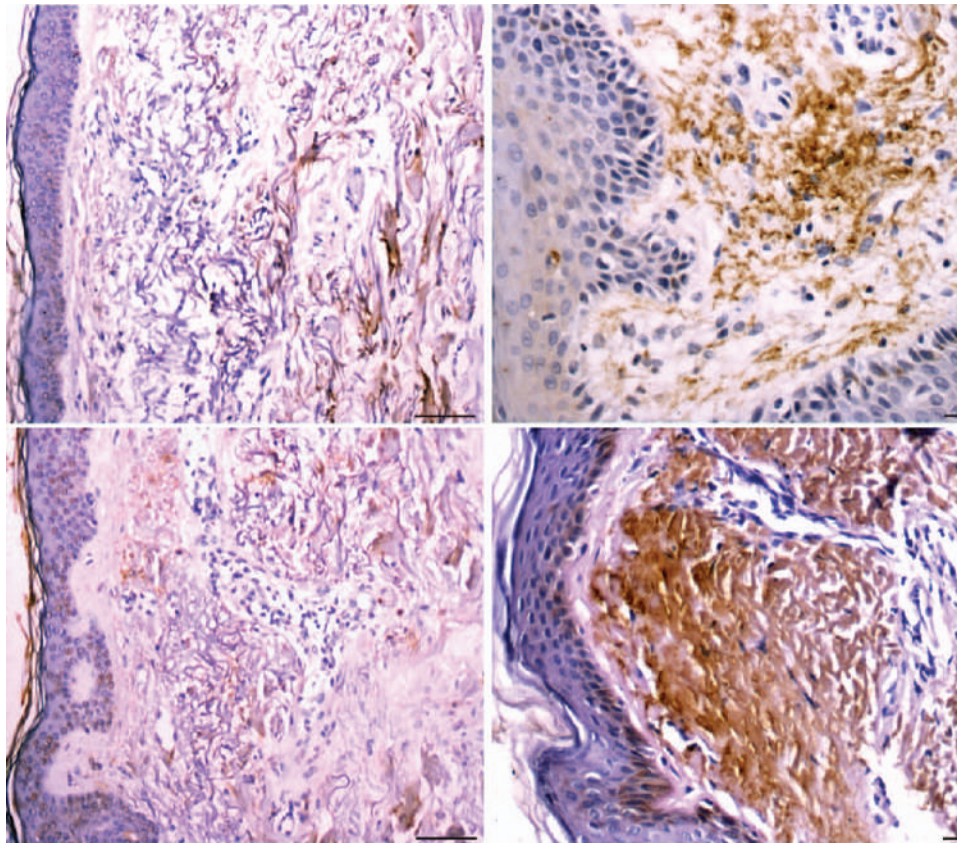
Fig. 7. Immunostaining. (*Above*, *left*) Fibrillin before treatment; (*above*, *right*) tropoelastin before treatment; (*below*, *left*) fibrillin after treatment; (*below*, *right*) tropoelastin after treatment. *Scale bars* = 70, 35, 70, and 35 μm, respectively.

in regenerative medicine.[27,28] They also have important paracrine actions, may stimulate angiogenesis, are antioxidant, and modulate immune tolerance and inflammation.[29,30] In part, these roles are attributed to the action of several growth factors released under specific conditions.[31–35]

The use of adipose-derived stem cells has been described in clinical studies (e.g., in cases of radiodermatitis, sequelae of burns, sclerodermas, sequelae of trauma, and others) with satisfactory results.[18] In contrast, numerous clinical studies suggest a role of mesenchymal cells in improving the quality of the skin in patients undergoing fat grafting and use of adipose-derived stem cells; however, to date, no studies have reported the histologic changes of human skin undergoing the action of adipose-derived stem cells.

Modification Induced by Treatment with Mesenchymal Stem Cells

Our study demonstrates that a modification of the aging skin can be obtained by injecting a population of expanded cells derived from autologous adipose tissue. It is interesting to note that the

effects of the treatment are visible in the epidermis also if the injection takes place in the subcutaneous region. There is evidence that this is attributable to a sequence of events, which requires detailed studies supported by histologic and ultrastructural analyses.

On light microscopy, the most relevant finding that characterizes the aged skin before treatment is the presence of a large amount of elastic fibers of large diameter in the deep portion of the reticular dermis, at the boundary with the subcutaneous fat. Our study has been specifically aimed at revealing the three-dimensional basis of this event using the scanning electron microscopic approach. If the most relevant finding on histologic evaluation is the increase of the elastic component that can lead to evident elastosis, the scanning electron microscopic study demonstrates that this is only an aspect of the process, that is also characterized by the formation of a collagenic scaffold that wraps the elastic fibers, which appear to be enmeshed in a network of reticular collagen. After treatment with expanded cells, an effect of the therapy seems to be a reorganization of the reticular dermis in which, in particular, the

Table 1. Modification of the Papillary Dermis Expressed Using a Semiquantitative Scale

Patient	Modification in the Papillary Dermis	Reduction of Large Fibers in the Reticular Dermis	Perivascular Infiltrate in the Papillary Dermis	Small Vessels at the Dermis-Hypodermis Junction
1	++++	+	−	+
2	+++	+	−/+	+
3	+++	+	−	+
4	++	−	+	+
5	+	−	+	−
6	+	−	+	−

network of collagen fibers is reduced and the elastic component is composed of fibers with a small diameter that represents an antiaging effect.

A similar rearrangement characterizes the uppermost layer of the dermis (i.e., the papillary dermis), which intertwines with the ridges of the epidermis and is usually composed of fine and loosely arranged collagen fibers. After treatment, within this layer, there is an evident proliferation of newly formed elastic fibers. Therefore, this study demonstrates that treatment causes a reabsorption of the elastic component in the reticular dermis and a new formation of elastic fibers in the papillary dermis. In both cases, the scanning electron microscopic examination demonstrates that the elastic fibers are relatively "young" and devoid of a strictly linked collagenic scaffold.

Modification Induced by Treatment with Fat and Stromal Vascular Fraction

It is interesting to note that treatment with the autologous fat and stromal vascular fraction induced modifications quite similar to those induced by the injection of expanded mesenchymal stem cells. To our knowledge, this is the first study aimed at analyzing this aspect. This result seems to suggest that the effect of a fat graft is caused, at least in part, by its stem cell component. This finding could have clinical relevance, because the possibility of using adipose tissue and thus avoiding in vitro expansion could make the rejuvenating treatment feasible on the same day on which liposuction is performed. The increase in the fat layer seems to be attributable to the fat graft itself.

Possible Determinants of the Cutaneous Changes

Evident limitations of the study are the difficulty of quantifying the rejuvenating effect precisely and the incomplete knowledge of the biological events that cause it. A suggestive hypothesis is the possibility that the cutaneous modification is linked to an angiogenic action operating at the junction between the dermis and the subcutaneous tissue.

Some indications in this sense are provided by the transmission electron microscopic studies. It is intriguing to imagine that, in the aging skin, a functional disconnection between the microcirculation of the dermis and that of the subcutaneous tissue exists. The hypothesis that the new formation of microvessels could play a role in the modification observed after treatment is suggestive but requires further study for definitive confirmation.

Despite the limitations of this preliminary experience, we can consider as the preferred technique for facial rejuvenation the use of fat grafting plus stromal vascular fraction instead of expanded adipose-derived stem cells only, for three main reasons: (1) it is an easier method; (2) it is cheaper; and (3) it avoids the complications of the regulatory issues that are applied to the cell culturing.

CONCLUSIONS

This study demonstrates that treatment with fat and stromal vascular fraction or with expanded mesenchymal stem cells modifies the pattern of collagen and elastic fibers in the dermis. These approaches appear to be very promising for facial antiaging surgical techniques.

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