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Dermal morphological changes following salicylic acid peeling and microdermabrasion

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Summary

Background: Microdermabrasion and chemical peeling are popular, inexpensive, and safe methods for treatment of some skin disorders and to rejuvenate skin.

Objectives: To study the alterations of the dermal connective tissue following salicylic acid peeling and microdermabrasion.

Methods: Twenty patients were participated in our study. All participants underwent facial salicylic acid 30% peel or microdermabrasion (10 cases in each group) weekly for 6 weeks. Punch biopsies were obtained from the clinically normal skin of the right postauricular region 1 week before treatment (control group). Other punch skin biopsies were obtained 1 week after the end of the treatments from the left postauricular area. This region was treated in a similar way to the adjacent lesional skin (treated group). We used routine histological techniques (H&E stain), special stains (Masson trichrome and orcein stains), and image analyzer to study the alterations of the dermal connective tissues.

Results: Our study demonstrates variations in the morphological changes between the control and the treated groups, and between chemical peels and microdermabrasion. Both salicylic acid 30% and microdermabrasion were associated with thickened epidermal layer, shallow dermal papillae, dense collagen, and elastic fibers. There was a significant increase among those treated sites vs control regarding epidermal thickness and collagen thickness. Also, there was a highly statistically significant increase among those treated with salicylic acid vs microdermabrasion regarding the epidermal, collagen, and elastin thickness.

Conclusions: Both methods stimulate the repair process. The mechanisms underlying these variations are open for further investigations.

KEYWORDS
chemical peeling, collagen fibers, elastic fibers, microdermabrasion, salicylic acid peel

1 | INTRODUCTION

Microdermabrasion, a nonchemical noninvasive resurfacing modality, is largely used by plastic surgeons, dermatologists, and estheticians. The technique has been initially developed in Italy in 1985 and has been proved to be clinically effective.[1] Currently, microdermabrasion devices are used to renew and rejuvenate damaged skin. The repetitive intraepidermal injury (during microdermabrasion) causes gradual improvement in the damaged skin by stimulating fibroblast proliferation and collagen production leading to new collagen deposition in the dermis. Microdermabrasion is used in the management of fine rhytides, photoaging, solar lentigines, striae, ~~melisma~~, and mild surgical and acne scars.[2] The microdermabraders use a negative pressure or suction force to propel crystals of sodium bicarbonate, aluminum

oxide, or salt at varying velocities. The latter impinge on the epidermis, creating an injury with variable depth that is determined by several factors including the rate at which the handpiece is moved over the skin, the spot size, the velocity, and the volume flow of the crystals.[1]

Chemical peeling is a popular, relatively inexpensive, and generally safe method for treatment of some skin disorders as well as for refreshing and rejuvenating the skin. Chemical peels are used to create a skin injury of a specific depth with the goal of stimulating new skin growth and improving surface texture and appearance. Chemical peels are classified by the depth of action into superficial, medium, and deep peels. The depth of the peel is correlated with clinical changes and the greatest change being achieved by deep peels. However, the depth is also associated with longer healing times and the potential for complications. The exfoliative effects of the chemical peels stimulate new epidermal growth with more evenly distributed melanin and collagen deposition. Superficial peels, penetrating only the epidermis, are used to enhance treatment for a variety of conditions including acne, melasma, dyschromias, photodamage, and actinic keratoses. Medium-depth peels, penetrating to the papillary dermis, may be used for dyschromia, multiple solar keratoses, superficial scars, and pigmentary disorders. Deep peels, affecting reticular dermis, may be used for severe photoaging, deep wrinkles, or scars.[3]

Salicylic acid is a beta-hydroxy acid. It is a hydroxyl derivative of benzoic acid that represents a carboxylic acid attached to an aromatic alcohol, phenol. It is the only member of the beta-hydroxy acid family, and it is named so because the aromatic carboxylic acid has a hydroxy group in the beta position.[4] It has an excellent keratolytic effects. It functions through solubilization of the intercellular cement, so reducing corneocyte adhesion. Because of its lipophilic nature, it has a strong comedolytic effect. It affects the arachidonic cascade and thus exhibits anti-inflammatory capabilities.[5] Also, it has the added advantage of having a whitening effect.[6]

The application of chemical peelings and microdermabrasion is associated with morphological changes in the skin including alterations of the stratum corneum, cellular epidermis, epidermal thickness, collagen, and fibroblasts.[7-10] We previously reported that skin treated with microdermabrasion or chemical peels (glycolic acid 70% or Jessner's solution) showed the immunohistological features of wound healing and ultrastructural changes of cell injury. Also, we observed that the dermal microvessel density and the cell proliferation index have increased following treatment with glycolic acid, Jessner's solution, and microdermabrasion compared to untreated skin.[10]

To date, our knowledge about the changes in the dermal connective tissue (collagen and elastic fibers) following salicylic acid peeling and microdermabrasion is limited. We carried out this investigation to examine this issue.

2 | MATERIALS AND METHODS

This investigation was carried out at the Departments of Dermatology, Venereology and Andrology, Assiut University, and the Department of Histology of Sohag University, Egypt.

2.1 | Patients

A total of 20 healthy adult patients with cosmetic problems (mild-to-moderate acne and melasma) participated in this study. All the participants were in need of cosmetic treatment and therefore had undergone facial chemical peelings (salicylic acid 30% solution) and microdermabrasion (10 cases in each group) weekly for 6 weeks. The participants were initially introduced to the concept, advantages, and complications of the peeling procedures. Informed consents were obtained from the patients. Punch biopsies were obtained from the clinically normal skin of the right postauricular region 1 week before treatment. Other punch skin biopsies were obtained 1 week after the end of the treatments from the left postauricular area. This region was treated in a similar way to the adjacent lesional skin of the face. The specimens obtained before the start of treatment served as the control group, while samples obtained at the end of treatment represent the treated groups (salicylic acid solution-treated and microdermabrasion-treated groups). No biopsies were obtained from the lesions.

Salicylic acid 30% solution was used as a peeling agent. It was applied by cotton balls to the face and postauricular area till a white frost appears. Microdermabrasion was performed using Reviderm skin peeler professional (Germany). Two centimeter squared area of the postauricular skin was treated with microdermabrasion. The microdermabrasion device was set to a setting of (−8 psi), and three passes with the microdermabrasion handpiece were performed. Each subject was instructed to apply sunblock daily to minimize the risk of sun exposure and to prevent postinflammatory hyperpigmentation. After the last treatment, 2-mm punch biopsy specimens were obtained from the treated postauricular site.

2.2 | Histological and histochemical evaluations

Specimens were placed immediately in 10% neutral buffered formalin solution and processed for paraffin embedding. Histological examination of sections was carried out utilizing hematoxylin and eosin (H&E) as well as Masson trichrome and orcein stains. Sections were examined by light microscopy, and the following histopathological changes were assessed: the epidermal changes and the alterations of the dermal connective tissue fibers (collagen and elastic fibers). These changes were examined in at least five different sections in each case, by two observers.

2.3 | Quantification of the epidermal thickness and mitotic figures

The whole epidermal thickness was measured from the basal cell layer to the surface horny layer (using image analyzer). This was performed in 10 selected HPFs in 10 different sections for each case. The results were reported as mean±SD.

2.4 | Quantification of the percent area of the dermis stained with orcein and Masson trichrome

The percent area of the dermal fibers stained with Masson trichrome and orcein was determined by image analyzer. Two observers recorded the average percentage area of the papillary and the upper layer of reticular dermis stained with Masson trichrome and orcein stains. This was performed in 10 selected HPFs in 10 different sections for each case. The results were presented as mean (±SD) of the positive stained percent area.

2.5 | Statistical analysis

Statistical comparison among different groups was evaluated using analysis of variance (ANOVA). Calculations were performed with the statistical package IBM SPSS Statistics for Windows, Version 19.0. IBM Corp, Armonk, NY, USA. Statistical significance was defined as $P<.05$.

3 | RESULTS

3.1 | Histological features of the untreated skin

In the untreated skin, the epidermis is formed of stratified squamous epithelium with thin layer of keratin. It rests on an irregular basement membrane forming pegs and papillae. The average epidermal thickness as measured from the epidermal grooves to the surface was 44.00±3.99. The dermis is formed of fibrous connective tissue with thin layer of papillary dermis and a thicker layer of reticular dermis. Sebaceous glands are of moderate size and open by short ducts into the hair follicles (Figure 1A and Table 1). The collagen fibers appeared thin and few in the papillary dermis. They form a dense network of thin fibers in the upper reticular dermis and a less dense network of thicker fibers in the deep reticular dermis. The percentage area of collagen fibers in the papillary and upper reticular dermis was 45.35±2.46 (Figure 2A and Table 1). The elastic fibers form few thin branched individual fibers located perpendicular to the long axis of the epidermis in the papillary dermis and form patchy areas of networks in the upper layer of the reticular dermis and patches of dense network of thin fibers in the deep reticular dermis. The percentage area of elastic fibers in the papillary and upper reticular dermis was 33.05±1.80 (Figure 3A and Table 1).

3.2 | Alterations of the epidermal thickness and dermal connective tissue following chemical peelings (salicylic acid 30% solution)

The average epidermal thickness as measured from the epidermal grooves to the surface was significantly increased in the salicylic acid-treated group vs control (57.20±7.79 vs 44.00±3.99; $P<.001$). The dermal papillae appeared deep compared to the control group. The dermis was composed of loose connective tissue. The sebaceous glands appeared surrounded by numerous inflammatory cells (Figure 1B and Table 1). Masson trichrome-stained sections revealed that the percentage area of collagen fibers in the papillary and upper reticular dermis was significantly increased (63.72±8.76 vs 45.35±2.46 in the control group; $P<.001$; Figure 2B and Table 1). Orcein-stained sections revealed that the percentage area of elastic fibers in the papillary and upper reticular dermis was significantly increased (39.79±3.45 vs 33.05±1.80 in the control group; $P<.001$; Figure 3B and Table 1).

3.3 | Alterations of the epidermal thickness and dermal connective tissue following microdermabrasion

In microdermabrasion-treated group, the epidermal layer appeared thick at focal areas with relatively thickened overlying keratin. The dermal papillae appeared shallow compared to the control group. The sebaceous glands were large and surrounded by numerous inflammatory cells. The average epidermal thickness as measured from the epidermal grooves to the surface was significantly increased in microdermabrasion-treated group vs control (48.16±3.90 vs 44.00±3.99; $P<.05$; Figure 1C and Table 1). Masson trichrome-stained sections revealed that the collagen fibers appeared forming patchy small areas of thin and short networks scattered throughout the dermis. The percentage area of collagen fibers in the papillary and upper reticular dermis was 50.79±2.95 with statistically significant increase in microdermabrasion-treated group vs the control group (45.35±2.46; $P<.05$), (Figure 2C and Table 1). Orcein-stained sections revealed that the percentage area of elastic fibers in the papillary and upper reticular dermis was slightly decreased (31.04±2.18 vs 33.05±1.80 in the control group). The fibers appeared widely dispersed as individual fragmented fibers without forming any networks at most areas (Figure 3C and Table 1).

3.4 | Alterations of the epidermal thickness and dermal connective tissue were more pronounced following chemical peelings as compared to microdermabrasion

When we compared the treated groups, we found that alteration of epidermal thickness and dermal collagen and elastic fibers were more pronounced in the salicylic acid-treated group as compared to microdermabrasion group ($P<.001$).

4 | DISCUSSION

Skin rejuvenation technologies include many physical (eg, microdermabrasion) and chemical (eg, salicylic acid solution) intervention methods. These techniques induce tissue damage and provide beneficial biochemical stimuli for skin re-epithelization and rejuvenation.[11] When peel agents reach the dermis, important wound-healing activities occur that cause skin remodeling and smoothing.[12] To the best of our knowledge, there are no available reports to evaluate the histological changes after the application of 30% salicylic acid as a

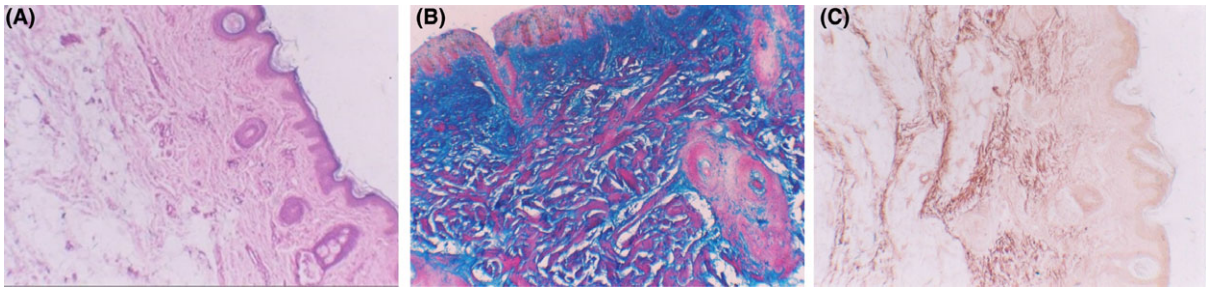

FIGURE 1 A: A photomicrograph of a part of control skin stained with H&E showing The epidermis (E) formed of stratified squamous epithelium with thin layer of keratin lying on an irregular basement membrane. The dermis is formed of fibrous connective tissue thin layer of papillary dermis (P) and a thicker layer of reticular dermis (R). Sebaceous glands (S) are moderate in size and open by short ducts into the hair follicles. B: A photomicrograph of a part of control skin stained with Masson trichrome showing thin and few collagen fibers in the papillary dermis (P) a dense network of thin fibers in the upper reticular dermis (R) and a less dense network of thick fibers in the deep reticular dermis (D). C: A photomicrograph of a part of control skin stained with orcein showing thin long branched elastic fibers in the papillary dermis (P) and in the upper layer of the reticular dermis (R) and patches of dense network of thin fibers in the deep reticular dermis (D)

TABLE 1 Histological features following salicylic acid peeling and microdermabrasion

Aspects	Epidermal thickness	Masson trichrome-stained area	Orcein-stained area
Control skin	44.00±3.99	45.35±2.46	33.05±1.80
Salicylic acid	57.20±7.79	63.72±8.76	39.79±3.45
Microdermabrasion	48.16± 3.90	50.79±2.95	31.04±2.18
P value	<.05	<.05	<.05

peeling agent on human skin. Nor have the variations in the morphological changes between salicylic acid peels vs microdermabrasion been investigated. In the present study, we used histochemical stains and image analyzer to address these issues. Our study revealed three important findings: (i) alterations of the epidermal thickness and dermal connective tissue following chemical peelings (salicylic acid 30% solution), (ii) alterations of the epidermal thickness and dermal connective tissue following microdermabrasion, and (iii) alterations of the epidermal thickness and dermal connective tissue were more pronounced following chemical peelings as compared to microdermabrasion.

4.1 | Alterations of the epidermal thickness and dermal connective tissue following chemical peelings (salicylic acid 30% solution)

Our findings following the application of salicylic acid 30% not only concur with previous studies.[10,13] but also suggest that both methods seem to stimulate the repair process so that skin defects are replaced with organized tissue. Imayama et al.[13] examined the histological changes following the administration of salicylic acid in hairless mice. They reported loss of cornified cells followed by the activation of the epidermal basal cells and the underlying fibroblasts. The authors proposed that the epithelial cells secrete factors that regulate the formation of connective tissue component as collagen and modulates construction of collagen fibers by arranging glucosaminoglycans along the basement membrane. The dynamic and proliferative changes in the overlying epidermis can stimulate the underlying fibroblasts.[13] Hussein et al. reported an increased thickness of the epidermis and density of the collagen fibers, angiogenesis, prominent fibroblasts, and high dermal lymphohistiocytic cell infiltrate in the skin treated by chemical peeling agents such as glycolic acid 70% or Jessner's solution. Similarly, Butler et al.[14]

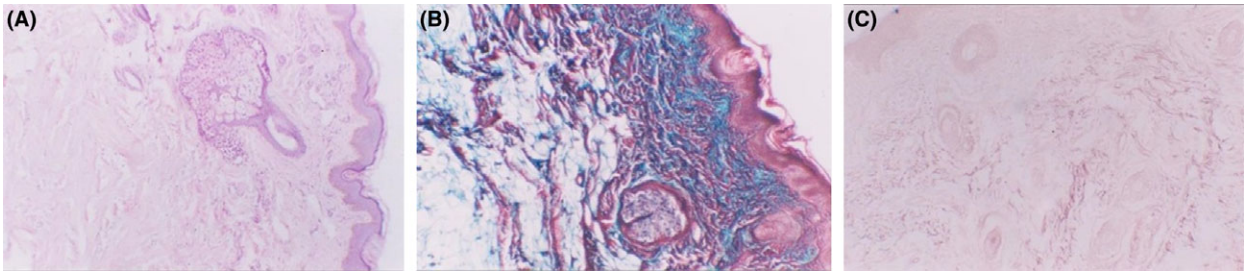

FIGURE 2 A: A photomicrograph of a part of microdermabrasion treated skin stained with H&E showing thickened epidermal layer (E) at focal areas with relatively thickened overlying keratin and relatively shallow dermal papillae. The sebaceous gland (S) is large and surrounded by numerous inflammatory cells B: A photomicrograph of a part of microdermabrasion treated skin stained with Masson trichrome showing relatively fewer number of collagen fibers in the papillary (P) upper reticular (R) and deep reticular (D) dermis. C: A photomicrograph of a part of microdermabrasion treated skin stained with orcein showing relatively fewer number of elastic fibers in the papillary (P) and in the upper reticular (R) dermis and few and fragmented fibers in the deep reticular (D) dermis

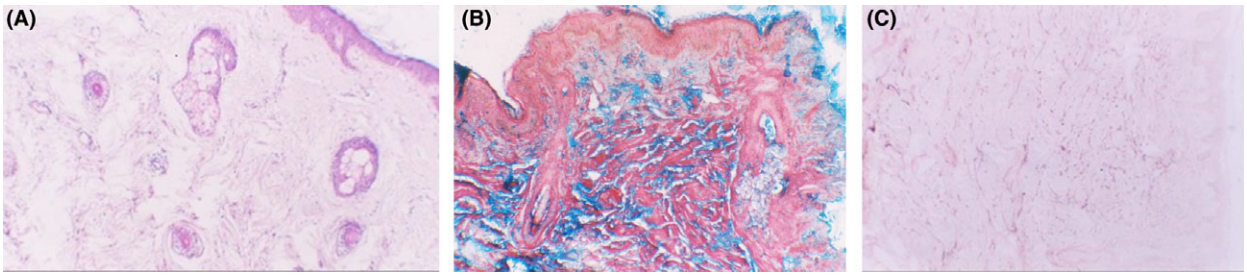

FIGURE 3 A: A photomicrograph of a part of salicylic acid solution treated skin stained with H&E showing shallow dermal papillae and loose connective tissue dermis ~~(D).~~ The sebaceous glands (S) are surrounded by numerous inflammatory cells. B: A photomicrograph of a part of salicylic acid solution treated skin stained with Masson trichrome showing patchy small areas of thin, few and short scattered networks of collagen fibers throughout the papillary ~~(P),~~ upper reticular ~~(R)~~ and in the deep reticular ~~(D)~~ dermis. C: A photomicrograph of a part of salicylic acid solution treated skin stained with orcein showing few number of widely dispersed individual and fragmented elastic fibers in the papillary ~~(P),~~ upper reticular ~~(R)~~ and deep reticular ~~(D)~~ dermis.

observed an increase in the dermal thickness and collagen content following the application of chemical peeling using 30% and 50% trichloroacetic acid and phenol solution. ~~El-Samahy et al., 1998, reported several histological and ultrastructural changes following peeling (using 70% glycolic acid and 35% trichloroacetic acid) including markedly decreased epidermal intracytoplasmic vacuoles, decreased elastic fibers, increased activated fibroblasts, and organized parallel arrays of collagen fibrils.~~[14]

4.2 | Alterations of the epidermal thickness and dermal connective tissue following microdermabrasion

In the current study, we observed epidermal thickening, shallow dermal papillae, and dermal inflammatory cell infiltrate with significant increase in collagen fibers and slight decrease in elastin fibers following microdermabrasion. These results are in agreement with other groups.[15-19] El-Domyati et al. ~~2016~~ reported a significant increase in epidermal thickness and increased collagen bundle density following the application of four sessions of microdermabrasion.[19] These changes were reasoned to the induction of type I and type III procollagen expression. The repetitive injury stimulates new epidermal growth and collagen, and activation of transcription factors, activator protein-1 (AP-1) and nuclear factor (NF)-kB that play critical role in regulation of expression of many genes involved in wound healing, growth, and differentiation.[20,21] Freedman et al. ~~2002~~ reported epidermal thickening with basal cell hyperplasia and increased mitotic activity, flattening and widening of the rete pegs, papillary dermal thickening with deposition of collagen and elastic fibers, and perivascular inflammation in the dermis after six rounds of treatments with microdermabrasion. Coimbra et al. examined the degree of visible improvement in photodamaged skin and fine rhytides following a series of microdermabrasion. In agreement with our results, they reported an increase in the epidermal thickness and organized collagen in the treated skin relative to the control group.[16] These findings suggest that the repetitive intraepidermal and dermal injuries that occur due to microdermabrasion enhance fibroblast proliferation and collagen production, leading to new collagen deposition in the dermis.[20,22]

Here, we report the dermal morphological changes following salicylic acid peeling and microdermabrasion. Our study indicates that the use of these agents is associated with changes consistent with wound healing and cell injury. Future studies are needed to elucidate the molecular events underlying these changes.

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