Short and long term clinical and histological evaluation of medium-depth chemical facial peel with 35% trichloroacetic acid on photo damaged skin

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Chemical peeling involves the topical application of a wounding agent with the goal of effecting an organized regeneration of the skin. The histological features of photo aged skin include structural abnormalities that disrupt normal epidermal and dermal architecture.

In the present prospective study we compared short and long term clinical and histological changes that occur after three sessions of enhanced medium depth chemical facial peel with 35% trichloroacetic acid on ten patients with photo aged skin changes. Biopsy specimens were taken pre-peel (control), and 3, 12, 48, months post-peel, for histological, and immunohistochemical analysis to assess total collagen type I content throughout the study that reach up to four years. Histological evaluations during the course of treatment have revealed an array of time-dependent morphologic changes that were classified into early, intermediate, and late. Clinical resolution of actinic damage corresponded with restoration of epidermal and dermal polarity. Characteristic histological and immunohistochemical analysis post-peel include decreased elastic fibers, increased activated fibroblasts and organized parallel arrays of collagen fibrils. As treatment is continued beyond 12 months, the neocollagen synthesis and organization continues to increase and elastosis continues to decrease. Besides, increases in epidermal and dermal mucin and decreases in epidermal melanin. We concluded that although there was no direct correlation between histological changes and clinical improvement, the major structural changes appear to be directed at restoring the skin to the pre-sun-damaged state.

Key words: *Chemical peel- photodamaged skin- histology- long term.*

Introduction:

Aging is defined as the process of becoming older. This traditional definition was recently challenged in the new Handbook of the Biology of Aging (Academic Press, 2006), as the process of system's deterioration with time. This definition allows for existence of non-aging systems (when "old is as good as new"), and anti-aging interventions (when accumulated damage is repaired). Certainly, facial skin deterioration is one of the most apparent examples of aging. For this reason combined with the social perception of youthfulness as a measure of outer beauty, it is not a surprise that people are seeking more avenues for anti-ageing interventions. This demand has prompted tremendous growth in the cosmetic industry, with numerous over-the-counter products available to "reverse aging changes." Whether to reverse the signs of aging or to treat cutaneous lesions, ablative skin resurfacing is an integral part of the practice of facial plastic surgery and dermatology.[1]

Chemical peeling is a technique that removes superficial lesions and improves the texture of skin by the application of a chemical defoliant. Peeling produces a controlled, partial-thickness chemical burn of the epidermis and the outer dermis. Regeneration of peeled skin from follicular and eccrine duct epithelium results in a fresh, orderly, organized epidermis. In the dermis, a new 2 to 3 mm band of dense,

compact, orderly collagen is formed between the epidermis and the underlying damaged dermis, which results in effective ablation of the fine wrinkles in the skin and a reduction of pigmentation.[2] These clinical and histological changes are long-lasting (15 to 20 years) and may be permanent for some patients. Medium-depth chemical peels are defined as the application of a chemical cauterant to the skin to produce wounding to the level of the upper reticular dermis.[3] although medium-depth chemical peels have been used to treat a myriad of cutaneous disorders; they are most commonly used to improve photodamaged skin by removing solar keratoses, softening mild rhytides, and evening pigmentary dyschromias. Reepithelialization of the skin after medium-depth wounding occurs from adnexal structures and by the stimulation of new collagen formation. Trichloroacetic acid (TCA) is a chemical cauterant that coagulates the proteins of the skin. During the application of TCA to the skin, a transient gray-white color change known as "frosting" occurs on the treated skin. Trichloroacetic acid peels are used to treat actinically damaged skin, resulting in necrosis of the epidermis and the upper dermis, followed by replacement with new epidermis and dermal connective tissues. The depth of penetration of TCA into the skin increases as the concentration of TCA is increased, as does the potential for scarring.[4] Traditionally, medium-depth chemical peels were performed using 40-60% trichloroacetic acid (TCA). However, these higher concentrations of TCA carried with them an increased risk of scarring.[5] In 1986, Brody and Hailey introduced the concept of combination medium- depth chemical peels whereby two superficial agents, solid carbon dioxide and 35% TCA, were used in succession to reach the same histological depth as a single agent while decreasing the risk of scarring[1,3]. In 1989, Monheit described the use of Jessner's solution (JS: resorcinol, salicylic acid, and lactic acid) as a keratolytic agent to increase the absorption and penetration of 35% TCA and thus produce a medium-depth chemical peel for photodamaged skin.[6] Coleman and Eutrell described a new medium-depth chemical peel: the glycolic acid-trichloroacetic acid (GA-TCA) peel. This peel employed 70% glycolic acid instead of Jessner's solution as the initial agent prior to the application of 35% TCA. It was theorized by the authors that pretreatment of the skin with glycolic acid would produce more even penetration of the TCA 35%. Although the use of glycolic acid in superficial chemical peels is now well established, its role in medium-depth chemical peels has yet to be fully elucidated.[7] Tretinoin (Retin -A), or all-transretinoic acid, is a synthetic vitamin A analogue used to treat a variety of dermatoses including photoaged skin.[8] Interest has focused on tretinoin as a wound-healing promoter. In animal studies, topical retinoid have enhanced the healing of full-thickness skin wounds and corneal epithelial wounds.[9,10] In an open non placebo-controlled study, tretinoin pretreatment of the skin for 2 weeks prior to dermabrasion was shown to accelerate healing.[11] Since tretinoin may alter the epidermal barrier to allow enhanced percutaneous absorption of other chemicals,[12] tretinoin pretreated skin might be expected to increase the depth of TCA penetration into the skin. Since, all patients in our study were pretreated for 2 weeks with retina -A 0.1% prior to chemical peel with 35% TCA to achieve safe medium depth peel. The histological changes of the aging skin are typical of actinic changes which are the photochemical effects of solar radiation exposure. These changes include a loss of orderly differentiation in the epidermis and degeneration of the elastic network, along with some mottled pigmentation and lymphocytic infiltration. There is a decrease in collagen as well as disordered degeneration of the dermal fibers, a flattening of the dermal-epidermal junction, and multiple actinic keratoses with atypia seen.

The number of melanocytes was increased in this actinic skin, but they were unevenly distributed and contained variable amounts of melanin.[13] Collagen I predominates in human dermis, accounting for 85 percent of the total, whereas collagen III accounts for only 10 percent.[14] Thus, studies of collagen

in human skin are more easily accomplished if directed at collagen I. The biosynthesis of collagen I in skin begins with the formation of procollagen I within dermal fibroblasts.[15,16] After its secretion from fibroblasts, procollagen I is enzymatically cleaved of its aminopropeptide and carboxypropeptide in a one-to-one ratio; the presence of propeptide provides an index of collagen I synthesis.[17] Therefore, an antibody that can recognize the aminopropeptide portion of procollagen I or its distal derivatives[18] will both identify precursors of collagen I and provide an indirect measure of collagen I formation **Figure(1)**. Using such an antibody we determined whether the formation of collagen I is reduced in photodamaged human skin and is increased by intermediate depth peel with 35% TCA. The finding of increased collagen I formation in photodamaged human skin treated with chemical peel suggests that peeling promotes clinical improvement by repairing dermal collagen.

The finding of increased collagen I formation in photodamaged human skin treated chemical peel suggests that it promotes clinical improvement by repairing dermal collagen.

The purpose of our study was to compare short term, long term relationship between, clinical improvement and histological changes that occur after medium-depth chemical facial peel with 35% TCA for patients with photoaging skin.

Patients and methods:

The present work was conducted in the Plastic Surgery and Histology Departments of Ain Shams University during the period from January 2007 to January 2012. Ten patients (three men and seven women) with photodamaged facial skin were evaluated. All patients had Fitzpatrick skin types IV–V. Their age ranged from 40 to 65 years (mean age 52 years). No patient had a previous chemical peel or dermabrasion, or used topical tretinoin in the year before entering the study. Informed consent prior to entering the study was obtained after the risks and benefits were explained. Each subject underwent three sessions of 35% TCA peel preceded by pretreatment with Tretinoin (Retin-A) 0.1% for 2 weeks.

The entire face was cleansed with alcohol followed by acetone to degrease the skin vigorously until it became very dry to the touch. 35 % TCA was then applied using two cotton tipped applicators until a uniform white frost was achieved. This was followed by tap water soaks for 10–20 minutes. Post-peel, patients were instructed to use sunscreen 1-2 weeks after the procedure, retin-A was resumed after 3months and continued for one year and beyond. None of the patients was treated with hydroquinone pre or post-peel except for those who developed post-inflammatory hyperpigmentaion. Clinical improvement was assessed by three independent evaluators using photographic analysis. Right and left profile photographs of each patient were taken pre and post peel at the time of taking biopsy specimen. All photographs were taken with the same camera under similar parameters, and magnification. Clinical parameters evaluated included, clearing of actinic keratoses, lightening of solar lentigines, and lessening of rhytides. Complications such as postinflammatory hyper or hypopigmentation, persistent erythema, persistent erosions, and milia were also assessed. A numerical scale was used to grade clinical improvement (0 = no response; 1 = fair; 2 = good; 3 = excellent) and complications {0 = none; 1 = slight; 2 = moderate; 3 = significant). The data were tabulated.

To evaluate histological changes, Full-thickness 5mm punch biopsy were taken from the preauricular or temporal area one month pre-peel (control) and at 3 month, 12 month, 48 month post-peel. The biopsy specimens were divided into two parts: one was fixed in 10% formalin and stained with haematoxylin and eosin stains, Verhoeff's elastic stain and Masson trichrome stain and processed for histological analysis by light microscopy. The second group of specimens was frozen in liquid nitrogen and stored at -80 °C. The samples were processed for staining with an immunoperoxidase technique (Vectastain

ABC Kit, Vector Laboratories, Burlingame, Calif). For the immunohistologic analysis to detect monoclonal antibody of type I collagen, we used the term "collagen I immunostaining" to refer to tissue staining observed with this antibody. The intensity and extent of staining were assessed with a six-point scale in which a score of 0 indicated no staining, a score of 1 minimal staining, a score of 2 low-to-moderate levels of staining, a score of 3 moderate staining, a score of 4 high levels of staining, and a score of 5 maximal staining. The patterns of collagen I immunostaining were extracellular, in a papillary dermal band (a narrow zone immediately beneath the epidermis) and intracellular, within fibroblasts. Four high-power fields from each section were graded under light microscopy.

Results:

Clinically, the enhanced medium-depth chemical peel with 35% TCA peel was effective in treating photodamaged skin. The peeling effect occurred between the third and fourth days after the application of the peeling agent. Erythema, crusting and swelling occurred within 24 h with crusting and swelling subsiding by 7 days post-peel. The time to complete re epithelialization occurred within 7–10 days. Once the scaling subsided, the skin had a more turgent and juvenile appearance, the skin color appeared to be more uniform and improvement in surface texture was noted in most patients. All treated patients showed statistically significant improvement and lessening of fine rhytides, pinkness, roughness and lightening of solar lentigines after a period of 6 months. When the study period was extended to a total of 12 months and beyond, the clinical improvements in wrinkling were sustained, and most of the change occurred during the first 6-12 months, with little additional improvement discernible after this period. No or little improvement of deep rhytides. No complications, except for rebound hyperpigmentation that occurred in 35% of cases especially with type V skin but subsided after use of bleaching creams. The clinical results and complications are summarized in **Table(1)**, **Figures(2-5)**.

Histologically, before peeling, structural changes varies in severity according to the degree of photodamage but overall parameters showed that photodamged skin had solar elastosis, strands of elastic fibers coursed through the papillary dermis dermo-epidermal junction showed a clear flattening, resulting in a decrease in the junction surface area with a corresponding decrease in interdigitating papillae and loss of rete ridges, vascular ectasia, and a fragmented array of disordered and poor striated collagen bundle **Figures(6a,7a)**.

At 3 months post-peel biopsy, the histological changes were most noticeable in the epidermis, as there were increases in epidermal thickness, decreased melanocytes, compaction of the basket-weave pattern of the stratum corneum, and deposition of alcian-blue staining/material (mucin) in the stratum corneum of 90% of specimens. A more organized epidermis overlay a thicker papillary dermis composed of more parallel homogenized layers of collagen showed in 20% of specimens The Verhoeff's elastic stain showed minimal development of mid-dermal elastic fibers and papillary dermal elastic fibers were decreased compared with the skin before peeling in 70% of specimens **Figures(6b,7b)**.

12 month post peel biopsy: Compaction of the stratum corneum was found in 30% of 12 months biopsies, which is less than the 90% observed at 3 months biopsies. The conversion of the stratum corneum from the basket-weave pattern to the compact appearance, melanin pigment continued to decrease as well as other epidermal changes, was presumed to have been a major contributing factor to the clinical improvement. However, these results bring into question the degree of correlation between clinical and histological changes in the epidermis. Organized bundles of collagen were evident in the papillary and reticular dermis in 70% of specimens **Figure(7c)**.

48 months post peel biopsy: There was a striking return to normal appearance in two-thirds of the samples, although there had been no change after only 6 months, yet at

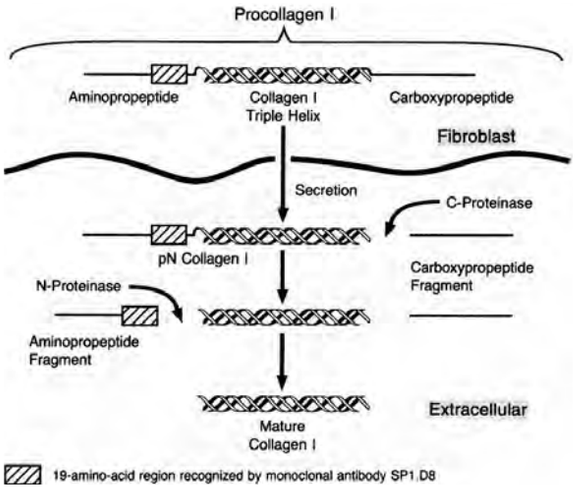


Figure (1): Biosynthesis of collagen I.

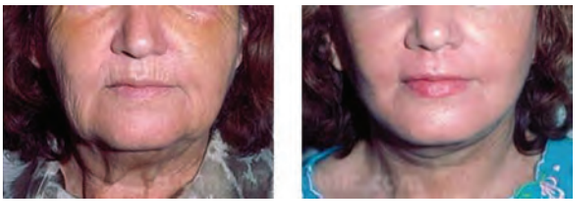


Figure (2): 60 year patient 2 years post 3 times peel showed marked improvement of fine wrinkles, skin texture and lightness of solar lentigins.

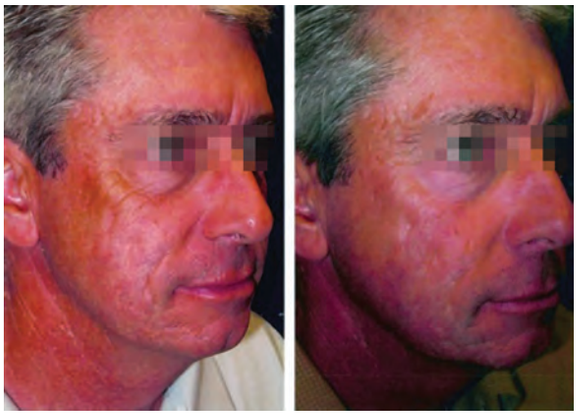


Figure (3): 55 years male patient after 1 year post peel show improvement of photoaging changes with no response on deep rhytides.

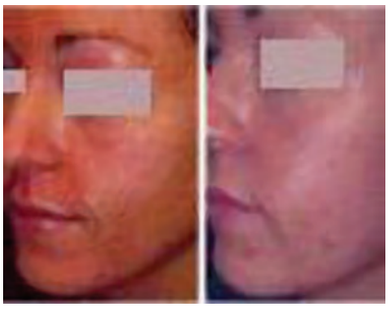


Figure (4): 40 years patient 6 months post peel show good response of solar lentigens with improvement of skin texture.

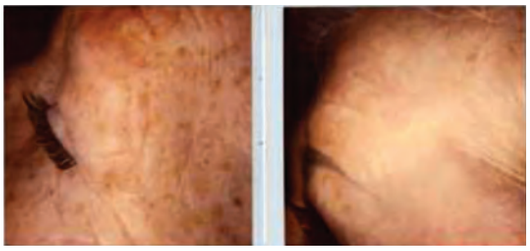
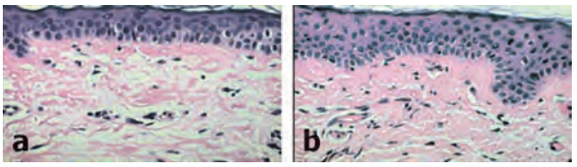


Figure (5): 65 year female patient 24 months post peel show marked improvement of solar lentigens and fine wrinkles of the forehead.



Figures (6a, 6b): The histology taken before chemical peel treatments shows loose, haphazard collagen bundles, typical of photodamaged skin (above left). 3 months post-peel treatments increases in epidermal thickness, more organized epidermis with ret-ridges thicker papillary composed of more parallel homogenized layers of collagen. (Hematoxylin and eosin stained, 450X).

21 months the epidermal thickness returned to baseline, the granular cell layer number declined, and the basket-weave pattern of the stratum corneum returned to baseline in 80% of samples. Melanin continued to decrease and correlate with an observed improvement in pigmentation in addition to persistent and more organized increase in papillary and reticular dermal collagen in 70% of samples **Figure(7d)**.

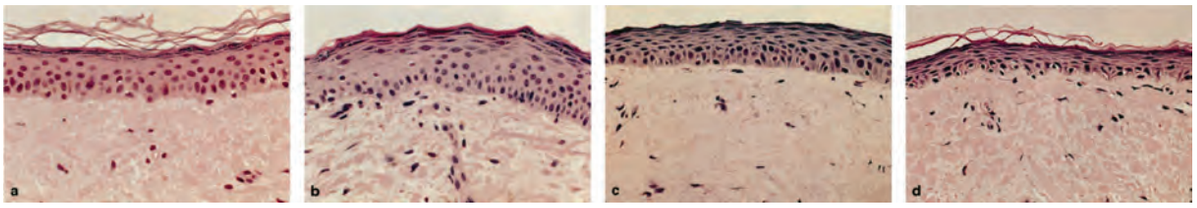


Figure (7): Facial biopsies of the same pre-peel (a), 3months (b), 12 months (c), and 48 months (d) post-peel. (Hematoxylin and eosin stained, 340X).

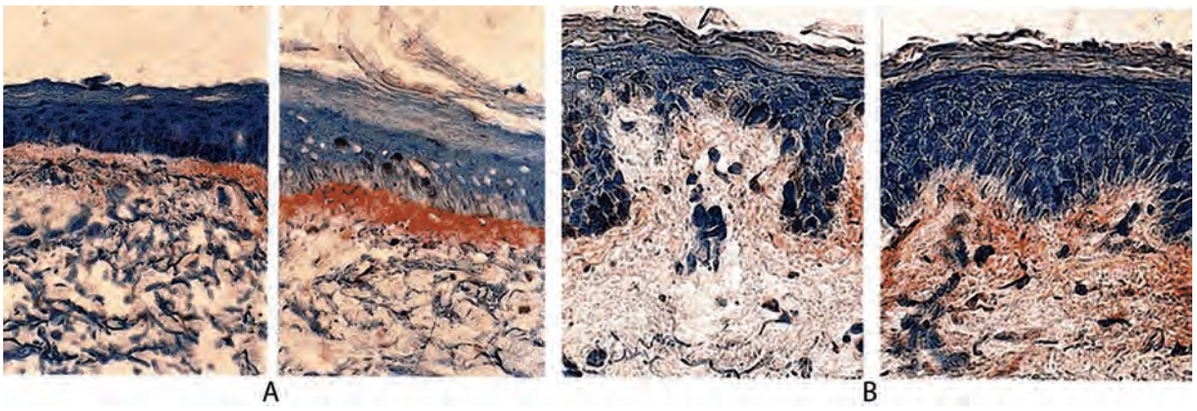


Figure (8): Collagen I immunostaining in photodamaged skin from two patients after 12 months with intermediate depth peel. In Panel A, there is minimal (grade 1) extracellular staining within the papillary dermis of photodamaged skin at base line (left- panel) and moderate (grade 3) staining after 12 months of treatment with chemical peel (right panel) (x90). In Panel B, there is minimal (grade 1) extracellular staining within the papillary dermis of photodamaged skin at base line (left panel) and slightly more staining (grade 2) after 12 months of treatment (right-panel) (x90). There is also an increase in fibroblast staining from grade 1 at base line to grade 2 after 12 months of treatment with chemical peel.

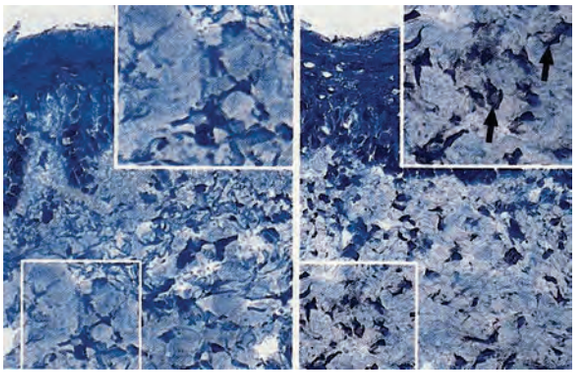


Figure (9): Collagen I immunostaining of fibroblasts in photodamaged skin from a patient treated with intermediate depth peel (x100). Fibroblast staining within the dermis of photodamaged skin is virtually absent prepeel (left panel) but is markedly increased (grade 5) after 12 months post-peel (right panel).

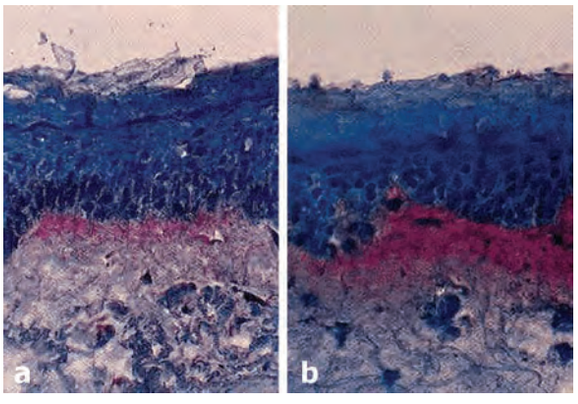


Figure (10): Collagen I immunostaining (x60) from a single patient skin 12 month, 48 months post-peel shows increase of extracellular collagen I staining form grade 1 (left panel) to grade 5 (right panel) extracellular staining within the papillary dermis.

Immunohistochemical analysis of dermal collagen:

The short term immunohistochemical study (3months) showed no evidence of qualitative or quantitative changes in dermal collagen after 4 months of treatment in 80% of

Table1. Clinical Efficacy of enhanced intermediate depth peel at different time interval.

Parameter	Early (3–6 months)	Intermediate (12–24 months)	Late (24–48 months)
Actinic keratoses	Good	Excellent	Excellent
Solar lentigens	Fair	Good	Good
Fine wrinkles	Fair	Good	Excellent
Deep wrinkles	No Response	Fair	Fair
Postinflamatory hyper pigmentation	Poor	Fair	NR
Postinflamatory hypopigmentation	Moderate	Slight	NR
Persistent erythema	Non	Non	NR
Persistent erosions	Non	Non	NR
Milia	Non	Non	NR

Table 2: Histological results of intermediate depth peel with 35% TCA on photodamaged skin. NR, not reported.

Parameter	Early (3–6 months)	Intermediate (12-24)	Late (24–48 months)
Stratum corneum morphology	Compact (altered)	Basket-weave (normal)	Basket-weave (normal)
Granular layer thickness	Increased	Normal	Normal
Epidermal thickness	Increased	Normal	Normal
Keratinocyte atypia	None or decreased	NR	None or decreased
Melanocyte atypia	None or decreased	NR	None or decreased
Epidermal mucin	No change to increased	Increased	Increased
Epidermal melanin	Decreased	Decreased	Decreased
Papillary dermal collagen	No change	Increased	Improved organization and new synthesis
Elastosis	No change	NR	Decreased
Vascularity	No change or slight increase	NR	No change

samples, only 20% showed grade 1 increase in collagen immunostain in the papillary dermis. After 12–24 months collagen synthesis was significantly increased as there was increase in extracellular collagen I immunostaining within the papillary dermis and reticular dermis by 75 % (figure 8) and 80 %increase in intracellular collagen I immunostaining in fibroblasts **Figure (9)** as compared with pre-peel specimens in 70% of samples. The histological results are summarized in **Table (2)**.

Discussion:

Whether for transient cosmetic improvement or for prophylaxis against skin malignancy, the chemical peel stimulates the repair process so that defects are replaced with organized tissue.[19] Histological studies have reported the presence of a subepidermal

band of new collagen or 'repair zone' in the papillary dermis following chemical peels.[20] The enhanced medium depth peel includes the use of glycolic acid (70% solution) as a dyscohesive agent to further the absorption and penetration of a 35% TCA peel. In our study we used tretinoin (Retin –A) two weeks before 35% TCA peeling as it alters the epidermal barrier to allow enhanced percutaneous absorption of other chemicals to increase the depth of TCA penetration into the skin. The results of our study showed that Retin-A when used 2 weeks before 35% TCA, produced a medium depth chemical peel that was effective in treating photodamaged skin, evening pigmentary dyschromias and lessening fine rhytides. This finding is in accord with that of Tse et al[21] who found that a GA–TCA peel was efficacious in removing actinic keratoses and lightening solar lentigines but was not successful in lessening coarse rhytides. Most clinical studies on chemical peel had been conducted on light-skinned after single session of peeling for each patient, with the exception of two published studies on black people.[22] Information from other racial groups, such as Orientals, is limited. Our study have been conducted on type IV, V skin so we enforced to perform three sessions of 35% TCA peel with 3 months apart for each patient to achieve satisfied clinical results, in spite of that rebound hyper pigmentation was the only complication that occurred up to 60% of cases. So patients with that type of skin must use sunscreen post-peel of high SPF and avoid prolonged sun exposure. Many clinical and animal studies showed detailed histological improvements in the epidermis, pigmentation and dermal matrix of photodamaded skin after chemical peel, either light microscopic, electron microscopy and even immunohistochemistry, laser electrophoresis to asses improvement in dermal matrix collagen, elastic fibers and melanin. But all these studies have been conducted on early phase (3-6 months) after single cession of chemical peel by whatever chemical. In a double-blind, vehicle-controlled study on photo damaged skin after chemical peel, all treated patients showed statistically significant improvement in wrinkling, pinkness, and roughness after a period of 16 weeks.[9] The histological changes that were most noticeable were increases in epidermal thickness, decreased melanocyte hypertrophy hyperplasia, compaction of the basket-weave pattern of the stratum corneum, and deposition of (mucin) in the stratum corneum. There were no dermal changes observed by light microscopy. This finding is in accord with our results in early period (3-6 months) post peel I as we concluded that the majority of the clinical improvement in hyper pigmentation pinkness, and wrinkling/ texture was a direct result of the changes in epidermal components, and that the absence of dermal changes was due to the short treatment duration. The first studies attempted to correlate clinical improvement with underlying histological changes after chemical peel specifically designed for the treatment of acne. No reported study explored long term (>12 months) relationship between clinical improvement and histological changes after multiple cessions of chemical peel on oriental races. So little knowledge had been accumulated on: (I) whether clinical and histological improvement could be sustained; (ii) what underlying factors were responsible for the clinical changes; and (iii) to what extent these were reversible or permanent. In our study we tried to answer these questions to achieve the goal of our study. We found that, after 12 months of treatment, some of early changes were no longer evident.

The epidermal thickness returned to baseline, the granular cell layer number declined, and the basket-weave pattern of the stratum cornea returned to baseline in many samples. There were also observed changes in the structural organization of the dermo-epidermal junction and appearance of ret-ridges although the flat profile was unchanged at 3 month biopsy. Melanin continued to decrease and to correlate with an observed improvement in pigmentation. Additional changes also became apparent at this stage, epidermal and dermal cumin were increased, elastic tissue decreased, and dermal collagen increased with organized bundles of

collagen evident in the papillary and reticular dermis as shown by light microscope and immunohistochemical analysis of dermal collagen. After 12 months, these findings were inconsistent with epidermal and dermal alterations being responsible for the clinical improvements that were sustained during that period of treatment as lessening of fine wrinkles, lightening of solar lentigens and improvement of skin texture.

In 48 months biopsies, there was a striking return to normal appearance in two-thirds of the samples, although there had been no change after only 6 months, at 21 month, the area of dermal elastosis was decreased by 34%, consistent with a trend noted at earlier time points, epidermal mucin continued to increase, melanin continued to decrease, and other histological alterations, such as epidermal thickness continued to revert to baseline levels with restoration of basket-weave pattern of the stratum corneum in 70% of samples. The whole mark of this stage is persistent increase of well organized papillary and reticular collagen dermal as compared with 3 month and 12month biopsy in 80% of samples.

The subdivision into early, intermediate, and late is, and is based largely on the results of the largest clinical trials conducted up to this point.

Conclusion:

Many histological markers of photo damaged skin have been identified. Intermediate depth chemical peel treatment induces a continuous course of clinical and improvement that is most rapid in the first 6–12 months. Some histological changes (increase in epidermal mucin and decrease in melanin) parallel clinical changes, and seem to be responsible for the continuing improvements in, for example, skin texture and pigmentation. Other changes (in the epidermis) are transient, and still others (dermal collagen synthesis) occur only after prolonged periods, and their relationship to clinical parameters is not clear. Nevertheless, the major histological changes induced by tretinoin appear to be specific and directed at restoring the skin structure and function.

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