Research Article

Formation of New Collagen after Dermal Roller in Atrophic Acne Scars

Reham Ezz El-Dawela*, Mohammad Abel Kerim* and Fatma El-Z. Salah El-D. Yassin**
* Departments of Dermatology, Venereology and Andrology, Faculty of Medicine, Sohag, Egypt.
**Department of Pathology; Faculty of Medicine, Sohag, Egypt.

Abstract
Background: Despite the many advances in the treatment of post acne scarring, it still does not have a fully satisfactory treatment. Percutaneous collagen induction (PCI) through dermal roller breaks old collagen strands that connect the scar with the upper dermis, promote removal of damaged collagen and induce more collagen immediately under the epidermis. The purpose of the current study is to evaluate the clinical and histologic facial skin responses to the Dermal Roller device as a minimally invasive treatment of atrophic acne scars. **Methods:** Total study duration was ٢٦ weeks in which twelve patients received seven sessions of PCI at ٣-weeks intervals, ٣- mm punch biopsy specimens of untreated scars were obtained from the twelve patients before treatment ,and again after the seventh session (at ١٨ weeks). Assessments were extended for ٨ weeks after the final session. **Results**: PCI induced notable improvement in the appearance of acne scars with significant reductions in the the scores associated with the clinical evaluation scale for acne scarring (ECCA) from (١٢٣.٣±٢٤.٥→ ٧٤.١٦±١٦.٤٩) (p=.٠.٠٠) after ٢٦ weeks. Histopathologic evaluations revealed increase in collagen production and deposition of newly synthesized collagen following PCI treatments detected mainly by picrosirius red staining. **Conclusions:** Skin needling is a simple and minimally invasive procedure with rapid healing and low downtime with minimized risks of hypo or hyperpigmentation. Although a single treatment may not give the smoothing that is seen with laser resurfacing the epidermis remain virtually normal. More than one session is needed because greater improvement is achieved after multiple sessions.
Key words: Acne scars, Dermal roller, ECCA scale, New collagen.

Introduction
Acne vulgaris is a common disease affecting ٨٠٪ of individuals between ١١ and ٣٠ years old and ٥٪ of elderly people[١,٢]. It is a multifactorial disease[٣]. Although the combination of hyperkeratini-zation, sebum production and propionibac-terium acnes are the proposed factors, the principle abnormality is comedone for-mation by hyperkeratinization[٤]. Facial acne scar-ring affects approximately ٩٥٪ of acne patients and is related to excessive inflam-mation[٥], initial acne severity and the time lapsed before seeking adequate treatment[٢]. Atrophic acne scar is one of the most dramatic consequences of inflammatory acne that can be debilitating to patients**.** Due to the physical disfigurement and psychological burden of these scars are noticeable, patients frequently seek medical treatment[٦].

Despite the many advances in the treatment of post acne scarring, it still does not have a fully satisfactory treatment[٧]. Various therapeutic options have been described with variable clinical outcomes and complications, such as surgical techniques (punch graft, punch excision, subcision), resurfacing techniques (dermabrasion, ablative

laser treatment, chemical peels), nonablative laser treatment, autologous fat transfer, and injection of dermal fillers[٨].

Treatment options like ablative laser resurfacing or dermabrasion that offer significant improvement in facial scars are invariably associated with considerable morbidity and downtime interference with the daily activities of the patients in the post treatment period[٩,١٠]. On the other hand, treatments like microdermabrasion and non ablative laser resurfacing that are associated with a minimal or no downtime, do not show the same level of efficacy as the traditional, ablative resurfacing techniques[١١,١٢]. Moreover, although the recently developed technique of fractional laser skin resurfacing has been reported to allow the recontouring of scars[١٣,١٤], the risk of hyperpigmentation in patients with darker skin types often precludes sufficient treatment[١٥].

As an alternative to laser treatments, microneedling therapy, also known as percutaneous collagen induction (PCI), collagen induction therapy (CIT) with Dermaroller (a needling tool) is an addition for managing postacne scars. It protects the epidermis and stimulates natural collagen synthesis. The needles break old collagen strands that connect the scar with the upper dermis, promote removal of damaged collagen and induce more collagen immediately under the epidermis[١٦].

The treatment is an office procedure[١٧]. Falabella and Falanga, ٢٠٠١ considered that skin needling induces normal wound healing, developing in three phases: Phase I: (inflammation), which starts immediately after injury; platelets, neutronphils and fibroblasts have the major role in this phase. Phase II: proliferation (tissue formation) which starts after about ٥ days and lasts about ٨ weeks; in which macrophages and several growth factors, mobile keratinocytes and fibroblasts promote collagen deposition. Phase III: (tissue remodeling), from ٨ weeks to about one year, mainly achieved by the fibroblasts: collagen type III is laid down in the upper dermis and is gradually replaced by collagen type I over a period of a year or longer[١٨].

Another hypothesis has been proposed by Liebl, (٢٠٠٩) to explain the PCI mechanism of action. The formation of new tissue (wound healing: inflammation–proliferation –maturation) is a complex series of reactions and interactions among cells and mediators. But it seems that these processes are somewhat cut short, when the skin is treated with needles. The fine microneedles do not create a wound in classic sense but according to bioelectricity theory (demarcation current) which triggers a cascade of growth factors immediately to the maturation phase. This demarcation current is additionally increased by the needles' own electrical potential. Cell membranes react to the local change in electrical potential with increased cell activity and with the release of potassium ion, proteins, and growth factors. the final result is deposition of new collagen in the upper dermis[١٩].

The purpose of the current study is to evaluate the clinical and histologic facial skin responses to the Dermal Roller device as a minimally invasive treatment of atrophic acne scars.

Subjects and Methods

Study design and patients

This prospective study was coducted from January ٢٠١٢ to October ٢٠١٢ at the Department of Dermatology, Sohag University and was approved by the Ethics of Research Committee, of Sohag university. Written informed consent was obtained from all participants before enrollment. Total, fourteen patients (٦ men, ٨ women) with different types of atrophic acne scars were enrolled in this study. Twelve of them completed the study (٥ men, ٧ women). Inclusion criteria were age ≥١٨ years and diagnosis of any type of facial atrophic scar (icepick,boxcar and rolling). Exclusion criteria were systemic retinoids or immunosuppressive drug intake during the previous ٦ months, coagulation defects or blood diseases, evidence or history of keloid scars and history of facial laser treatment or surgical procedure or dermabrasion ٦ months before study enrollment, presence of skin cancers, warts, solar keratoses or any skin infection, patients with

a medical condition that might have influenced the wound healing process, pregnancy and unrealistic expectations. Patients were allowed to continue previous acne medications during the study period, except isotretinoin. PCI treatment was performed for all patients. Each patient received seven sessions of treatment at ٣ weeks intervals. Patients were instructed to avoid medications such as aspirin and non-steroidal antiinflammatory drugs (NSAIDs) for at least ١ week before the session and to start using topical retinoid ٢ weeks before each session, stopping ٢ days before the session to avoid irritation to the skin.

Skin needling procedure:

Local anesthetic cream (eutectic mixture of prilocaine and lignocaine) was applied to the face under occlusion for approximately ٤٥ to ٦٠ minutes before the procedure. Patients were treated using the needling tool (Dermaroller MF ٨, Horst Liebl CEO, Fresenheim, France), which is a sterile plastic cylinder with needles protru-ding from the surface that rolls vigorously over the skin. The tool consists of ٢٤ circular arrays of eight needles (١,٥٠٠ μm long each) (total ١٩٢ needles) in a cylindrical assembly. The needles are made of stainless steel, which is mechanically strong and nontoxic.

The face was sterilized with povidone-iodine and alcohol. (Proper wiping of povidone-iodine is necessary to prevent foreign body granuloma formation) Cold compresses were used throughout the procedure to minimize pain. The treatment was then performed by rolling the needling tool over the areas affected by acne scars five times in the four directions (vertical, horizontal, and diagonal) without pressing too hard (lips and eyelids were avoided). In patients with deep scars, an assistant stretched the skin perpendicular to the Dermaroller movement to reach the base of the scar. The skin bled for ٣٠ seconds to ٢ minutes, which was less than normal clotting time, and wet gauze swabs were used to soak up any fluid ooze. After each session, all patients were instructed to minimize sun exposure, avoid trauma, and tension at the scar site and to apply sunscreen daily with a sun protection factor of ٥٠ or more.

Outcome assessments:

Patient follow -up was scheduled at ٣-week intervals during the ١٨-week treatment period and at ٤-week intervals for ٨-weeks after the final session (total study duration, ٢٦ weeks from treatment commencement). Acne scar improvements were quantified by assessing the degrees of improvement according to scar types, and the *echelle d'evaluation clinique des cicatrices d'acne* [clinical evaluation scale for acne scarring] (ECCA) scores by two dermatologists. ECCA grading scales are based on semiqu-antitative, weighted assessments of ٦ types of acne scars, namely, V-shaped atrophic scars (icepick), U-shaped atrophic scars (boxcar), M-shaped atrophic scars (rolling), hypertrophic inflammatory scars, keloid scars, and superficial elastolysis[٢٠]**.** Standar-dized digital facial photographs were obtained at baseline prior to dermal roller sessions (preoperative) and subsequent follow-up photographs were taken using identical camera settings of Panasonic digital camera (DMC-FX٣٦, Osaka, Japan). Degrees of improvement according to each subtype are presented as percentage impro-vement (٠-١٠٠٪). Patient subjective satisf-action scores were determined by a quartile grading scale (٠, slight improvement, <٢٥٪; ١, moderate improvement, ٢٥–٤٩٪; ٢, significant improvement, ٥٠–٧٤٪; ٣, marked improvement, ≥٧٥٪) to assess their percentage of improvement on a question-naire completed at the end of the study[٢١]. Any side effects observed were recorded at each treatment session and follow-up visit, and pain was graded on a scale of ٠ (none) to ٩ (maximum).

For histopathological evaluation, ٣-mm punch biopsy specimens of untreated scars were obtained from the twelve patients before treatment commencement and again after the seventh session (at ١٨ weeks).A third biopsy was taken from two of the patients (volunteers) at ٨ weeks after final treatment (after the ٢٦ weeks of the study) to assess the collagen maturation. Biopsy specimens after treatment were taken from a site near the pretreatment biopsies. All

histopathological and histochemical staining and evaluation were carried out in department of pathology, faculty of medicine, Sohag University. Biopsy specimens were fixed in a solution of ١٠٪ neutral buffered formalin, embedded in paraffin and sectioned at thickness of ٥um, the following stains were used; standard hematoxylin and eosin, Massion Trichrome (BioTek, code KT٠٣٤) and picrosirius red (Via Marche, ١٩, DDK Italia S.r.l) .

Massion Trichrome (to assess total collagen amount):

Sections were deparaffinized, hydrated and mordant in Bouiǹs fluid for one hour, rinsed in tap water then stained in Weigerts Hematoxylin, sections were put in the followings solutions; Biebrich Acid Fuschsin, phosphotungstic Acid, Aniline Blue Stain, and ١٪ Acetic acid respectively, then sections were dehydrated, cleared in Xylene and mounted with DPX.

Picrosirius Red staining: (to assess newly formed collagen):

The sections were deparaffinized, hydrated, stained with Weigerts hematoxylin for ٨ minutes (for nuclei), stained with picrosirius red for one hour, dehydrated in ٣ changes of ١٠٠٪ ethanol, cleared in xylene and mounted with DPX.

The stained sections were analyzed using an Olympus micro-scope (U- MDOB٣, USA) equipped with filters to provide circularly polarized illumination. The lower filter (٣M circular polarizer; Edmud Industrial optics, Barrington, NJ, USA) was placed above the microscopes field iris diaphragm, while the upper filter was constructed from a combination of a quarter – wave plate (U-TP١٣٧, Olympus) placed below a linear polarizer aligned such that its transmission axis was at ٤٥ to the fast axis of the wave plate[٢٢]. These two filters were aligned so that the background in the field of view was dark as possible (the filters were crossed), tissue images were obtained with a ١٠, ٢٠ and ٤٠ objective lenses and recorded on a digital Camera (E٣٣٠-DC ٧.٤V, Olympus).

Statistical analysis

Statistical analysis were performed with Wilcoxon signed rank test (for comparison of before and after dermal roller treatment) and student T test using SPSS software (version ١٦, SPSS Inc, Chicago, IL). Data are expressed as means plus or minus standard deviations, and P values less than ٠.٠٥ were considered statistically significant.

Results

Twelve patients (٥ men,٧ women) enrolled in this study, completed the ٢٦ weeks study without any significant adverse effect. Age of patients ranged from ٢٠ to ٣١ years mean±SD (٢٥.٩±٤.٣). Two patients (١٦.٦٪) were skin type III, Six patients (٥٠٪) were skin type IV and four patients (٣٣.٣٪) were skin type V. The mean duration of acne scars was ٤.٣ years (range ٢–٨ years). Detailed ECCA scores of all patients are listed in table ١.

Dermal roller treatment induced a statistically significant improvement in acne scars. Consistently, ECCA scores were significantly reduced following PCI after the ٢٦ weeks (١٢٣.٣±٢٤.٥→٧٤.١٦±١٦.٤٩) (*p*= ٠.٠٠). There was a highly significant decrease in the means of all types of atrophic acne scars after PCI treatment (table ٢). Wilcoxon signed rank test showed highly significant decline in icepick, boxcar and rolling scars, total ECCA grading score) at ٢٦ weeks treatment (*P*=٠.٠٠١&٠.٠٠٥ & ٠.٠٠٥ & ٠.٠٠٢ respectively) with variable degree of improvement but no statisti-cally significant difference in mean percentage of improvement between different types of atrophic scars (icepick, boxcar or rolling) (*p*= ٠.١٦), (Table ٢, Figure ١ & ٢). No significant difference between males and females regarding the severity score (ECCA) either before or after treatment (p=٠.٦٥ and ٠.٤٥ respectively). No significant correlation was found between the degree of improvement and scar duration.

Seven patients reported significant improvement (٥٠–٧٤٪) of their acne scars, while three reported moderate impovement (٢٥-٤٩%) and only two reported marked improvement (٧٥٪) and all the patients were satisfied with their treatment results. Mean of patients pain score during the sessions was (٥.٤±١.٩). Adverse events were only transient pain, erythema and edema in

treated areas. Mean of post erythema days was (٣.٠±٠.٨) and mean of downtime days was (٢.٩ ± ٠.٨) days. None of the patients developed post-operative hyperpigmentation.

Histopathological evaluation:

Hematoxylin and eosin staining demonstrated relatively thickened epidermis with more developed dermal papillae (rete ridges) post operatively, the epidermis remained intact except for minute holes. No remarkable changes were found in melanocytes after PCI. The superficial dermis showed very thin newly formed capillaries and discrete inflammatory cells (fig.٤).

Massion Trichrome showed a considerable increase in collagen deposition at ١٨ weeks postoperatively. The collagen appeared to have been laid down in a normal lattice pattern, rather than in parallel bundles as seen in scar tissue (fig. ٣ & ٤). However, Massion Trichrome fails to reveal very thin collagen fibers.

The color of collagen fibers stained with **Picrosirius red** (fig.٣ & ٤ & ٥) and viewed with polarized light depends upon fibers thickness and their maturation; as fiber thickness increases and matures the color changes from green to yellow to orange to red. Postoperatively after ١٨ weeks and after ٢٦ weeks (in two biopsies); the thin recent collagen fibers stained green to yellow, while the old thick mature collagen fibers (preoperatively) appeared orange to red.

Table ١: Types of postacne atrophic scars and Total (ECCA) grading score severity before and after PCI in all patients.

Patients	Acne Scar Severity Score (ECCA grading score)								
	Ice Pick		Box car		Rolling (M shape)		Total Score		Percentage Improvement Mean ± SD ٣٩.٨ ±١١.٩
	Before	After	Before	After	Before	After	Before	After	
١	٤٥	٣٠	٤٠	٢٠	٥٠	٢٥	١٣٥	٨٥	٣٧٪
٢	٤٥	٣٠	٤٠	٢٠	٥٠	٠	١٣٥	٥٠	٦٣٪
٣	٤٥	٣٠	٤٠	٤٠	٥٠	٢٥	١٣٥	٩٥	٢٩.٦٪
٤	٤٥	٣٠	٦٠	٤٠	٥٠	٢٥	١٥٥	٩٥	٣٨.٧٪
٥	٤٥	٣٠	٤٠	٢٠	٢٥	٢٥	١١٠	٧٥	٣١.٨٪
٦	٤٥	٣٠	٦٠	٤٠	٥٠	٠	١٥٥	٧٠	٥٤.٨٪
٧	٤٥	٣٠	٤٠	٢٠	٥٠	٢٥	١٣٥	٧٥	٤٤.٤٪
٨	٤٥	٣٠	٤٠	٤٠	٥٠	٢٥	١٣٥	٩٥	٢٩.٦٪
٩	٤٥	٣٠	٤٠	٤٠	٠	٠	٨٥	٧٠	١٧.٦٪
١٠	٣٠	٣٠	٤٠	٢٠	٥٠	٢٥	١٢٠	٧٥	٣٧.٥٪
١١	٤٥	٣٠	٤٠	٢٠	٠	٠	٨٥	٥٠	٤١.٢٪
١٢	٤٥	٣٠	٠	٠	٥٠	٢٥	٩٥	٥٥	٤٢.١٪

Table ٢: Means of ECCA grading scores and percentage improvement of each atrophic acne scar type before and after PCI .

Type of atrophic acne scar	Before PCI Mean±SD	After PCI Mean±SD	Percentage improvement(%)
Ice pick	٤٣.٧ ±٤.٣	٣٠.٠± ٠.٠	٣١.٤٪
Box scar	٤٠.٠± ١٤.٧	٢٦.٦±١٣.٠٢	٣٣.٥٪
M shape (Rolling)	٣٩.٥٨±١٩.٨٢	١٦.٦٦±١٢.٣	٥٧.٩٪
Total score (ECCA)	١٢٣.٣±٢٤.٥	٧٤.١٦±١٦.٤٩	٣٩.٨٪

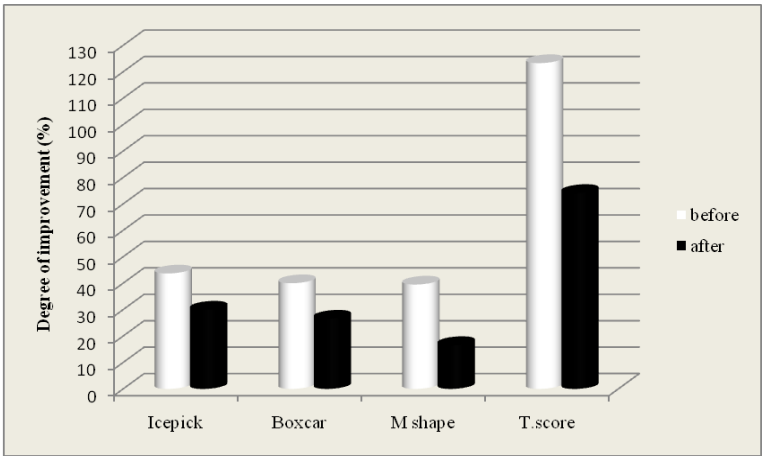

Fig. ١: Degree of improvement in different types of atrophic acne scars and total ECCA grading scores after PCI.

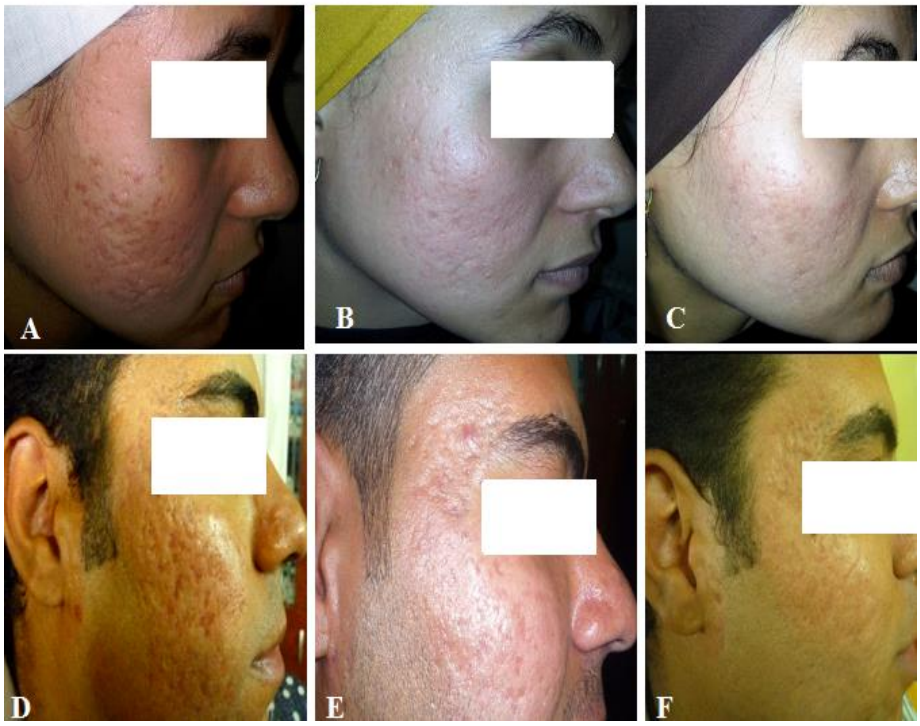

Fig. ٢: Atrophic acne scars of representative patients showed notable improvement in all types ٨ weeks after completion of PCI treatment (at ٢٦ weeks). A and D before treatment (baseline), B and E after ١٨ weeks of PCI treatment and C and F after ٢٦ weeks (at the end of study).

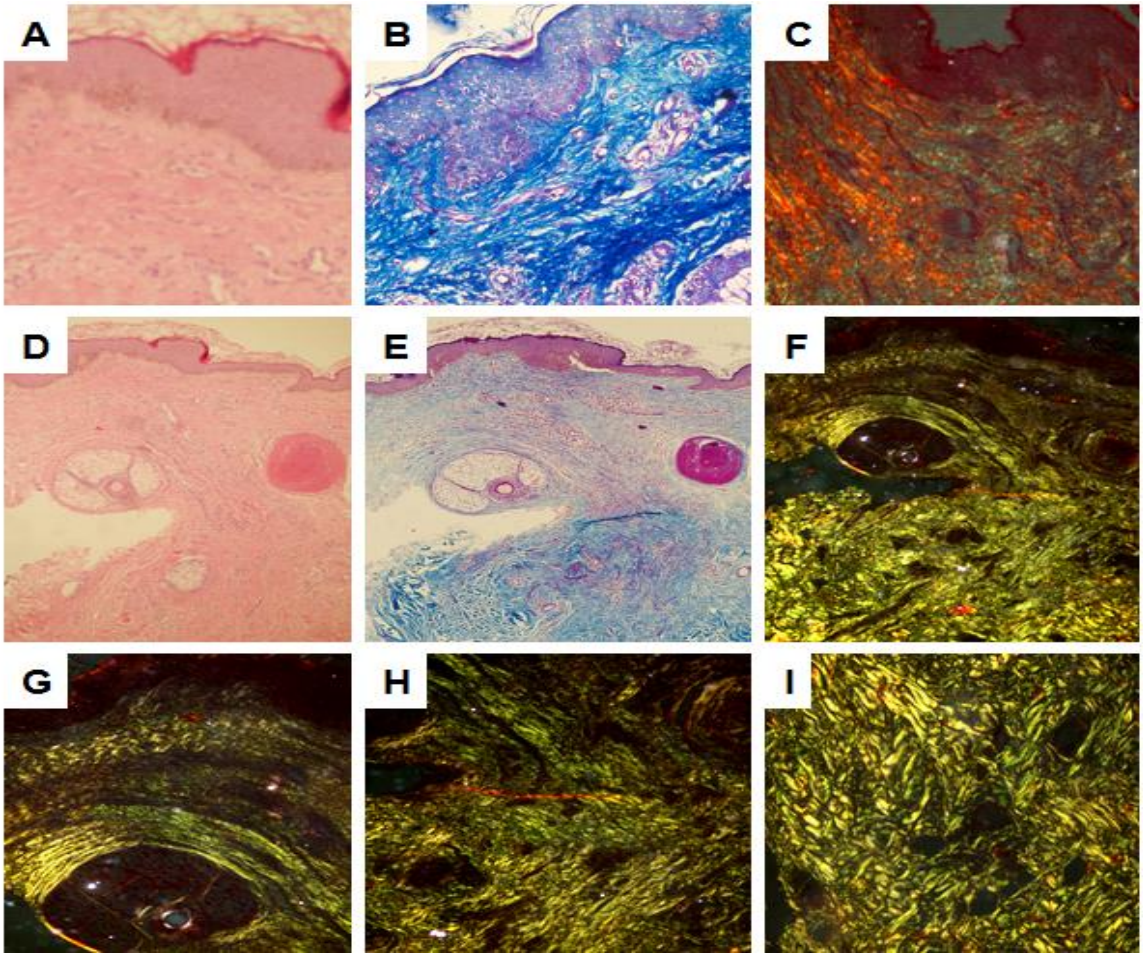

Fig. ٣: A B, and C: Pre-operative biopsy of acne scar; A (H & E original magnification X١٠٠), B (Massion Trichrome original magnification X٢٠٠) C (Picrosirius red original magnification X١٠٠ D-I; post-operative biopsy; D &E (H&E and Massion Trichrome X١٠٠, ١٠٠): revealed increase the dermal content of collagen fibers, F-I (Picrosirous red stained sections X١٠٠,٢٠٠,٢٠٠,٢٠٠ original magnifications) both superficial and deep dermis showed newly formed collagen fibers, appear green- yellow birefringence by polarizing light, (H) showed very thin recently formed green- yellow collagen fibers in superficial dermis, (I) showed thick green- yellow collagen fibers in deep reticular dermis with focal knobby pattern.

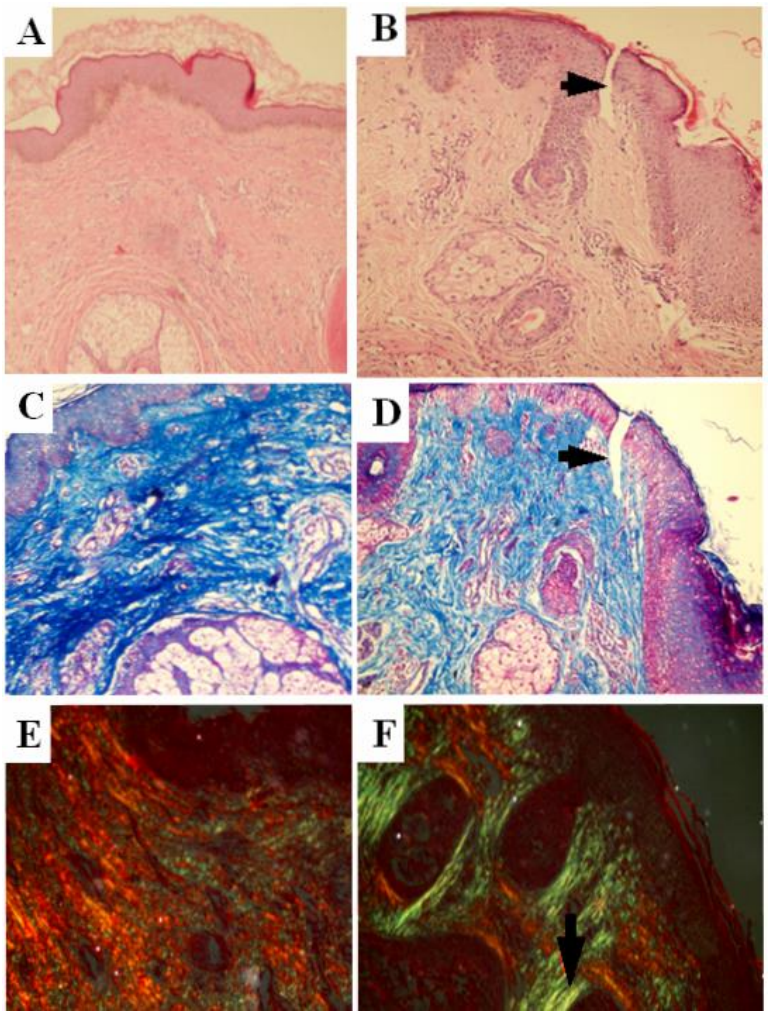

Fig. ٤: A, C, E: Pre-operative biopsy; A (H&E, original magnification X١٠٠) showed thin epidermis, flat rete ridges, C (Massion Trichrome, original magnification X٢٠٠) the superficial dermis showed dense collagen bundles of old acne scar, E (Picrosirius Red, original magnification X١٠٠) the same bundles showed old red- orange birefringence. B, D, F: Post-operative biopsy; B (H & E, original magnification X١٠٠) showed thick epidermis, newly formed rete ridges and pin hole is cutting the epidermis (arrow), D (Massion Trichrome, original magnification X ١٠٠) showed recent collagen bundles laid down in lattice pattern with more newly formed capillaries and inflammatory cells, F (Picrosirius Red, original magnification X١٠٠) revealed recent collagen fibers of green birefringence (arrow) dissecting the old red- orange ones.

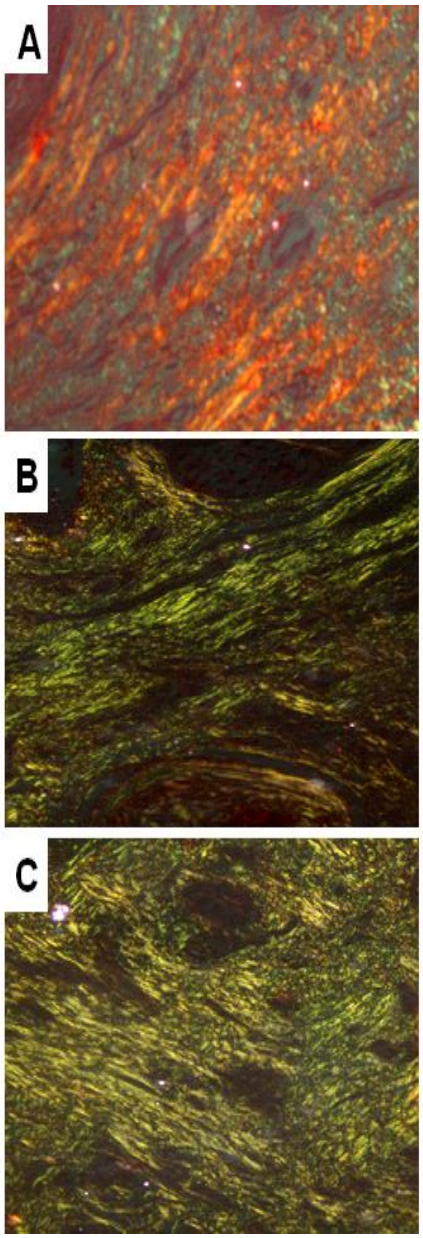

Fig.٥: A, B, C) Picrosirius red stained sections (original magnification x ٢٠٠), (A) preoperative biopsy showing old orange-red birefringence collagen fibers, (B) ١٨ weeks postoperative showing very recently formed green birefringence collagen fibers, while (C) ٨ weeks after end of treatment (after ٢٦ weeks) showing more mature yellow birefringence collagen fibers(maturation of collagen in dermis).

Discussion

Acne vulgaris depending on its severity, can end with a variety of scars. The affected skin of postacne scarring has an abnormal contour, with most scars being depressed below the adjacent normal skin[٢٣]. Many treatment modalities for scar improvement, such as dermabrasion, microdermabrasion, chemical peeling, laser and cosmetic surgery had disadvantage of either being too mild and ineffective or too aggressive and complicated[٧].

The epidermis is a complex, highly specialized organ that, although only ٠.٢mm thick, is our first layer of protection from the environment. We should never damage the epidermis unless the risks associated with leaving it intact are greater than those associated with its removal. As an alternative to laser treatments, skin needling has been used, which protects the epidermis and stimulates natural collagen synthesis[١٦]. When thousands of fine pricks are placed close to each other thus creates thousands of microclefts through the epidermis into the papillary dermis and produces a confluent zone of superficial bleeding that is a powerful stimulus to initiate the normal process of wound healing[٢٤].

The present study revealed a statistically significant overall improvement of atrophic acne scars in all patients as mean of ECCA scores was significantly reduced following PCI after the ٢٦ weeks of the study (١٢٣.٣±٢٤.٥→٧٤.١٦±١٦.٤٩) (*p*=.٠.٠٠) with ٣٩.٨% percentage improvement. This was in agreement with Majid (٢٠٠٩) who reported excellent response in ٧٢.٢٪ of patients after four sessions of PCI[١٧]. These results were consistent also with Fabbrocini et al., (٢٠٠٩) who found that the severity of the acne scars in all patients was greatly reduced after only two sessions with an ٨-week interval, without any side effects apart from redness and swelling, which disappeared in ٢ to ٣ days[٢٤]. Also with Leheta et al., (٢٠١١) who revealed that PCI improved atrophic acne scars in ١٠٠٪ of patients, with overall scar improvement of up to ٩١.٧٪ (mean ٦٨.٣±١٩.٣) after four sessions with an ٤ week interval[٢٥].

The present study showed highly significant decline in icepick, boxcar, rolling scars and total ECCA scores at ٢٦ weeks of treatment. Rolling scars showed ٥٨٪ improvement after PCI, followed by ٣٣.٥٪ in Boxcar and ٣١.٤٪ in icepick scars. In comparable to these results, Majid (٢٠٠٩) reported excellent response in rolling and boxcar scars, while moderate response in icepick scars[١٧]. Fabbrocini et al., (٢٠٠٩) reported that the relative rolling scar depth was significantly reduced after PCI[٢٤]. In a comparative study done by Leheta et al, (٢٠١١) they reported significant improve-ment of rolling acne scars after PCI compared to ١٠٠٪ TCA (chemical recons-truction of skin scar) CROSS. They recommended ١٠٠٪ TCA CROSS over PCI for treatment of boxcar and ice pick acne scars. They also stated that PCI is a valuable mean of treating boxcar and ice pick scars for patients with a history of skin dyschromia because of a higher incidence of consequent post inflammatory hyper-and hypopigmentation with ١٠٠٪ TCA CROSS [٢٥].

After PCI, our patients experienced transient erythema and edema that lasted for a mean of (٣.٠± ٠.٨) days, and overall mean downtime was (٢.٩±٠.٨) days, which was consistent with other studies[١٧,٢٤,٢٥]. None of our studied patients developed post-operative hyperpigmentation.

Histopathological examination in the present study showed normal epidermis with flat rete ridges at baseline, the epidermal thickness increased at ١٨weeks postoperatively with well developed rete ridges. This agreed with Fernandes and Signorini, (٢٠٠٨) who showed that the skin became thicker with well formed rete ridges after PCI[١٦]. Also with Aust et al., (٢٠٠٨) who showed that PCI left the epidermis intact without any damage to stratum corneum or any other layer of the epidermis or the basal membrane[٢٦].

As regard to increased epidermal thickness, Fernandes (٢٠٠٥) explained the difference between standard wounds healing and healing after dermal roller. In standard

wounds, the main cells are the keratinocytes, which change in morphology and become mobile to cover the gap in the basement membrane. Peripheral cytoplasmic actin filaments also are developed to pull keratinocytes together to close the wound. These actin filaments, however, are not an important factor in PCI because re-epithelialization, or the closure of needle holes, occurs within a few hours after needling because the gap is small. A day or two after PCI, the keratinocytes start proliferating and act more in thickening the epidermis than in closing the defect[٢٧].

The current study showed no remarkable changes in melanocytes, as melanocytes number neither increased or decreased. This agreed with Aust et al., (٢٠٠٨) who reported that, no change in the number of melanocytes after PCI. In their study, DNA microarray experiments demonstrated that suppressor factor (interleukin-١٠) is increased during the first ٢ weeks after PCI and melanocyte stimulating hormone gene is dowenregulated which suggests that needling reduces risk of dyspigmentation through significant downregulation of melanocyte stimulating hormone in the postinflammatory response[٢٦]. As melanocytes are not negatively impacted after PCI, risks of postinflammatory hypo- or hyperpigmentations are minimized, so it can be safely performed on Asian and darker skins[٢٨].

The current study compared collagen deposition of acne scar pre and post-operatively at the end of treatment (١٨ weeks) and at (٢٦ weeks in two biopsies). Pre-operative collagen fibers arranged in parallel pattern, lack of newly formed thin capillaries and fibroblasts that observed post-operatively in which collagen deposition was increased and appeared to be laid down in normal lattice pattern as evidenced by H & E and Massion Trichrome staining. This came in agreement with Fernandes and Signorini, (٢٠٠٨) who reported greater collagen deposition ٤ months post-operatively[١٦]. Aust et al., (٢٠٠٨) and Fabbrocini et al., (٢٠٠٩) also showed consi-derably greater collagen deposition ٦ months post-operatively[٢٩,٢٤].

Scar tissue is the exact same protein (collagen) as the tissue that it replaces, but the fiber composition of the protein is different; instead of a random basket weave formation of the collagen fibers found in normal tissue, in fibrosis the collagen cross-links and forms a pronounced alignment in a single direction. This collagen scar tissue alignment is usually of inferior functional quality to the normal collagen randomised alignment[٣٠].

With microneedling, one gets a purer stimulus for collagen synthesis without the heavy inflammatory reaction because subdermal fat is certainly not damaged at the same time. It is believed that because the epidermis is intact, this might favour predominantly transforming growth factor β٣ (TGF-β٣) rather than TGF-β١ *and* –β٢, which are associated with scar collagen deposition. TGF-β٣ is implicated in scarless healing and normal lattice weave collagen deposition so PCI seems to induce normal lattice weave rather than scar collagen[٣١]. On the other hand, ablative laser resurfacing and deep peeling injure the skin and subsequently cause fibrosis of the papillary dermis which initiates an inflammatory response that propels the fibroblasts to produce scar collagen in parallel orientation rather than in the normal lattice network of normal skin. They lighten scars because generally, they destroy the epidermis, and very importantly, its basement membrane, which is replaced by an epidermis that no longer has dermal papillae and is thinner than before[٣٢].

We further assessed the effect of PCI on new collagen formation, and whether the increase in collagen level as observed by masson trichrome was a result of the enhancement of newly synthesized collagen formation. Detection of newly synthesized collagen with picrosirius red stain under polarized lens, microscopic examination showed increase of newly synthesized collagen at the end of treatment (١٨ weeks) and at (٢٦ weeks in two biopsies). Picrosirius red staining confirmed the maturation process of collagen fibers as old fibers of pre-operative acne scar appeared orange birefringence while the recent ones

appeared as green to yellow birefringence postoperatively; these results came in accordance with Rich and Whittaker, (٢٠٠٥)[٣٣].

El-Domyati et al., (٢٠١١) evaluated the histologic changes of the nonablative Radiofrequency device in photoaging with picrosirius red stain and showed signifi-cantly increased newly synthesized collagen at the end of treatment, and at ٣ months posttreatment, compared with baseline[٣٤].

Additionally, Picrosirius red staining in the present study could differentiate between very thin recent green- yellow collagen fibers (type III) in superficial dermis and thick bundles of collagen fibers (type I) in deeper dermis, so we can use Picrosirius red stain as qualitative method rather than specific to differentiate between type III and I collagen fibers instead of immune-histochemical staining.

Major advantages of PCI are that, patients had no open wound and consequently require only a short healing phase, which is encouraging for many patients. Because the epidermis and stratum corneum were never removed, there was no risk of photo-sensitivity or any postinflammatory dysch-romia. Disadvantages were the surgeon's exposure to blood, the need for complete anesthesia of the skin when performing needling, swe-lling and bruising for the first few days, and that the final result took a long time because new collagen continues to be laid down for approxi-mately ٣ months[٢٩].

Conclusions

The findings obtained enabled us to draw the following conclusions:

(١) Skin needling is a simple and minimally invasive procedure with rapid healing and low downtime.

(٢) It has undisputable advantages compared with conventional methods. The most important is that the epidermis remains intact because it is not damaged, eliminating most of the risks and negative side effects of chemical peeling or laser resurfacing.

(٣) As melanocytes are not negatively impacted, risks of postinflammatory hypo- or hyperpigmentations are minimized, so it can be safely performed on Asian and darker skins, as well as skins that have been previously treated with laser or had dermabrasion.

(٤) Histologic improvements, as evidenced by Massontrichrome staining and picrosirius red, were found to concur with clinical efficacy observed by physicians and patients and

(٥) Acne scars can be evaluated quickly and reliably using the ECCA grading scale.

Aknowledgement:
We thank all the patients who participated in this study.

References

١. Jacob CI, Dover JS and Kaminer MS. Acne scarring: a classification system and review of treatment options. J Am Acad Dermatol ٢٠٠١;٤٥(١):١٠٩-١٧.

٢. Layton AM, Henderson CA and Cunliffe WJ. A Clinical evaluation of acne scarring and its incidence. Clinical and Experimental Dermatology ١٩٩٤;١٩(٤): ٣٠٣-٨.

٣. Norris JF and Cunliffe WJ. A histological and immunocytochemical study of early acne lesions. Br J Dermatol. ١٩٨٨; ١١٨(٥): ٦٥١-٩.

٤. Hirsch RJ. The future of acne treatment with lasers. Cosmet Dermatol. ٢٠٠٢; ١٥:٧٣-٤.

٥. Holland DB, Jeremy AH, Roberts SG, Seukeran DC, Layton AM and Cunliffe WJ. Inflammation in acne scarring: a comparison of the responses in lesions from patients prone and not prone to scar. Br J Dermatol. ٢٠٠٤;١٥٠:٧٢-٨١.

٦. Sadick NS and Schecter AK. A preliminary study of utilization of the ١٣٢٠-nm Nd: YAG laser for the treatment of acne scarring. Dermatol Surg. ٢٠٠٤;٣٠(٧):٩٩٥-١٠٠٠.

٧. Ramesh M, Gopal MG, Sharath kumar and Ankur Talwar. Novel technology in treatment of acne scars: The matrix tunable radiofrequency technology. Journal of cutaneous and aethetic surgery. ٢٠١٠;٣(٢):٩٧-١٠١.

٨. Kim HJ, Kim TG, Kwon YS, et al. Comparison of a ١,٥٥٠ nm erbium: glass fractional laser and a chemical reconstruction of skin scars (CROSS) method in the treatment of acne scars: a

simultaneous split-face trial. Lasers Surg Med. ٢٠٠٩;٤١:٥٤٥–٥٤.

٩. Alster TS. Cutaneous resurfacing with $CO_٢$ and Erbium:Yag lasers: Preoperative and postoperative considerations. Plast Reconstr Surg. ١٩٩٩; ١٠٣: ٦١٩-٦٣٢.

١٠. Alster TS, Lupton JR. An overview of cutaneous laser resurfacing. Clin Plast Surg.٢٠٠١,٢٨:٣٧-٥٢.

١١. Ang P, Barlow RJ. Non ablative laser resurfacing: A systemic review of the literature. Clin Exp Dermatol. ٢٠٠٢; ٢٧:٦٣٠-٦٣٥.

١٢. Shim EK, Barnette D, Hughes K, Green-way HT. Microdermabrasion: A clinical and histopathologic study. Dermatol Surg. ٢٠٠١;٢٧:٥٢٤-٥٣٠.

١٣. Lee HS, Lee JH, Ahn GY, Lee DH, Shin JW, Kim DH, et al., Fractional photothermolysis for the treatment of acne scars: a report of ٢٧ Korean patients. J Dermatol Treat.٢٠٠٨;١٩:٤٥-٤٩.

١٤. Alster TS, Tanzi EL and Lazarus M. The use of fractional laser photothermolysis for the treatment of atrophic scars. Dermatol Surg. ٢٠٠٧;٣٣:٢٩٥-٢٩٩.

١٥. Chan HH, Manstein D, Yu CS, Shek S, Kono T and Wei WI. The prevalence and risk factors of post-inflammatory hyper-pigmentation after fractional resur-facing in Asians. Lasers Surg Med. ٢٠٠٧;٣٩:٣٨١-٣٨٥.

١٦. Fernandes D and Signorini M. Combacting photoaging with percutaneous collagen induction. Clinics in Dermatology. ٢٠٠٨;٢٦,١٩٢-١٩٩.

١٧. Majid I. Microneedling therapy in atrophic facial scars: an objective assessment. J Cutan Aesthet Surg. ٢٠٠٩; ٢:٢٦–٣٠.

١٨. Flabella AF and Falanga V. Wound healing. In: The biology of the skin, Feinkel RK, Woodley DT, editors. New York: Parethenon. ٢٠٠١; P.٢٨١–٩٩.

١٩. Liebl H. reflections about collagen-inductiontherapy (CIT). A hypothesis for the mechanism of action of collagen induction therapy (CIT) using micro-needles (on-line). (accessed March ٣٠, ٢٠٠٩).

٢٠. Dreno B, khammari A, Orain N, Noray C, Merial-kieny C, Mery S, et al., EccA grading scale: an original validated acne scar grading scale for clinical practice in dermatology. Dermatology. ٢٠٠٧; ٢١٤: ٤٦-٥١.

٢١. Cho SB, Lee JH, Choi MJ, Lee KY, OH Sh. Efficacy of the fractional photothermolysis system with dynamic operating mode on acne scars and enlarged facial pores. Dermatol.Surg ٢٠٠٩; ٣٥(١):١٠٨-١١٤.

٢٢. Whittaker P, Kloner RA, Boughner DR and Pickering JG. Quantitative assessment of myocardial collagen with picrosirius red staining and circularly polarized light. Basic Res.Cardiol. ١٩٩٤; ٨٩:٣٩٧-٤١٠.

٢٣. Goodman GJ. Postacne scarring: a review of its pathophysiology and treatment. Dermatol Surg. ٢٠٠٠;٢٦:٨٥٧-٨٧١.

٢٤. Fabbrocini G, Fardella N, Monfrecola A, et al. Acne scarring treatment using skin needling. British association of dermatology. Clin Exp Dermatol. ٢٠٠٩;٣٤:٨٧٤–٨٣.

٢٥. Leheta T, EL tawdy A, Abdelhay R and Farid S. Percutaneous Collagen Induction Versus Full-Concentration Trichloroacetic Acid in the treatment of Atrophic Acne scars. DermatolSurgery. ٢٠١١;٣٧(٢):٢٠٧–٢١٦.

٢٦. Aust MC, Reimers K,Claudia R, Frank S, Sbrina J and Merlin G. Minimally invasive skin rejuvenation without risk of hyperpigmentation –Fact or Fiction? Plast Reconstr Surg ٢٠٠٨b;١٢٢(٥):١٥٥٣-١٥٦٣.

٢٧. Fernandes D. Minimally invasive percutaneous collagen induction. Oral Maxillofac Surg Clin North Am. ٢٠٠٥; ١٧:٥١-٦٣.

٢٨. Fabbrocini and M.P. De Padova. Combination of Chemical Peels and Needling for Acne Scars A. Tosti et al. (eds.), Color Atlas of Chemical Peels, Verlag Berlin Heidelberg, ٢٠١٢ p ٨٧.

٢٩. Aust MC, Fernandes D, Kolokythas P, et al., Percutaneous collagen induction therapy*:* an alternative treatment for scars, wrin-kles, and skin laxity. Plast Reconstr Surg. ٢٠٠٨;١٢١١٤٢١–١٤٣٠.

٣٠. McDougall, Dallon J, Sherratt J, Maini P. Fibroblast migration and collagen deposition during dermal wound healing: mathematical modelling and

clinical implications. Phil Trans. R. Soc. ٢٠٠٦;٣٦٤:١٣٨٥–١٤٠٥.

٣١. Orentreich DS and Orentreich N. Subcuta-neous incisionless (subcision) surgery for the correction of depressed scars and wrinkles. Dermatol Surg Clin North Am ١٩٩٥; ٢١:٦٥٤٣-٦٥٤٩.

٣٢. Matthias AC, Fernandes, D, kolokythas, PK, Hilton M, Vogt PM. Percutaneous collagen induction therapy: An alterna-tive treatment for scars, wrinkles and skin laxity. Plastic and reconstructive surgery ٢٠٠٨;١٢:(٤)١٤٢١-١٤٢٩.

٣٣. Rich L and Whittaker P. Collagen and Picrosirius red staining: A polarizes light assessment of fibrillar hue and spatial distribution. Braz .J. morphol. Sci. ٢٠٠٥;٢٢(٢):٩٧-١٠٤.

٣٤. El-Domyati M, El-Ammawi TS, Medhat W, Moawad O, Brennan D, Mahoney MG and Jouni U. Radio-frequency facial rejuvenation: Evidencebased effect. J Am Acad Dermat. ٢٠١١; ٦٤:(٣):٥٢٤-٥٣٥.