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Confocal Microscopy Images to Monitor Skin Needling in the Treatment of Acne Scars

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Abstract

Background: Acne is a common and generally self-limiting skin disease. However, the severe sequel of acne scarring can lead to long-term psychological impairment. Although the occurrence of post-acne scarring, especially in the cases of papulopustular and nodulo-cystic variants, remains unknown, its incidence is extremely common. There is a wide range of modalities used in the treatement of acne scars such as surgical techniques, subcision, chemical peels, ablative lasers, fractional lasers and collagen induction therapy. Reflectance confocal microscopy is a new noninvasive technique for the examination of the skin "*in vivo*", which can be useful to evaluate the effectiveness of different therapeutic approaches.

Aims: The aim of this study is to evaluate the modification and the improvement induced by skin collagen induction on acne scars through the use of confocal microscopy.

Methods: 30 patients, who had previously been diagnosed with acne scars, were selected. All patients were clinically evaluated according to the qualitative grading scale of acne scars proposed by Goodman and Baron. In addition, quality of life was also assessed using DLQI. Finally, all patients were evaluated using GAIS system. Skin collagen induction (Dermaroller®) was performed in one session, with follow up assessments at 30 (T1) and 60 days (T2). In 10 patients confocal microscopy was performed at T0 and T2.

Results: 28 out of 30 patients showed clinical improvement of acne scars and in all 10 patients confocal microscopy showed improvement of the texture and collagen neosynthesis induced by skin collagen induction.

Conclusions: Skin needling is a very effective treatment for acne scars. Confocal microscopy observation can better define the modification induced by skin needling in the treatment of acne scars and open new perspectives on this treatment.

Keywords: Acne; Acne scars; Skin collagen induction; Confocal microscopy

Introduction

Acne is an almost ubiquitous disease among teenagers. Post-acne sacrring is one of the most frequent sequel observed in patients affected by moderate and severe acne [1,2].

Although the occurrence of post-acne scarring, especially papulo-pustular and nodulo-cystic variants, remains unknown, its incidence is extremely common. There is a wide range of treatment approach used for acne scars such as surgical techniques, subcision, chemical peels, ablative lasers, fractional lasers and skin collagen induction (skin needling). In the last few years skin needling has proved to be one of the most effective treatments for roller and ice pick scars [3,4]. Since 1995 skin needling has been used to achieve percutaneous collagen induction (PCI) [5,6]. This technique involves puncturing the skin multiple times with a small needle to induce collagen growth. The needle breaks old collagen strands in the most superficial layer of the dermis. It is assumed that this process promotes the removal of damaged collagen and induces the synthesis of new one [7,8]. Reflectance confocal microscopy (RCM) is a novel non-invasive technique for “in vivo” examination of the skin. RCM is mainly constituted by a near-infrared diode laser, a light detector and a pinhole that allows the selection of “in focus” light wavelengths reflected from the tissue according to the different refractive indexes of skin structures. More detailed technical data have been previously reported in literature [9-11]. Formerly confocal microscopy was largely used to analyze melanocytic lesions; its use was later extended to non-melanocytic skin tumors and inflammatory skin diseases. More recently cosmetologic applications of RCM have also been examined [12-17]. The horizontal evaluation of tissue using confocal microscopy ranges from the stratum corneum to the upper dermis, where resolution of images is lost (approximately up to 250 microns). High-resolution images are employed for the evaluation of cyto-architectural features [18].

In order to evaluate the ability of RCM to show the microscopic improvement of collagen neosynthesis after PCI, we performed RCM

in a group of 10 patients affected by acne scars, treated with one session of skin needling.

Materials and Methods

30 patients (18 men and 12 women) with facial atrophic/hypotrophic acne scars were enrolled from the outpatient service of the Department of Clinical Medicine and Surgery, Division of Clinical Dermatology, University of Naples "Federico II". Age of patients ranged from 19 to 44 years (average age of 30.6 years, ± 7.97). All patients were photographed with Reveal device (The Reveal® Imager - Canfield's exclusive RBX Technology, 15 megapixel resolution, auto focus, flash cross-polarized light). This device allows the standardization of photographic documentation by capturing patient's images in 3 standard positions (45° to the left/0°/45° to the right). All patients were clinically evaluated according to the qualitative grading scale of acne scars proposed by Goodman and Baron (Table 1) [27,28,14]. Moreover, quality of life was assessed using DLQI (Table 2). Finally, all patients were evaluated using GAIS system (Table 3).

Types of scars (quantitative evaluation)	Point	Number of scars	Moltiplicator
Macular or mild atropic	1	1-10	1
Moderately atrophic	2	11-20	2
Punched out or linear-troughed severe scars	3	>20	3
Hyperplastic papular scars	4		
Types of scars (qualitative evaluation)	GRADE		
Erythematous hyper or hypopygmented marks	GRADE 1		
Mild atrophy, can be covered with make up or facial hair	GRADE 2		
Moderate scarring, not covered by make-up but can be flattened by manual stretching of the skin	GRADE 3		
Scarring not flattened with manual stretching of the skin	GRADE 4		

Table 1: Methods of evaluation of scar severity according to quantitative and qualitative global scarring grading system of Goodman and Baron.

Score	DLQI
0-1	No effect at all on patient's life
2-5	Small effect on patient's life
6-10	Moderate effect on patient's life
11-20	Very large effect on patient's life
21-30	Extremely large effect on patient's life

Table 2: DLQI evaluation.

All patients were treated with skin needling (Dermaroller® manufactured by Dermaroller GmbH, Wolfsburg, Germany) with a1.5 mm deep device. 10 of the 30 patients were evaluated using RCM (VivaScope 1500, LUCID) at baseline (T0) and after 60 days (T2) in order to correlate clinical information to microscopical data. After two months, all 30 patients received a re-evaluation of the treated area (Table 4). The inclusion criteria were: subjects of both sexes over the age of 18 years, subjects with atrophic/hypotrophic scars; while the exclusion criteria considered were: presence of skin tumors in the treated area, presence of skin infection in the treated area, acne in active phase, collagenopathies, diabetes, allergy to local or general anesthetics, therapies with antineoplastic drugs, corticosteroids and/or anticoagulants, pregnancy or breastfeeding.

Degree	Description
Score 1. Exceptional Improvement	Excellent corrective result compared with the original condition
Score 2. Very improved patient	Marked improvement of the appearance, but not completely optimal. A touch-up would slightly improve the result
Score 3. Improved patient	Improvement of the appearance, better compared with the initial condition, but a touch-up is advised
Score 4. Unaltered patient rade	The appearance substantially remains the same compared with the original condition
Score 5. Worsened patient	The appearance has worsened compared with the original condition

Table 3: GAIS evaluation system.

Method
T0
Assessment of acne scars by Goodman and Baron
Test of psychological assessment and appraisal of patients with acne scars
Digital photography with Reveal
Informed consent
Confocal microscopy
T1
Skin collagen induction
T2
Assessment of acne scars by Goodman and Baron
Test of psychological assessment and appraisal of patients with acne scars
Digital photography with Reveal
Informed consent Confocal microscopy
Assessment by Global Aesthetic Improvement Scale (GAIS)

Table 4: Schematic evaluation of patients.

In vivo confocal microscopy procedure

Oil (e.g. Crodamol oil) or water-based gel was applied as a support between the skin and the microscope. A metal tissue ring with a disposable adhesive window was attached on the skin to minimize artifacts. The skin area (or the area of interest in larger lesions) were positioned at the center of the adhesive ring. A dermoscopic image was captured through the ring applied on the skin with a specific camera connected to the confocal microscope (VivaCam®).

The dermoscopic image was used to select the exact area of confocal images acquisition. Ultrasound gel was used as an immersion medium for the confocal lens (refractive index=1.3). The RCM probe was then magnetically coupled to the adapter ring in order to display the image on the computer. During real-time imaging, basic confocal images were displayed on the screen (500 × 500 micron). Images were sequentially acquired in horizontal mosaics (6 by 6 mm) at different skin layers focusing on the upper dermis. In the areas of interest, vertical micro-tomography (called Stacks) was also performed moving from the stratum corneum to the upper dermis. A sort of virtual biopsy of the skin tissue was performed. Vivascope 1500 was applied at T0 (before treatment) and T2 (after 2 months of treatment) in 10 patients treated with skin needling.

Skin needling procedure

The procedure began with the accurate disinfection of the skin and proceeded with the occlusive application of topical anesthetic 5% lidocaine (EMLA) about 30-45 minutes before the session in the areas to be treated. Skin needling (Dermaroller® manufactured by Dermaroller GmbH, Wolfsburg, Germany) was performed on the face and rolled in all directions, about 10 times forwards and 10 times backwards, by applying a constant pressure. The tool used for our patients is a device with about 200 needles of 1.5 mm length, such as to induce a quick and uniform bleeding over the entire treated area. In patients with deep scars, we stretched the skin with the fingers in the direction perpendicular to the movement of the skin needling in order to reach the scar properly. Home therapy with topical application of antiseptics for 3-4 days and sunscreen for 2 weeks minimum after the treatment with skin needling were prescribed to all patients. Immediately after treatment slight bleeding and thick, minimum oozing, serious appearance of redness and swelling, rapid closure of the micropores were evident. Late effects of treatment were swelling and redness (for 1-2 days).

Results

All patients [with an average age of 30.6 years, ± 7.97] generally well tolerated the procedure, except for a bit more persistent presence of erythema (about 6 days) in one case; no adverse effects were reported. No patients were limited in their daily activities after treatment. Clinically, at T2, all patients treated with skin collagen induction showed significant improvement in skin texture according to Reveal photograph evaluation. Clinical images captured with Reveal showed a global improvement of all scars, reduced in number as in Figure 1 and reduced in width as in Figure 2.

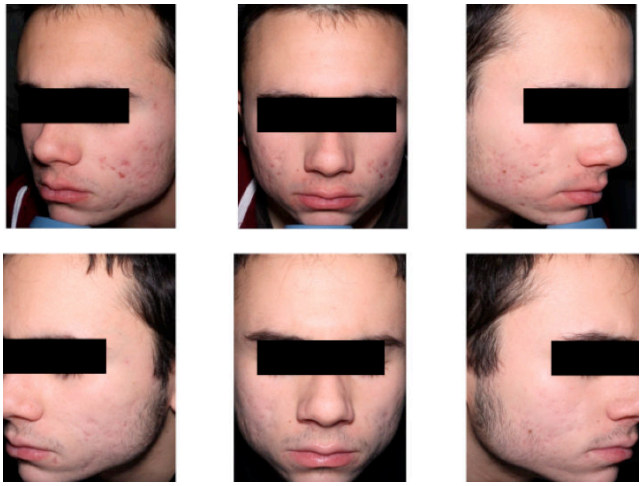

Figure 1: Dermaroller®.

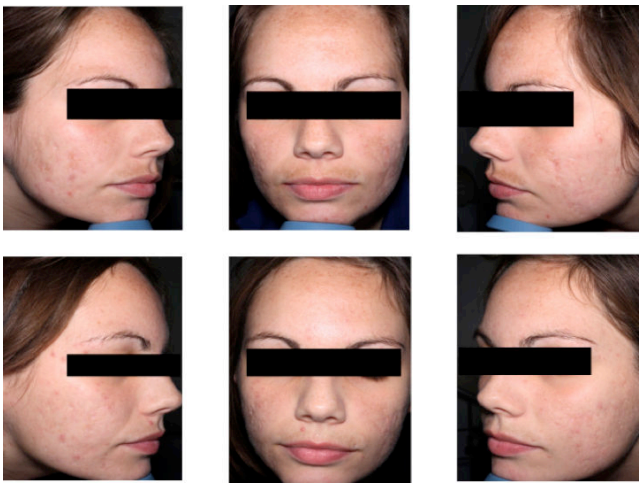

Figure 2: Types of scars.

Based on the quantitative evaluation of scars proposed by Goodman and Baron, 23 out of 30 patients showed significant improvement (Table 5) [19,20]. The GAIS assessment scale (Table 6) showed a significant clinical improvement in 28 out of 30 patients and also in 8 out of 10 patients treated with skin collagen induction after 2 months of therapy (21). The DLQI evaluation showed an overall high level of satisfaction related to the results obtained after the use of skin needling (Table 7).

PZ	T0	T1	PZ	T0	T1
1°	2	1	16°	2	2
2°	6	4	17°	6	4

3°	8	8	18°	3	2
4°	2	1	19°	2	1
5°	12	12	20°	12	9
6°	3	2	21°	3	2
7°	2	1	22°	12	9
8°	6	6	23°	3	2
9°	6	4	24°	2	1
10°	3	3	25°	6	4
11°	3	2	26°	12	12
12°	6	4	27°	2	1
13°	3	2	28°	3	2
14°	8	6	29°	12	9
15°	12	12	30°	6	4
T0 (qualitative evaluation)	**N° patients Grade**		**T1 (qualitative evaluation)**	**N° patients Grade**	
	8	Grade 2		6	Grade 1
				2	No modification
	10	Grade 3		8	Grade 2
				2	No modification
	12	Grade 4		9	Grade 3
				3	No modification

Table 5: Evaluation of scars by Goodman and Baron quantitative and qualitative system after skin needling.

N° patients	GAIS T1
13	1
10	2
7	4

Table 6: Improvement of GAIS system after skin needling.

N° pz	DQLI T0 (0-30)	DQLI T1 (0-30)
30	25	8

Table 7: Evaluation of DLQI after skin needling.

In the 10 patients evaluated with RCM, the procedure showed at T0 the presence of some areas at the level of the upper dermis (150-200 microns depth), with a variable diameter (T0 range diameter: 1.7 mm-3.5 mm) darker than the surrounding stroma, corresponding to scars.

Upper dermis of the scars was characterized by a highly refractive network of fibers seen as blurred (Figure 3a), thick and irregularly disposed (Figure 4a) (white arrows). At T2 RCM was performed in the same places of the first examination thanks to the accurate imaging performed at T0. Reduction of scars' diameter (T2 range diameter: 1.2 mm-2 mm) was observed (Figure 3b), in association with a more organized distribution of dermal fibers (Figure 4b), which were also seen as more fibrillar (Figure 4b) (red arrows). Moreover, an increase of adnexal structures was evident after 2 months from the treatment on RCM examination (Figures 5a and 5b) (red arrows). These modifications can be observed in all patients evaluated with RCM after 60 day of skin needling. These RCM images well correlate with the clinical improvement documented by clinical images. In order to support our visual evaluations with quantitative data we performed statistical analysis of scars'diameter using Paired T test. This test showed a statistically significant reduction of scars' diameter from a mean of 2.52 ± 0.587 (T0) to a mean of 1.56 ± 0.250 (T2) ($p<0.0001$).

Discussion

There are still no general guidelines available to optimize the treatment of acne scars. There are several therapeutic options, but sometimes the improvements induced are controversial [22-24]. Both non-invasive and invasive methods, already described in literature, are very expensive, and the improvement induced by different therapies was evaluated with difficulty [25].

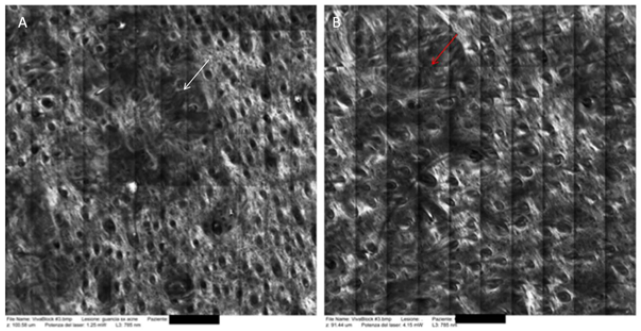

Figure 3: a) Upper dermis of the scar is characterized by a highly refractive network of fibers seen as blurred. **b)** After skin collagen induction: we observe a reduction of the diameter (from 2,5 to 1.5 mm) of the scar.

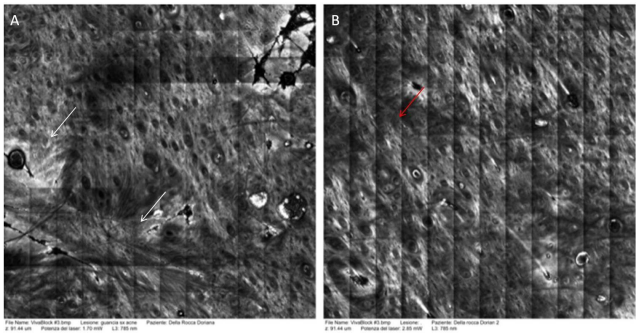

Figure 4: a) Before the treatment, upper dermis of the scar was characterized by a network of fibers that appear thick and irregularly disposed (white arrows). **b)** After the treatment, we observe a more organized distribution of dermal fibers which were also seen as more fibrillar (red arrows).

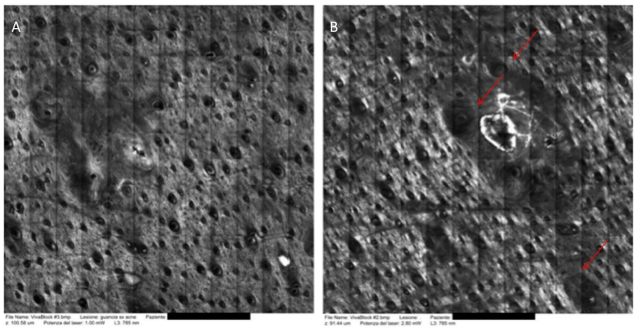

Figure 5: a: Before treatment; **b:** After treatment: an increase of adnexal structures is evident after 2 months from thetreatment on RCM examination (red arrows).

RCM can be a good tool to give an objective evaluation of acne scars improvement; it is particularly relevant to monitor timeline therapy. As a matter of fact, observation at 60 days after therapy shows that this examination is necessary to observe dense modification. The needling timeline cannot be proposed every week or every 15 days, but only after 60 days, and RCM is useful to evaluate if a new treatment is necessary. Here we propose to observe scars treated by skin collagen induction with confocal microscopy; the use of this diagnostic procedure allows to have a real quantification of the benefits of a specific technique and to better understand the mechanism of the treatment and the timing of recovery, as well as how and when the neocollagenesis is induced by this technique. Our study shows that after skin needling patients have a higher reflection of the skin expression of the new collagen synthesis and a new follicular growth that is an expression of the activity of the skin that moved from atrophy to normal condition. The modifications registered by confocal microscopy show that the technique can induce: increase in the number of fibrous bundles, a better distribution of collagen, higher organization and distribution of melanocytes, increase in the density and size of the hair follicles. All these aspects suggest that skin collagen induction can be a suitable technique for the treatment of acne scars, and confocal microscopy can better define the induced modifications and the improvement of the scars. This method could become an important instrument for diagnosis and follow-up.

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