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Original Article	**Evaluation of Two Medium-Depth Peels: Glycolic Acid 70% Versus Trichloroacetic Acid 35%: A Histological and Immunohistochemichal Study on Rat Skin**

Soheir A. Filobbos, Nagla M. Salama, Rahma K. Abo-Elnour and Eman A. Farag

Histology Department, Faculty of medicine, Cairo University

ABSTRACT

Background: Chemical peels represent useful tool for improving skin texture and the effects of ageing. With the advent of newer chemical peels, there is now a wide range of peeling agents that can be applied on specific patients.

Aim of the work: The present work was designed to evaluate and compare histologically between two of the most commonly used peeling agents in medium depth chemical peel: TCA (35%) and GA (70 %).

Materials and Methods: This study was carried out on forty adult male albino rats. They were divided into five groups, eight rats each. Group I: Control rats that received no peeling session. Groups Π and ΠΙ: Received a peel session of TCA 35% and skin specimens were taken after one week and three weeks respectively. Groups IV and Group V: Received a peel session of glycolic acid 70% and skin specimens were taken after one week and three weeks respectively.

Sections were prepared to be stained by H&E, Masson's trichrome, Orcein and immunohistochemichal staining for CD34, which is a marker for stem cells in hair follicle bulge region. Morphometric measurements of epidermal thickness, dermal thickness, area percent of collagen and elastic fibers, area percent and optical density of immunopositive cells for CD34 were done by image analyzer. Data obtained were statistically analyzed.

Results: The present study revealed that after one week of medium depth chemical peel (by TCA (35%) or GA (70 %), the skin showed increase in epidermal and dermal thickness, increase in elastic and collagen fibers which became more organized and regularly arranged. It also showed pronounced increase in CD34 immunopositive cells (hair bulge stem cells, spindle cells of the reticular dermis, endothelial cells of dermal vessels and sebaceous glands). Three weeks after the chemical peel, the skin showed further significant increase in epidermal thickness, dermal thickness, collagen and elastic fibers. However, there was marked decrease in area percent and optical density of CD34 immunopositive cells.

Conclusion: The present study revealed that both TCA (35%) and GA (70%) are effective in stimulation bulge stem cells and in improving the skin morphology with no significant difference between the two reagents.

Key Words: Chemical peel, glycolic acid, trichloroacetic acid, skin, cd34, hair follicles.

Corresponding Author: Dr. Nagla M. Salama, Histology department, Faculty of medicine, Cairo, University. Cairo, Egypt, Email: nag_salama@hotmail.com, Mobile: 01224262375

INTRODUCTION

Chemical peeling is a method by which concentrated chemicals are applied to the skin to peel off the epidermis and dermis. This results in regeneration of the dermis and epidermis with synthesis of new collagen and elastic fibers in the dermis *(Bukvić Mokos & Lipozencić, 2009)*.

Chemical peeling can be used to enhance treatment within a variety of conditions, including acne, melasma, dyschromias, photodamage and actinic keratoses. In addition, peels can be combined with other in-office procedures to optimize outcomes and enhance patient satisfaction *(Berson et al., 2009)*.

The explosion of interest in chemical peeling on the part of dermatologists has paralleled the general public's interest in acquiring a youthful appearance *(Monheit, 2004)*.

Chemical peeling agents were classified as very superficial (exfoliation), superficial (epidermal), medium (papillary dermal) and deep (reticular dermal) *(Wiest, 2004)*. Medium-depth chemical peeling is defined as controlled damage to the epidermis and papillary dermis *(Bukvić Mokos & Lipozencić, 2009)*.

Medium-depth chemical peeling can be achieved by using glycolic acid (GA) 70% or trichloroacetic acid (TCA) 35% *(Mendlsohn, 2002)*. Trichloroacetic acid is one of the commonly utilized agents for chemical resurfacing of the face *(Herbig et al., 2009)*. Glycolic acid is a member of the α-hydroxy acids (AHA) family, which occurs naturally in foods and has been used for a long time as a cutaneous rejuvenation treatment. It is used in many cosmetic products as an exfoliant and moisturizer *(Yener & Baitokova, 2006; Bhattacharyya et al., 2009)*.

The epidermis is a continuously renewing tissue that is replenished and repaired by epithelial stem cells (SCs). Several lines of evidence indicate that during high proliferative need, an important source of the proliferating cells in the epidermis are follicular epithelial SCs that reside within a specialized permanent segment of the outer root sheath of hair follicle known as the bulge *(Lavker & Sun, 2000; Ito et al., 2005)*. Bulge SCs have been viewed for years as the major source of cells not only for the regeneration of hair follicles but also for regeneration and repair of epidermis. These follicular bulge SCs play an important role in the epidermal regeneration after physical or chemical removal of epidermis, superficial and full thickness skin wounding, or burns *(Taylor et al., 2000; Ito et al., 2005; Levy et al., 2005)*.

CD34 antibody was used to immunostain keratinocytes stem cells. This antibody was shown to be the best marker for stem cells in hair follicle bulge region *(Trempus et al., 2003; Cotsarelis, 2006)*.

The aim of this study was to compare the histological effects of GA 70% and TCA 35% in Medium-depth chemical peeling on rat skin and compare between the abilities of TCA and GA to stimulate hair follicle bulge stem cells during wound healing.

MATERIALS AND METHODS

a) Drugs:

- **Trichloroacetic acid** was supplied by "Delasco Dermatology Lab and Supply" in the form of a bottle (60 ml) of (TCA solution 35%).

- **Glycolic acid** was supplied by "Delasco Dermatology Lab and Supply" in the form of a bottle (120 ml) of (GA solution 70%).

b) Animals:

This study included 40 adult male albino rats, 150-180 gram body weight. They were divided into five groups, eight rats each. They were housed in hygienic stainless steel cages and kept in clean well ventilated room in animal house according to the guidelines of Animal Ethics Committee. They were fed standard chow diet and allowed free access to water:

Group I (control group): Received a pre-treatment cleansing step only (no peeling session). Skin biopsies were taken from two rats with each experimental group.

Group II: Received a peel session of TCA 35% and skin biopsies were taken after 1 week.

Group III: Received a peel session of TCA 35% and skin biopsies were taken after 3 weeks.

Group IV: Received a peel session of glycolic acid 70% and skin biopsies were taken after 1 week.

Group V: Received a peel session of glycolic acid 70% and skin biopsies were taken 3 weeks thereafter.

- All animals received pre-treatment cleansing step after epilation of hairs. The skin was cleansed with alcohol then washed with water and dried.

- According to *Roberts (2004)*, TCA (35%) solution was applied, using cotton-tipped

applicator, to the dorsal left side of the rats (group II and group III) until appearance of level II frosting (white-coated frosting with erythema showing through) and then neutralized with copious amounts of water.

- GA (70 %) was applied to the dorsal left side of the rats (group IV and group V) until erythema appeared and then neutralized with a 5% solution of sodium bicarbonate followed by copious amounts of water.

Under chloroform anaethesia, Skin specimens from the dorsal left side were taken from rats of group II and group IV after one week and from rats of group III and group V after three weeks. The dimensions of the specimens were about 1x1x0.3 cm. They were fixed in 10% buffered formalin solution for 24-48 hours, dehydrated in ascending grades of ethanol and embedded in paraffin. Serial sections of 5 µm thickness were cut and were subjected to the following stains: H&E *(Kiernan, 2001)*, Masson's trichrome for collagen fibres *(Bancroft & Gamble, 2008)* and Orcein stain for elastic fibers *(Kiernan, 2001)*.

Immunohistochemistry:

Immunohistochemichal staining for CD34, which is a marker for stem cells in hair follicle bulge region of rats, was done according to *Bancroft and Cook (1994)*. The primary antibody used was a mouse monoclonal antibody (CD34 protein Ab-1) supplied by "Lab Vision Corporation laboratories, CA 94539, USA, catalogue number MS-363-R7" followed by antibody detection system (Histostain SP kit, Zymed Laboratories Inc, San Francisco, CA 94080, USA, catalogue number 95-9643). For negative control, the primary antibody was replaced by phosphate buffer saline. Positive tissue control was a specimen of human tonsil immunostained for CD34.

Morphometric study:

Using "Leica Qwin 500 C" image analyzer computer system Ltd. (Cambridge, England), measurements of epidermal thickness, dermal thickness, area percent of collagen and elastic fibers and area percent and optical density of immunopositive cells for CD34 were done. All measurements were taken in 10 non-overlapping randomly chosen fields for each animal.

Stastistical analysis:

Data obtained were analyzed using SPSS software version 9. Comparison between different groups was made using analysis of variance (ANOVA). Results were considered statistically significant when p was <0.05 *(Armitage & Berry, 1994)*.

RESULTS

Histological results:

A. **Hematoxylin and Eosin stained skin sections**

Group I (Control): Examination of skin sections from the control group (group I) revealed normal layers of the skin.The epidermis was composed of stratified squamous epithelium. The stratum basal was prominent formed of a single layer of basophilic columnar cells, whereas the stratum spinosum was formed of 2-3 layers of cuboidal cells with central rounded nuclei. The stratum granulosum was formed of flattened cell layer with flattened nuclei. Langerhans' cells with cytoplasmic halo were occasionally seen. The stratum corneum represented the superficial keratin layer. The border between the epidermis and dermis was clearly demarcated (Fig. 1).

The dermis consisted of densely packed, variably oriented, thick bundles of collagen. Clusters of hair follicles and associated sebaceous glands were evident.

Hair follicles were observed in mid- to upper dermis and also some follicles were seen resting within the stratum adiposum**.** They were cut in different directions and appeared composed of medullary and cortical remnant surrounded by a homogenous layer of the inner root sheath, followed by cells forming the broad outer root sheath. This outer root sheath was surrounded by a thick, vascular connective tissue sheath belonging to the dermis and separated from it by the glassy membrane (Figs. 1, 2).

The sebaceous glands were associated with hair follicles and consisted of lobules of epithelial cells that had foamy acidophilic cytoplasm with rounded central nuclei (Fig. 1).

Group II: (1 week after a peel session of TCA 35%): Examination of skin sections of group II revealed that after one week, the re-

epithelization process started and the epidermis started to regenerate. The underlying dermis showed normal thickness (Fig. 3).

Group III: (3 weeks after a peel session of TCA 35%): The epidermis showed further increase in the thickness of cellular layers and keratin layer as compared with that of the control group and group II. Langerhans cells with cytoplasmic halo were occasionally seen among the cells of the prickle and granular layers. The superficial ducts of the sweat glands opened by different openings within the epidermis. The underlying dermis showed marked increase in cellularity especially in papillary dermis with denser arrangement of its fibers. The striking finding was the presence of numerous blood capillaries both in papillary and reticular dermis (Fig. 4).

Group IV: (1 week after a peel session of glycolic acid 70%): Examination of skin sections of group IV revealed that after one week, the reepithelization process started and the epidermis started to regain its thickness. The underlying dermis showed increase in thickness (Fig. 5).

Group V: (3 weeks after a peel session of glycolic acid 70%): The epidermis showed further increase in the thickness of cellular layers and keratin layer as compared with that of the control group and group IV. Langerhans cells with cytoplasmic halo were occasionally seen among the cells of the prickle and granular layers. The underlying dermis showed marked increase in cellularity especially in papillary dermis with denser arrangement of its fibers (Fig. 6).

B. Masson's trichrome stained skin sections

In group I (Control), the dermis appeared of normal thickness formed of blue stained bundles of collagen fibers that interlaced with each other in a network manner (Fig. 7). In group Π, the dermis showed densely arranged collagen fibers that resulted in increase in dermal thickness as compared with the control group (Fig. 8). The dermis of group ΠΙ showed the most dense and more regularly arranged thick collagen fibers that resulted in marked increase in dermal thickness as compared with both the control group and group II (Fig. 9). In group IV, the dermis showed more densely arranged collagen fibers as compared with the control group (Fig. 10). The dermis of group V showed densely and regularly arranged thick collagen bundles that resulted in marked increase in dermal thickness as compared with the control group and group IV (Fig. 11).

C. Orcein stained skin sections

In group I (Control), elastic fibers appeared in the dermis as thin branching dark brown fibers (Fig. 12). The dermis of group II showed moderate increase in elastic fibers as compared with the control group (Fig. 13). The dermis of group III showed the most marked increase in elastic fibers as compared with the control group and group II (Fig. 14). In group IV, there was slight increase in elastic fibers as compared with the control group (Fig. 15). In group V, the dermis showed marked increase in elastic fibers as compared with the control group and group IV (Fig. 16).

Immunohistochemical results

Group I (Control): Moderate localized CD34 immunoreactivity was detected at the Outer Root Sheath (ORS) of hair follicles, in spindle cells interspersed between the collagen bundles of the reticular dermis and in endothelial cells of dermal vessels (Fig. 17).

Group Π: Dense CD34 immunoreactivity was detected throughout the outermost layer of the external root sheath of the hair follicles. Widespread CD34 immunoreactivity was also detected in spindle cells of the reticular dermis and endothelial cells of dermal vessels. Sebaceous glands showed dense CD34 immunostaining (Figs. 18, 19).

Group ΠΙ: Localized mild to moderate CD34 immunoreactivity was detected at the outermost layer of the external root sheath of the hair follicles. Mild positive immunoreactivity was also detected in the cells of sebaceous glands, spindle cells of the reticular dermis and in endothelial cells of dermal vessels (Fig. 20).

Group IV: Widespread dense immunoreactivity was detected at the outermost layer of the external root sheath of the hair follicles and in endothelial cells of dermal vessels. Many CD34 Immunopositive spindle cells were seen in the reticular dermis. Sebaceous glands showed dense positive CD34 immunoreactivity (Fig. 21).

Group V: Localized mild to moderate immunoreactivity was detected at the outermost layer of the external root sheath of the hair follicles. Mild to moderate positive immunoreactivity was also seen in the cells of sebaceous glands, spindle cells of the reticular dermis and in endothelial cells of dermal vessels (Fig. 22).

Morphometric results:

The means and standard deviation of all morphometric parameters are represented in Tables (1, 2). Analysis of morphometric data revealed the followings:

Trichloroacetic acid (35%) treated groups:

Compared to the control group, group II (1 week after TCA peeling) and group III (3 weeks after TCA peeling) showed significant increase in the epidermal thickness with more pronounced increase three weeks after TCA peeling. The difference between group II and group III was statistically significant.

The dermal thickness, the area percent of collagen fibers and the area percent of elastic fibers showed non significant increase in group II but significant increase in group III (as compared with the control group). The difference between group II and group III was statistically significant.

Regarding the area percent of CD 34 immunopositive cells, it showed significant increase in group II and non significant increase in group III as compared with the control group. The difference between group II and group III was statistically significant.

The optical density of CD 34 immunopositive cells showed significant increase in group II and in group III when compared with the control group. The difference between group II and group III was non significant.

Glycolic acid (70%) treated group:

The epidermal thickness, The dermal thickness, the area percent of collagen fibers and the area percent of elastic fibers showed non significant increase in group IV (1 week after GA peeling) but significant increase in group V (3 weeks after GA peeling) as compared to the control group. Apart from the area percent of elastic fibers, the difference between group IV and group V was statistically significant.

The area percent of CD 34 immunopositive cells showed significant increase in group IV and non significant increase in group V as compared with the control group. The difference between group IV and group V was statistically significant.

The optical density of CD 34 immunopositive cells showed significant increase in group IV and in group V when compared with the control group. The difference between group IV and group V was non significant.

- Comparing group II (1 week after TCA peeling) with group IV (1 week after GA peeling), there was non significant difference between the values of epidermal thickness, dermal thickness, area percent of collagen and elastic fibers and area percent of CD 34 immunopositive cells but significant difference in the optical density of CD 34 immunopositive cells.

- Comparing group III (3 weeks after TCA peeling) with group V (3 weeks after GA peeling), there was non significant difference between the values of epidermal thickness, dermal thickness, area percent of collagen fibers, area percent of CD 34 immunopositive cells and the optical density of CD 34 immunopositive cells but significant difference in the area percent of elastic fibers.

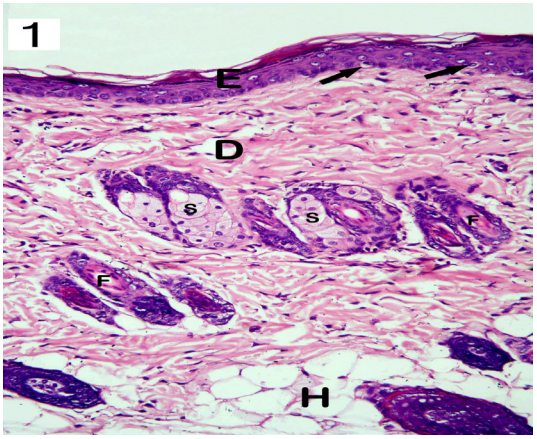

Fig. 1: Photomicrograph of a section in the skin of an albino rat from control group, the upper surface is covered by the epidermis (E) and Langerhans cells with cytoplasmic halo are seen (arrows). Many hair follicles (F), sebaceous glands (S) are seen within the dermis (D) and within the hypodermis (H). Hx.&E.; x200

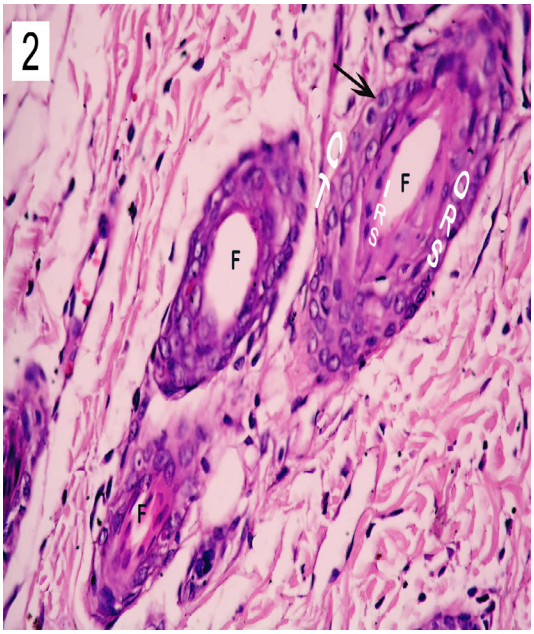

Fig. 2: Photomicrograph of a section in the skin of an albino rat from control group, showing an oblique section in the hair follicles (F). Note the broad outer root sheath (ORS) which is separated from connective tissue sheath (CT) by the glassy membrane (arrow). Part of the inner root sheath (IRS) is recognized by its homogenous appearance. Hx.&E.; x400

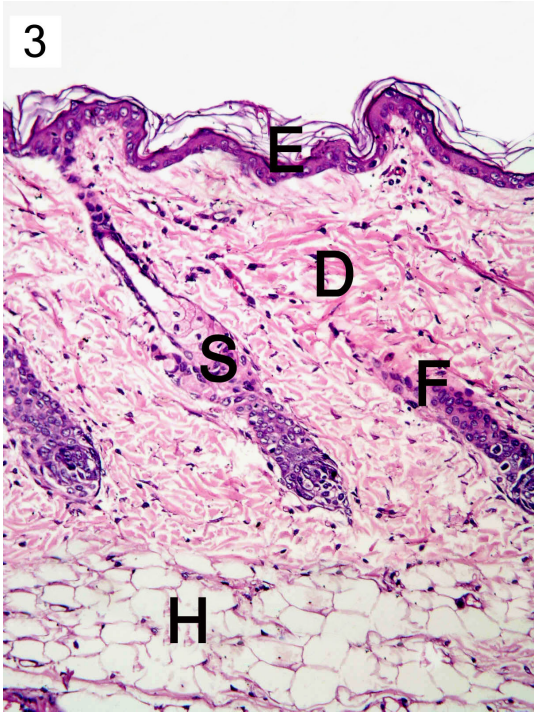

Fig. 3: Photomicrograph of a section in the skin of an albino rat from group Π (1 week after TCA 35% peel session) showing thin epidermis (E). Note the dermis (D) regained its normal thickness. The hypodermis (H), hair follicles (F) and sebaceous glands (S) are seen. Hx.&E.; x200

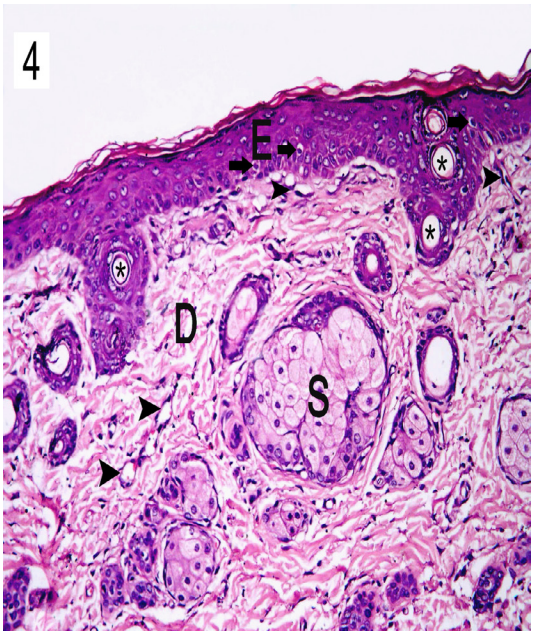

Fig. 4: Photomicrograph of a section in the skin of an albino rat from group III (3 weeks after TCA 35% peel session) showing marked increase in the thickness of the epidermis (E). Langerhans cells with cytoplasmic halo are seen (arrows). The dermis (D) shows denser and more regularly arranged connective tissue, numerous blood capillaries both in papillary and reticular dermis (arrow heads). Ducts of the sweat glands are seen in the epidermis (*) and sebaceous glands (S) are seen in the dermis. Hx.&E.; x200

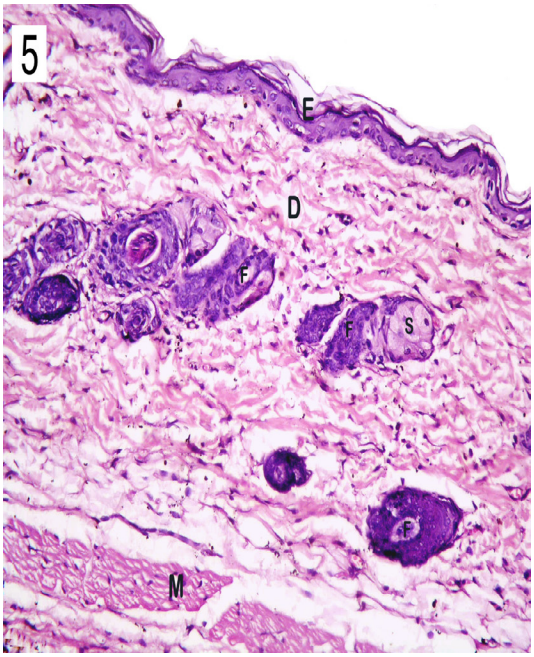

Fig. 5: Photomicrograph of a section in the skin of an albino rat from group IV (1 week after glycolic acid 70% peel session) showing thin epidermis (E). Note the dermis (D) regained its normal thickness. Hair follicles (F), sebaceous glands (S) and panniculus muscle (M) are seen. Hx.&E.; x200

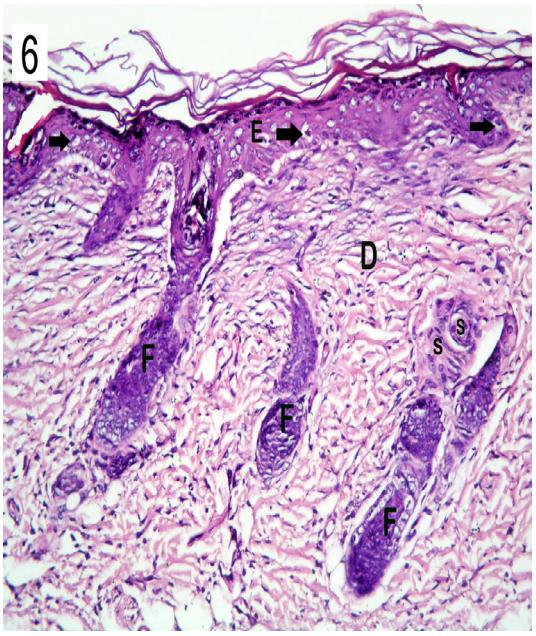

Fig. 6: Photomicrograph of a section in the skin of an albino rat from group V (3weeks after glycolic acid 70% peel session) showing marked increase in the thickness of the epidermis (E). Langerhans cells with cytoplasmic halo are seen (arrows).The dermis (D) shows denser and more regularly arranged connective tissue. Hypercellularity of papillary dermis is seen. Hair follicles (F) and sebaceous gland (S) are seen in the dermis. Hx.&E.; x200

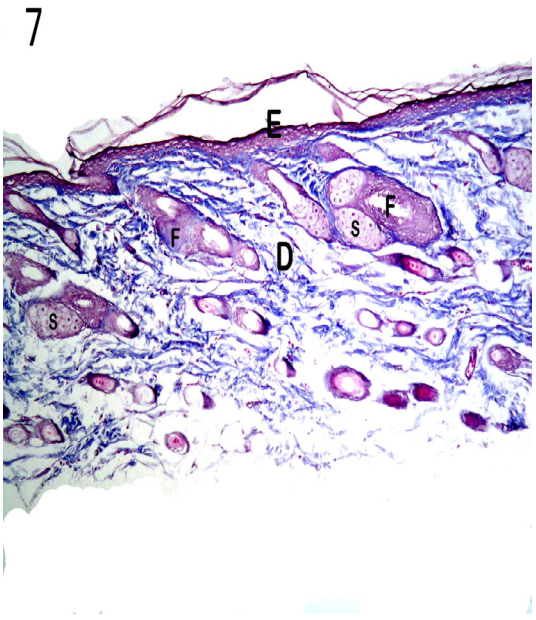

Fig. 7: Photomicrograph of a section in the skin of an albino rat from control group showing collagen fibers that form most of the thickness of the dermis (D). The upper surface is covered by thin epidermis (E). Sebaceous glands (S) are seen in contact with hair follicles (F). Masson trichrome; x100

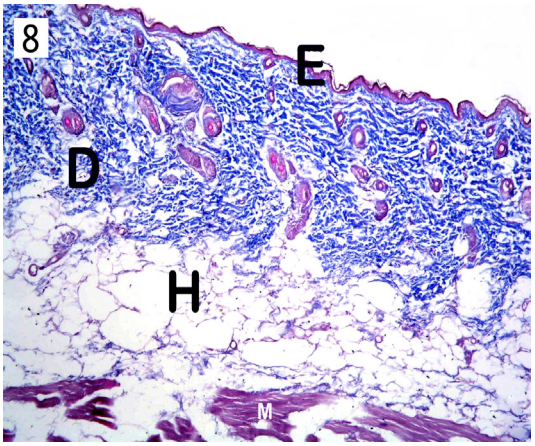

Fig. 8: Photomicrograph of a section in the skin of an albino rat (group II) showing increased thickness of the dermis (D) with densely arranged collagen fibers. The epidermis (E), hypodermis (H) and panniculus muscle (M) are seen. Masson trichrome; x100

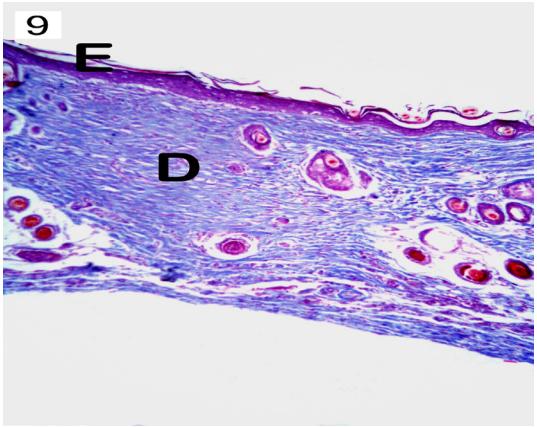

Fig. 9: Photomicrograph of a section in the skin of an albino rat (group III) showing more increase in the thickness of the dermis (D). It shows dense regularly arranged collagen fibers. The upper surface is covered by the epidermis (E). Masson trichrome; x100

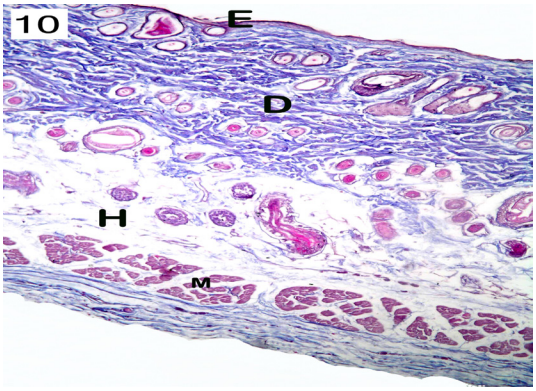

Fig. 10: Photomicrograph of a section in the skin of an albino rat (group IV) showing dense collagen of the dermis (D). The upper surface is covered by thin epidermis (E). The hypodermis (H) and panniculus muscle (M) are seen. Masson trichrome; x100

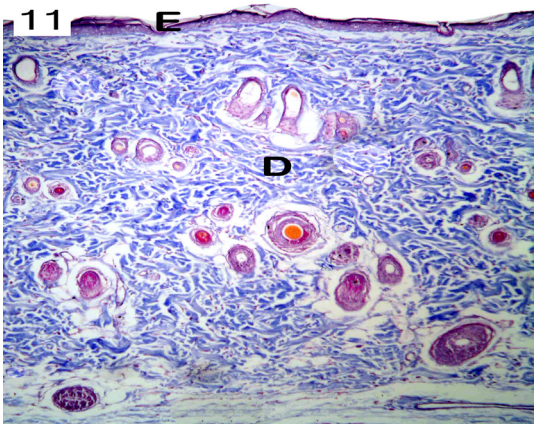

Fig. 11: Photomicrograph of a section in the skin of an albino rat (group V) showing increased thickness of the dermis (D) which is formed mainly of regularly arranged collagen bundles. The upper surface is covered by the epidermis (E). Many hair follicles (F) and Sebaceous glands (S) are seen in the dermis. Masson trichrome; x100

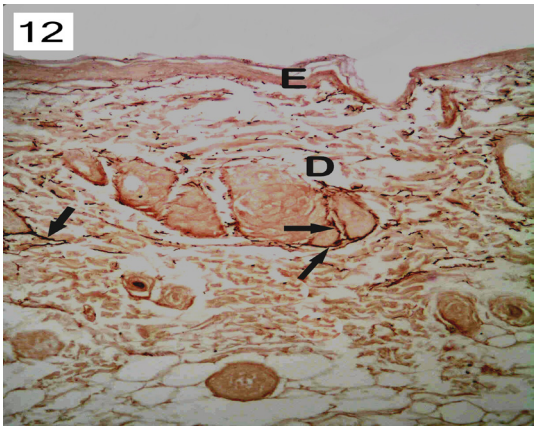

Fig. 12: Photomicrograph of a section in the skin of an albino rat from control group (group I) showing scattered elastic fibers (arrows) in the dermis (D). The upper surface is covered by the epidermis (E). Orcein; x200

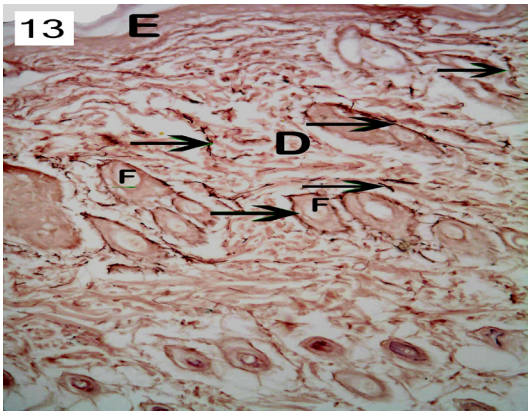

Fig. 13: Photomicrograph of a section in the skin of an albino rat (group II) showing increased elastic fibers (arrows) in the dermis (D). The upper surface is covered by the epidermis (E). Many hair follicles (F) are seen in the dermis surrounded by elastic fibers. Orcein; x200

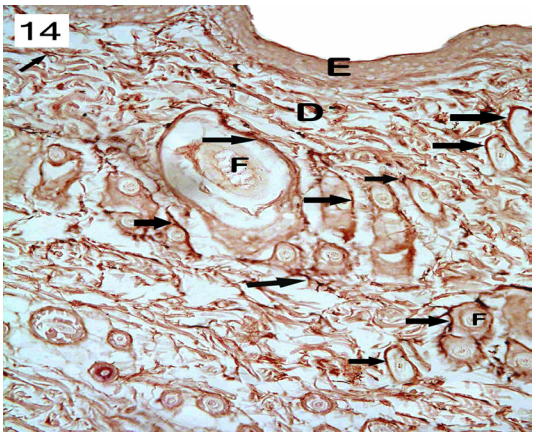

Fig.14: Photomicrograph of a section in the skin of an albino rat (group III) showing marked increase in elastic fibers (arrows) in the dermis (D). The upper surface is covered by the epidermis (E). Many hair follicles (F) are seen in the dermis surrounded by elastic fibers. Orcein; x200

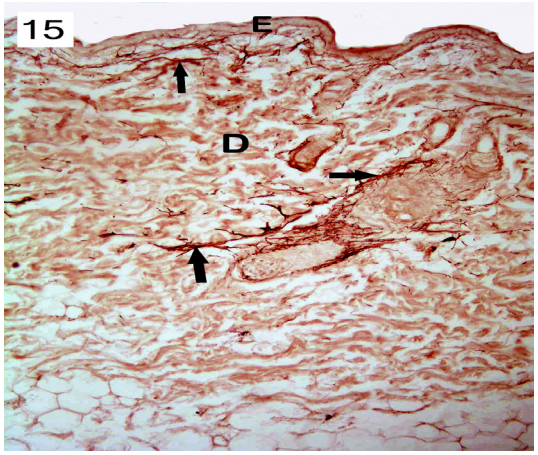

Fig. 15: Photomicrograph of a section in the skin of an albino rat (group IV) showing increased elastic fibers (arrows) in the dermis (D). The upper surface is covered by the epidermis (E). Orcein; x200

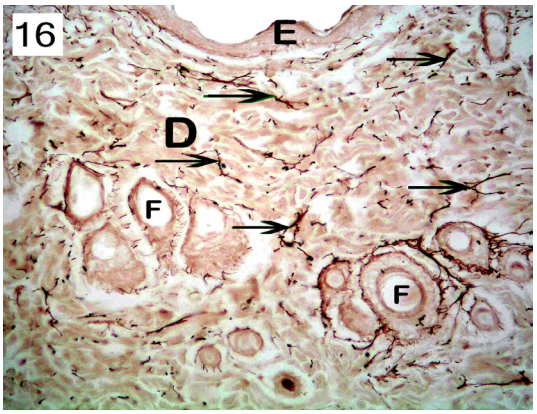

Fig. 16: Photomicrograph of a section in the skin of an albino rat (group V) showing more increase in elastic fibers (arrows) in the dermis (D). The upper surface is covered by the epidermis (E). Many hair follicles (F) surrounded by elastic fibers are seen in the dermis. Orcein; x200

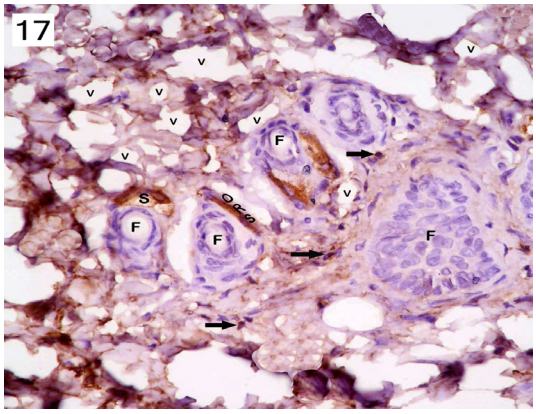

Fig. 17: Photomicrograph of a section in the skin of an albino rat (control group) showing moderate localized immunoreactivity in the cells of the outermost layer of the (ORS), endothelial cells of dermal vessels (V) and spindle cell of the reticular dermis (arrows). Immunohistochemical stain for CD 34; x400

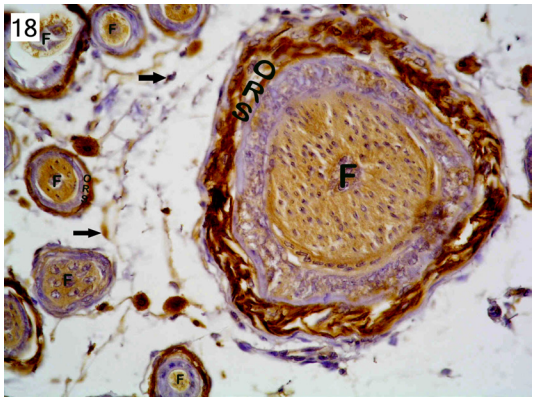

Fig. 18: Photomicrograph of a section in the skin of an albino rat (group II) showing many hair follicles (F). Widespread dense immunoreactivity is seen throughout (ORS) of almost all the follicles. Note the immunopositive spindle cells of the reticular dermis (arrows). Immunohistochemical stain for CD 34; x400

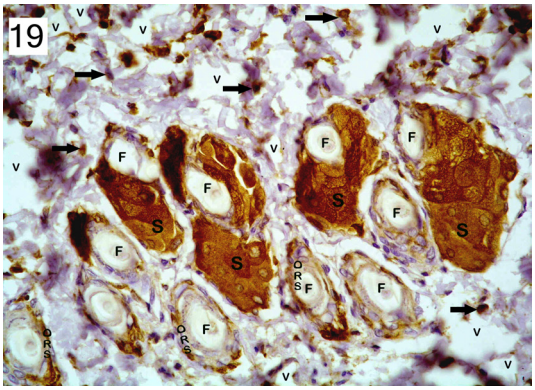

Fig. 19: Photomicrograph of a section in the skin of an albino rat (group II) showing widespread dense immunoreactivity is seen throughout (ORS) of nearly all the hair follicles (F). Note the marked increase in immunopositive spindle cells of the reticular dermis (arrows) and in endothelial cells of dermal vessels (V). Dense positive immunoreactivity is seen in the cells of sebaceous glands (S). Immunohistochemical stain for CD 34; x400

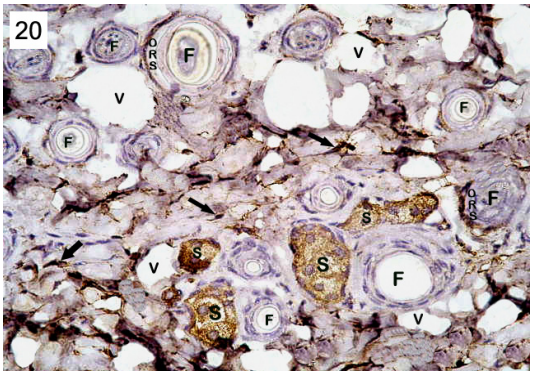

Fig. 20: Photomicrograph of a section in the skin of an albino rat (group III) showing multiple hair follicles (F) with localized mild immunoreactivity in the cells of the outer root sheath (ORS). Some immunopositive spindle cells of the reticular dermis (arrows) and endothelial cells of dermal vessels (V) are seen. Mild immunoreactivity is seen in the cells of sebaceous glands (S). Immunohistochemical stain for CD 34; x400

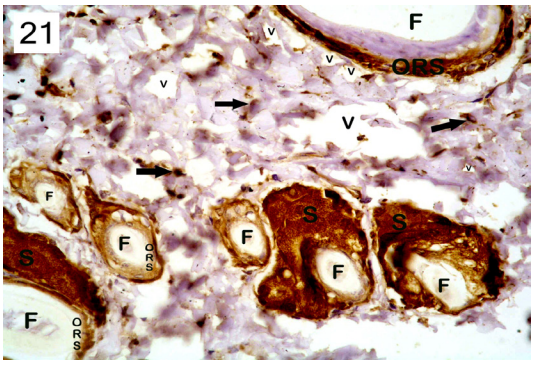

Fig. 21: Photomicrograph of a section in the skin of an albino rat (group IV) showing many hair follicles (F) with widespread dense immunoreactivity throughout (ORS) of nearly all the follicles. Note the marked increase in immunopositive spindle cells of the reticular dermis (arrows) and in endothelial cells of dermal vessels (V). Dense positive immunoreactivity is seen in the cells of sebaceous glands (S). Immunohistochemical stain for CD 34; x400

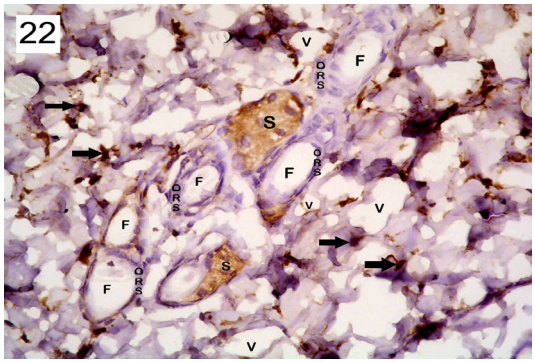

Fig. 22: Photomicrograph of a section in the skin of an albino rat (group V) showing hair follicles (F) with mild limited immunoreactivity in the cells the outer root sheath (ORS). Some immunopositive spindle cells of the reticular dermis (arrows) are seen. Mild immunoreactivity is seen in the cells of sebaceous glands (S) and in endothelial cells of dermal vessels (V). Immunohistochemical stain for CD 34; x400

Table 1: The mean±standard deviation of epidermal thickness, dermal thickness, area % of collagen and area % of elastic fibers in all studied groups.

Parameter	Group I	Group II	Group III	Group IV	Group V
Epidermal thickness (µm)	20.6±2.6	24 ±1.7*	27.1±3.9*	21.7±3.1	25.4±2.4*
Dermal thickness (µm)	719.9±23.6	742.3±29.4	1042.8±21.3*	740.2±13.4	1023.5±19.4*
Area % of collagen	20.3±4.1	23±4.9	35.1±9.4*	22.8±4.5	34.8±7.4*
Area % of elastic fibers	2.2±0.6	3.1±0.8	5.5±2.2*	2.6±1.1	3.9±1.4*

* Significant (P <0.05) (compared to control).

Table 2: The mean±standard deviation of area % and optical density of CD3 immunopositive cells in all studied groups.

Parameter	Group I	Group II	Group III	Group IV	Group V
Area % of immunopositive cells	1.6±0. 6	6.3±1.3*	3.4±1	6.4±3.1*	2.9±1.0
Optical density of immunopositive cells	0.7±0.2	0.9±0.1*	0.9±0.3*	1.1±0.3*	0.9±0.2*

* Significant (P <0.05) (compared to control).

DISCUSSION

Chemical peels are methods used to cause a chemical ablation of defined skin layers. This would induce an even and tight skin as a result of the regeneration process. The mechanical action of peeling, even when limited to the epidermis, is able to stimulate regeneration in the dermis via pathways that are not well understood *(Fischer et al., 2009)*.

This study revealed that both TCA (35%) and GA (70%) are effective as medium depth chemical peeling agents. They improve texture of skin by increasing the epidermal, dermal thickness as well as by increasing and reorganizing the dermal structural elements (collagen and elastic fibers) with no significant difference between the two reagents.

Glycolic acid and trichloroacetic acid were chosen in this work because they have become an increasingly popular method to treat a myriad of benign skin disorders *(Grover & Reddu, 2003)*. Concerning trichloroacetic acid, it is one of the most widely used peeling agents. It was used for the cosmetic treatment of photodamaged skin such as actinic lentigines and pitting scars due to acne *(Yonei et al., 2007)*.

The present work demonstrated that chemical peeling of the skin of albino rats with TCA produced significant increase in epidermal thickness after one week with progressive increase after three weeks. It also demonstrated significant increase in dermal thickness and area percent of both collagen and elastic fibers after three weeks.

Increased epidermal thickness might be attributed to re-epithelialization of skin from the adnexal structures that were spared from the chemical damage. This explanation is in accordance with *Deprez (2007)*.

Another possibility for increase in epidermal thickness, dermal thickness and area % of both collagen and elastic fibers was postulated by *Yonei et al. (2007)*. They demonstrated that the TCA-treated keratinocytes, before undergoing full necrosis, acted as a major source of platelets derived growth factor B (PDGF-B), which promote re-epithelialization and dermal regeneration without wound contraction and scarring. In other words, the TCA-treated epidermis acted as a "biological dressing" that not only protected the wound surface, but also released growth factors in the early phase.

Thus, TCA peeling might have an advantage as compared with other abrasive treatments like CO_2 laser, which immediately evaporates epidermal cells.

These findings are also in agreement with *Butler et al. (2001)*, who observed that the clinical effects of TCA were due to both reorganization in dermal structural elements and increase in dermal volume as a result of an increase in collagen content, glycosaminoglycan and elastin.

Concerning glycolic acid, it gained wide acceptance as skin rejuvenating consumer products. In clinical dermatology, GA was used as chemical peeling agents in the treatment of acne and in reversal of photo-aged skin. However, the exact mechanism of the therapeutic efficacy of GA is not known *(Tsai et al., 2000)*.

In the present study, it was observed that chemical peeling of the skin of albino rats with GA (70%) produced increase in epidermal thickness, dermal thickness, density of the collagen and elastic fibers one week after peeling that progressed after three weeks.

Increased epidermal thickness in this study might be attributed to the direct action of GA on the epidermis that resulted in thickening of the epithelium involving the basal and spinous layers. This explanation agrees with the results of *Rodrigues and Maia Campos (2002)* who studied the effect of GA on hairless mice epidermis. They attributed the cosmetic benefits of GA to its action on the epidermis. The previous finding was further supported by *Park et al. (2002), Sams et al. (2002) and Inan et al. (2006)*.

Another confirming study is that of *Denda et al. (2010)* who suggested that one of the mechanisms of GA-induced epidermal proliferation was a growth response of basal keratinocytes to the local elevation of hydrogen ion concentration by infiltrated GA. This response was mediated by release of chemical mediators such as ATP.

The increased area of the collagen and elastic fibers in the present study after three weeks might be attributed to the ability of the glycolic acid to accelerate collagen and elastic fibers synthesis by fibroblasts. This explanation agrees with *Dinardo et al. (1996) and Kim et al. (1998)*.

These findings are also in accordance with *Inan et al. (2006)* who reported increase in dermal thickness with GA peeling and explained it by increased synthesis of glycosaminoglycans, collagen and elastic fibers. *Omi et al. (2010)* results are also in agreement with these findings. They found that GA peel of the skin resulted in increase in vimentin filaments within fibroblasts and endothelial cells; hence they directly induced collagen formation within the dermis.

The present study revealed non-significant increase in dermal thickness as well as collagen and elastic fibers content at the end of the first week. These might be due to the time needed for collagen and elastic fibers formation. These findings are also in accordance with *Butler et al. (2001)* who found that collagen content increased initially three days after GA peel and peaked on day 28.

Isoda et al. (2001) findings are also in agreement with these findings. They found that skin treated with glycolic acid showed a peak increase in dermal thickness 14 days after treatment. However, *Inan et al. (2006)* demonstrated different findings. They found significant increase in dermal thickness and densely arranged thick collagen fiber bundles in the dermis by the end of the first week.

Remodeling of collagen fibers noticed in this study is consistent with the results of *Rakic et al. (2000), Ahn et al. (2002) and Okano et al. (2003)* who suggested that GA not only directly accelerated collagen synthesis by fibroblasts, but it also modulated matrix degradation and collagen synthesis through keratinocytes-released cytokines. They found that GA-treated keratinocytes released IL-1a which is one of the primary mediators for matrix degradation.

One of the objectives of this study was to detect and compare between the abilities of TCA and GA to stimulate stem cells during wound healing after peeling. CD34 antibody was used to immunostain keratinocytes stem cells. This antibody was shown to be the best marker for stem cells in hair follicle bulge region *(Trempus et al., 2003; Cotsarelis, 2006)*.

By analyzing the skin peeled with TCA or GA immunohistochemically with antibodies to CD34 antigen, the present work demonestrated a significant increase in the area percent and optical density of CD34+ve cells in peeled skin compared to normal control skin after one week. This increase could be explained by stimulation of the bulge cells by peeling induced denuded skin as the outer root sheath cells could be used to cure lesions like ulcers and that keratinocytes from hair follicles could re-populate wounds. These findings are in accordance with *Limat et al. (2003) and Cotsarelis (2006)*.

These findings are also in agreement with *Ito et al. (2005) and Levy et al. (2005)*. They demonstrated that bulge cells contributed to wound repair but do not contribute to normal epidermis homeostasis in non wounded skin. Once the epidermis was damaged, the bulge cells and their progeny migrated out into epidermis to repair the wound *(Ohyama, 2007)*.

The present work demonstrated high microvessels density in the peeled skin especially after one week. This finding could be explained by stimulation of inflammatory angiogenesis by wounded skin to contribute in wound healing. These findings are in accordance with *Bluff et al. (2006)*.

Moreover, *Hussein et al. (2008)* in their comparative study between GA chemical peelings and microdermabrasion on human skin. They reported increased CD34 immunopositive endothelial cells in skin treated with GA.

The present work demonstrated dense immunoreactivity in cells of sebaceous glands in TCA and in GA-treated skin that was more marked after one week of treatment. This proposal could be explained by multipotency of the follicle bulge cells. This finding agrees with the data of *Oshima et al. (2001)* who demonstrated that bulge keratinocytes of vibrissa follicles could produce the hair follicle, sebaceous gland and the interfollicular epidermis. Another confirming study is that of *Blanpain et al. (2004)* who found that single bulge cell, after the expansion *in vitro*, was able to re-populate hair follicles, sebaceous gland and epidermis as well as bulge cells.

The present work demonstrated increase in CD34 immunoreactive spindle cells (CD34SC) of the reticular dermis in TCA and in GA-treated skin after one week of treatment which might be attributed to their stimulation to contribute in healing of peeling induced lesion.

This finding agrees with those of *Erdag et al. (2008)* who investigated the role of CD34SC in wound healing after excision of cutaneous lesions. They suggested that CD34SC might have a role in regulation of collagen synthesis**,** wound healing, scar formation and remodeling of scarred skin. More confirmation comes from-*Nickoloff (1991)* who speculated that these cells might be translocated stem cells that could interact with the epithelial stem cells of the bulge.

The increase in area % of CD34+ve cells was not maintained but declined by the end of the third week in all groups. This decrease is also consistent with the finding of *Limat et al. (2003) and Cotsarelis (2006)*, as after regeneration of peeled skin there was no need any more to the previous increase.

On comparing the histological effects of TCA and GA in the present study, there was slightly more increase in epidermal thickness, dermal thickness, area percent of collagen and elastic fibers in TCA-treated groups (group II and group III). These results are consistent with the results of *Butler et al. (2001)* who compared between the histological effects of TCA (30%), GA (50%) and phenol peel on the skin of photo-aged mice. They demonstrated an increase in collagen content per unit volume, increase in dermal thickness, re-organization of the collagen and elastic fibers in all chemical peel groups with more increase in TCA and phenol peel than in GA.

Accordingly, it could be concluded that both TCA (35%) and GA (70%) were effective as medium depth chemical peeling agents. The mechanism of inducing resurfacing of skin after peel may be through stimulation of bulge stem cells in the external root sheath of the hair follicle. It is suggested that bulge cells may be used as an excellent stem cell source for cutaneous regenerative medicine. Patient's bulge stem cells can be tried in treating alopecia

as they have the potential to form new hair follicles. They can also be used in covering denuded skins as in ulcers and major burn.

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تقييم التقشير متوسط العمق باستخدام حمض الجليكوليك(٧٠٪) مقابل ثلاثي كلور حمض الخليك (٣٥%): دراسة هستولوجية وهستوكيميائية مناعية على جلد الفأر

سهير أسعد فيلبس- نجلاء محمد سلامة- رحمة كمال الدين أبو النور- إيمان عباس فرج

من قسم الهستولوجيا - كلية الطب - جامعة القاهرة

ملخص البحث

يمثل التقشير الكيميائي أداة مفيدة لتحسين نسيج الجلد وآثار الشيخوخة، ونتيجة ظهور مواد أحدث للتقشير الكيميائي أصبح هناك الآن مجموعة واسعة من مواد التقشير التي يمكن استخدامها لنفس المريض.

وتهدف هذه الدراسة إلى اجراء تقييم ومقارنة هستولوجية بين اثنين من مواد التقشير الأكثر شيوعا في التقشير الكيميائي المتوسط العمق وهما ثلاثى كلور حمض الخليك (٣٥٪) مقابل حمض الجليكوليك (٧٠٪).

وقد أجريت هذه الدراسة على أربعين من ذكور الفئران البالغين والمقسمة إلى خمس مجموعات يضم كل منها ثمانية فئران: المجموعة الأولى وتضم الفئران الضابطة التي لم تتلق جلسة تقشير. المجموعة الثانية والثالثة وتضم الفئران التي تلقت جلسة تقشير بثلاثى كلور حمض الخليك (٣٥٪) وتم أخذ عينات الجلد منها بعد أسبوع وبعد ثلاثة أسابيع على الترتيب. المجموعة الرابعة والخامسة وتضم الفئران التي تلقت جلسة تقشير بحمض الجليكوليك (٧٠٪) وتم أخذ عينات الجلد منها بعد أسبوع وبعد ثلاثة أسابيع على الترتيب. وتم إعداد الشرائح وصباغتها بالهيماتوكسيلين والإيوسين، وصبغة ماسون ثلاثي الألوان وصبغة الأورسين والصبغة الهستوكيميائية المناعية ضد س د ٣٤ الذي يعد علامة على الخلايا الجذعية الموجودة في انتفاخ بصيلة الشعر. وقد تم قياس سمك طبقة البشرة وسمك طبقة الأدمة والمساحة المئوية لألياف الكولاجين والألياف المرنة، وكذلك تم قياس المساحة المئوية وشدة الكثافة الضوئية للخلايا المتفاعلة مع الصبغة الهستوكيميائية المناعية س د٣٤ باستخدام جهاز تحليل الصورة. و تم تحليل البيانات الناتجة إحصائيا.

وقد أظهرالجلد بعد أسبوع من التقشير بثلاثى كلور حمض الخليك (٣٥٪) أو بحمض الجليكوليك ٧٠٪) زيادة في سمك طبقة البشرة وسمك طبقة الأدمة وزيادة الألياف المرنة وألياف الكولاجين والتي أصبحت أكثر ترتيبا وانتظاما. كما أظهر الجلد أيضا زيادة واضحة في الخلايا الموجبة مناعيا ضد س د٣٤ والتي تضم الخلايا الجذعية في بصيلات الشعر، والخلايا المغزلية، والخلايا المبطنة للأوعية الدموية الموجودة في طبقة الأدمة و أيضا خلايا الغدد الدهنية.

وبعد ثلاثة أسابيع من التقشير أظهرالجلد زيادة أكثر وضوحا في سمك طبقة البشرة وسمك طبقة الأدمة وفي ألياف الكولاجين والألياف المرنة بينما كان هناك انخفاض ملحوظ في المساحة المئوية والكثافة الضوئية للخلايا الموجبة مناعيا ضد س د٣٤.

هذا وقد أظهرت الدراسة كفاءة كل من ثلاثى كلور حمض الخليك (٣٥٪) و حمض الجليكوليك (٧٠٪) في تحفيز الخلايا الجذعية في انتفاخ بصيلات الشعر وفي تحسين بنية الجلد دون فروق ذات دلالة احصائية بين تأثيركل منهما.