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Clinical and Histological Evaluation of Er:YAG Ablative Fractional Skin Resurfacing

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ABSTRACT

Ablative fractional skin resurfacing represents a new possibility that allows for shorter downtime and minimizes the risk of possible side effects compared to classical full ablative Er:YAG and CO_2 treatment.

To evaluate the potential benefits of fractional treatment with Er:YAG for skin resurfacing, we performed a clinical and histological comparison of the healing process after fractional and non-fractional Er:YAG laser treatment using parameters with comparable ablation and coagulation depths. The treated area of three healthy volunteers with Fitzpatrick skin types II-IV were clinically evaluated until complete healing was achieved. Histological comparison of wound healing between both treatments was performed on 4 mm punch biopsies.

Clinical as well as histological results demonstrated that the wound healing process after the fractional treatment was significantly shorter compared with the non-fractional treatment, resulting also in milder side effects.

Key words: histology, Er:YAG, biopsy, fractional treatments

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I. INTRODUCTION

Skin resurfacing has long been considered as the most desired aesthetic improvement. Ablative skin resurfacing with Er:YAG and CO_2 lasers is still recognized as the gold standard treatment for aging skin [1–4]. The results of ablative skin resurfacing are effective although they are often associated with long healing times as well as possible side effects such as long-lasting erythema, which may lead to hyperpigmentation and scaring, thus representing a significant drawback for patients [5–7]. Patients' requirements for relatively painless procedures with short downtime prompted researchers to develop new, safer therapies with shorter downtimes. Recently fractional ablative treatments have been presented as alternatives to classical full-ablative methods, allowing less aggressive treatment with faster healing and a significant reduction of recovery time as well as minimal risk of complications [8–17]. These are thus more desirable treatments for patients, but there are only a few clinical studies evaluating the safety, efficacy and healing profiles of fractional treatments.

To evaluate the potential benefits of fractional treatment with Er:YAG before non-fractionated treatments for skin resurfacing, we performed a clinical and histological study of the healing process, comparing fractional with non-fractional treatments using a 2940 nm Er:YAG laser.

II. MATERIALS AND METHODS

Three healthy volunteers aged between 45-60 years with Fitzpatrick skin types II-IV were included in the study. Before participating in the study, patients were informed about potential risks and benefits and informed consents were signed by all participants. The study was performed according to principles of good clinical practice and the Declaration of Helsinki. In order to histologically and clinically assess the wound healing process *in vivo,* an abdominal area of human skin was treated with an Er:YAG laser system (SP Dynamis, Fotona, Slovenia) using the Fotona F-22 fractionated scanner and non-fractionated Fotona R11 handpiece. Parameters with comparable ablation and coagulation depths were used with two different fluences (Table 1) allowing 80 μm and 400 μm ablation depths. No anesthesia or air cooling was used during the treatment.

Table 1: Parameters with comparable ablation depths

Handpiece	Fluence J/cm²	Pulse duration	Spot size (mm)	Depth (μm)
R-11	27.4	185 μs	4	80
	108	300 μs	3	400
F-22	24	185 μs	0.25 with 5% coverage	80
	110	300 μs	0.25 with 10% coverage	400

a) Clinical evaluation:

The treated area was clinically evaluated every day by three independent dermatologists until complete healing was achieved. The intensities of mean erythema, localized tissue edema, bleeding, crusting or scarring were evaluated using a 10-point scale. The healing period was determined to be complete when no more crusting was observed. Photographs were taken using the same camera settings, light and position of the treated area until the completion of the healing process. The pain was also evaluated by the patients on a 10-point scale during and after the treatment. The collected data were statistically evaluated and data were summarized as mean ± SD.

b) Histological evaluation:

4 mm punch biopsies were taken immediately, 12 hours, 24 hours, 3 days (68 hours),7 days (168 hours) and 14 days after the treatment. Altogether 12 biopsies were taken to monitor the healing process of fractionated in comparison to full-beam skin resurfacing. Immediately following excision, each sample was fixed in 10 % of neutral formalin buffer overnight and then embedded in paraffin. The samples were vertically sectioned on a microtome. 5 to 7 μm thin slices have been further histologically processed using Hematoxylin Eosin staining and have been examined under light microscope using objectives for 2X, 4X, 10X and 20X magnifications.

III. RESULTS

a) Clinical evaluation

The healing process after fractional treatment is significantly faster compared with non-fractional ablative treatment, as can be seen from Figure 1 and Table 2. The mild erythema was observed in both fractional as well as full-beam treatments and disappeared 14 days after the treatment. After fractional treatment, no other side effects except erythema and minimal crusting, which disappeared in 7 days, have been observed. On the other hand extensive crusting after the treatment with the non-fractionated handpiece was observed even after 14 days developing into a scar which was still visible 1 month after the treatment. Pain evaluated by the patients was milder (2±0.82) when using fractionated handpieces in comparison with full beam (3.67±0.47) and disappeared the second day after the treatment. No moisturizing cream was used during the healing process, resulting in a longer healing period.

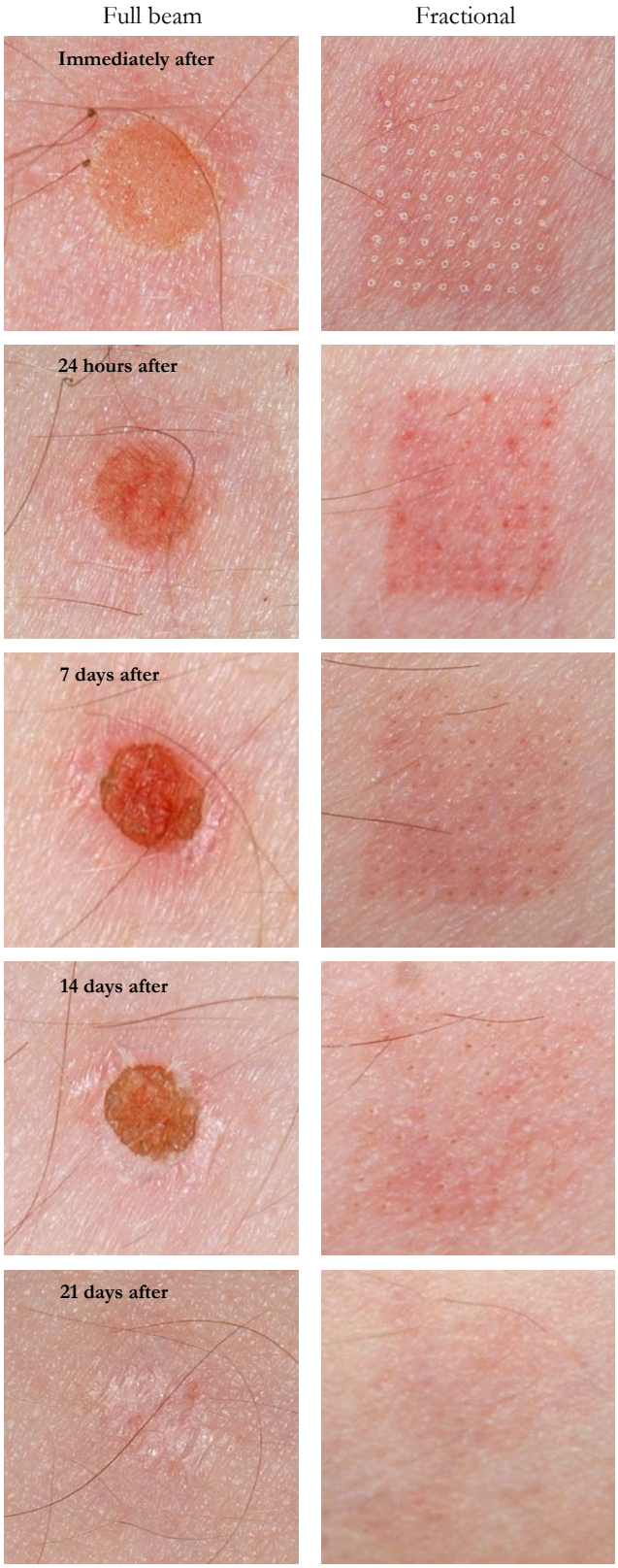

Figure 1: Comparison of the wound healing process using the non-fractional treatment and the fractional treatment immediately after, 24 hours, 7 days, 14 days and 21 days after the treatment. Treatments were performed with a Fotona Dynamis R11 handpiece for full-beam and a Fotona Dynamis F-22 scanner for fractional treatment.

Table 2. Clinical evaluation of the wound healing process using the non-fractional and fractional treatment.

	Treat -ment	Erythema	Swelling	Crusting	Scaring
Imme-diately after	full-beam	3±0	2.67±0.47	2±0	/
	Fract.	3±0	0.67±0.94	1.33±0.47	/
1 day after	full-beam	4±0	2±0	4±0.82	/
	Fract	4±0	1±1.41	2±0.82	/
3 days after	full-beam	6±1.4	1.67±0.94	6±0.82	/
	Fract	3±0.82	0.33±0.47	0.67±0.47	/
7 days after	full-beam	5±1.4	2±1.41	5.67±2.1	3.67±2.36
	Fract	2±0	/	/	/
14 days after	full-beam	2.67±0.47	0.67±0.94	3,67±2.36	4±0.82
	Fract	1±0	/	/	/
21 days after	full-beam	/	/	/	4.33±0.47
	Fract	/	/	/	/
28 days after	full-beam	/	/	/	3.67±0.47
	Fract	/	/	/	/

b) Histological evaluation

In the central part of both histological samples on Fig. 2, a well-controlled ablation penetrating approximately 400 μm deep through the epidermis into the underlying papillary and reticular dermis can be seen immediately after full-beam as well as fractional treatment. A thin area of coagulation with more basophilic appearance surrounding the ablation area was observed after both treatments (Fig. 2a and 2b).

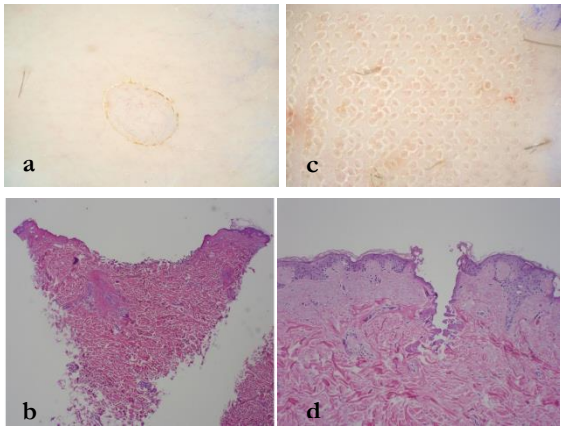

Figure 2: Macroscopic and histological comparison of full-beam treatment (a, b) and fractional treatment (c, d) immediately after the procedure. All histological pictures shown were taken under 2x objective magnification for full-beam treatment (b) and 4x objective magnification for fractional treatment (d). Treatments were performed with a Fotona Dynamis R11 handpiece for full-beam and a Fotona Dynamis F-22 scanner for fractional treatment.

12 hours after the full-beam treatment, some fibrin could be observed in the bottom as well as in the lateral parts of the ablative zone (Fig. 3a). While in the case of fractional treatment, histologically smaller damages were completely filled with fibrin, and an infiltration of a large number of inflammatory cells – neutrophil granulocytes – could already be observed 12 hours after the treatment (Fig 3b).

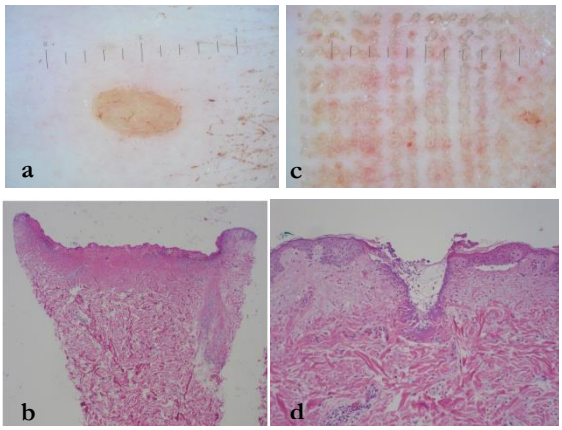

Figure 3: Macroscopic and histological comparison of full-beam (a, b) and fractional treatment (c, d) 12 hours after the procedure. Full beam treatment was performed with Fotona Dynamis R11 handpiece and fractional treatment was performed with Fotona Dynamis F-22 scanner.

24 hours after the full-beam treatment, almost the entire depth of the defect was filled with a dense fibrin plug (Fig. 4a), unlike the fractional treatment, where the serum and fibrin plug was already seen 12 hours after the treatment. 24 hours after the fractional treatment, the injury was filled with fibrin and densely infiltrated with neutrophil granulocytes and their debris (Fig. 4b).

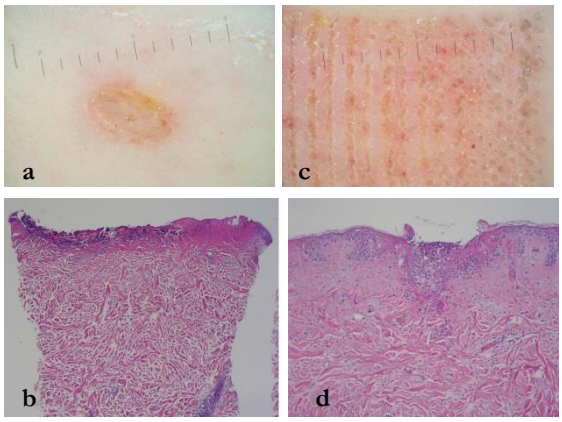

Figure 4: Macroscopic and histological comparison of full-beam (a, b) and fractional treatment magnification) (c, d) 24 hours after the treatment. Treatment was performed with a Fotona Dynamis R11 handpiece for full-beam and a Fotona Dynamis F-22 scanner for fractional treatment.

68 hours after full-beam treatment the floor of the injury was still covered with a fibrin plug accompanied with irregular basophilic areas as the result of neutrophil degranulation (Fig. 5a) while in

the case of fractional treatment, extensive re-epithelialization was already seen. Keratinocytes from lateral compartments of the epidermis had migrated into the area of the upper fibrin plug and practically covered it (Fig. 5b). The wound was clinically already re-epithelialized. That the process was still in the stage of formation was visible by the presence of parakeratosis in the stratum corneum, on the top of which serous crust with neutrophil debris could be seen. The coagulation of collagen was still present around the area of the defect, but without a pronounced inflammatory infiltrate of the surrounding dermis. The inflammatory reaction of the rest of the dermis was also minimal.

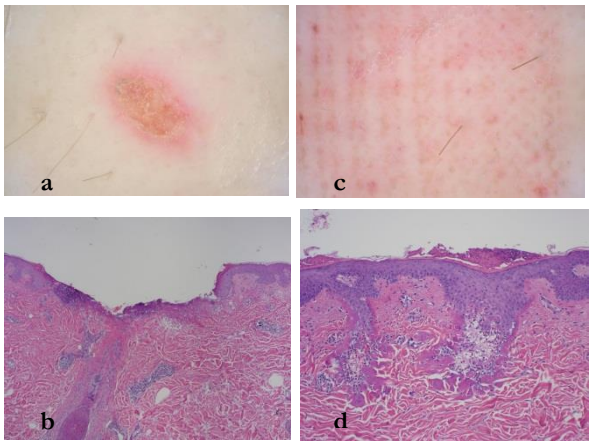

Figure 5: Macroscopic and histological comparison of full-beam (a, b) and fractional treatment) (c, d) 68 hours after the treatment. Full-beam treatment was performed with a Fotona Dynamis R11 handpiece and fractional treatment was performed with a Fotona Dynamis F-22 scanner.

7 days after the full-beam treatment, only partial re-epithelialization with a hyper-proliferative epithelium, especially from the side, was observed (Fig. 6a), while after fractional treatment, re-epithelialization was complete and normal maturation of the epidermis, manifested by the presence of stratum corneum and orthokeratosis, was observed (Fig. 6b). Under the area of re-epithelialization the smaller area of subepidermal fibrin and a few neutrophil infiltrations could be observed 7 days after the fractional treatment. The coagulation of collagen was still present. The wound after full-beam treatment had not been completely healed 7 days post-treatment and was covered with extensive crust, but was smaller in diameter - as a result of re-epithelialization from the edges toward the center. Adherent serous crust could be observed over the epithelium, as well as over the injury with plenty of neutrophil infiltration and debris (Fig. 6a). Just below the *re-epithelialized* epidermis, indication of some granulation tissue could be observed. A stronger inflammatory infiltrate at the central part of the injury was also present.

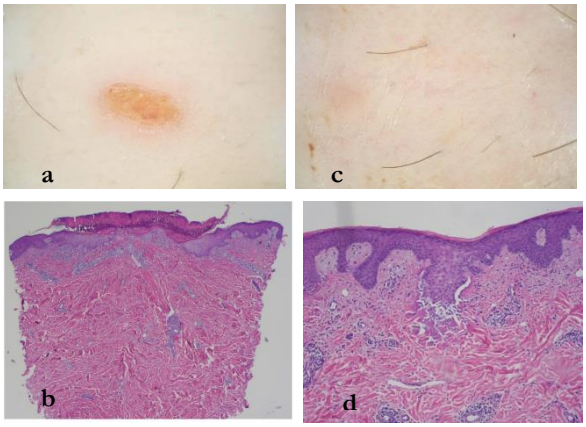

Figure 6: Macroscopic and histological comparison of full-beam (a, b) and fractional treatment (c, d) 7 days after the treatment. Treatments were performed with a Fotona Dynamis R11 handpiece for full-beam and a Fotona Dynamis F-22 scanner for fractional treatment.

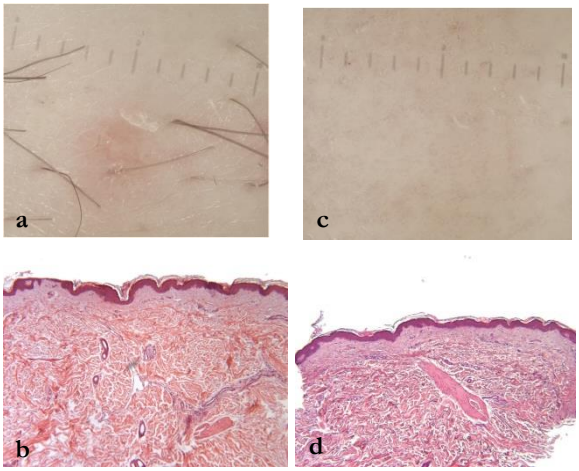

Figure 7: Macroscopic and histological comparison of full-beam (a, b) and fractional treatment (c, d) 14 days after the treatment. Treatments were performed with a Fotona Dynamis R11 handpiece for full-beam and a Fotona Dynamis F-22 scanner for fractional treatment.

14 days after the full-beam treatment, complete re-epithelialization with normal maturation of the epidermis was observed (Figs. 7a-b). The number of fibroblasts in the dermal layer was suggestive of the initial phase of neocollagenesis, although new collagen fiber could not be detected (Fig. 7b). In contrast to the fractional treatment, a slightly denser perivascular inflammatory infiltrate was still present in the middle part of the dermis (Fig. 7b), which was clinically manifested as slight erythema (Fig. 7a).

14 days after the fractional treatment (Figs. 7c-d), complete re-epithelialization with normal maturation of the epidermis was observed, manifested by normal-width orthokeratosis, the presence of stratum

corneum, and stratum granulosum. Just below the epidermis some melanocytes could be observed. In addition to numerous fibroblasts indicating the beginning of neocollagenesis, tiny new collagen fibers were already visible parallel to the dermoepidermal junction (Fig. 7d).

IV. DISCUSSION

To understand the clinical potential of ablative fractional Er:YAG treatment, knowledge of the wound healing process on a histological level is essential. Recently, numerous studies have demonstrated shorter healing times and other benefits of fractional resurfacing by overcoming the high risk of side effects caused by full-ablative techniques [8–17]. But just a few studies have compared the histological and clinical effects of fractional and full-ablative treatments [13,16]. Our comparative study clearly demonstrates the differences in the healing process between full-ablative and fractional-ablative Er:YAG treatments.

Since the ablation depth and thermal injury are evidently correlated with the parameters used [18–20], energies which allow us to achieve 400 μm depth of ablation were chosen. After the treatment with the full-beam technique, we created a 3 mm wide ablation area, while with the fractional technique, many 250 μm wide ablation zones were formed. Smaller ablation zones created with fractional Er:YAG were surrounded by healthy tissue, providing an environment for faster healing.

In our study the re-epithelialization of the wound after fractional treatment started after 12 hours, and after three days complete epidermal wound healing and cell reorganization was achieved. In contrast, only partial re-epithelialization of the wound created by the full-beam treatment was observed after 7 days and complete re-epithelialization was not observed until after 14 days. Full-ablative treatment also induced more intensive inflammation response histologically seen as denser neutrophil infiltrate accompanied by superficial and deep perivascular inflammatory infiltrate, clinically manifested as erythema that lasted 14 days. Inflammatory infiltration was significantly less pronounced after the fractional-ablative treatment. While the extensive crust was observed even 7 days after the full-beam treatment, the tiny crusts were hardly seen 3 days after the fractional treatment.

Our findings are also consistent with other studies of Er:YAG and CO_2 fractional-ablative skin resurfacing [11,13,16], although there was a broader coagulation zone observed after CO_2 fractional treatment. Actually, there are some differences between the two most frequently used laser treatments for skin resurfacing. While fractional CO_2 lasers usually cause increased thermal injury which could result in scarring [21,22], fractional Er:YAG lasers take advantage of the high water absorption coefficient and short pulse durations, allowing formation of clear ablative areas with minimal or no coagulation zones [23,24], which was also observed in our case. Clinical and histological findings of Dierickx et al. demonstrated that with controlled ablation depth and a variable extent of surrounding coagulation, new treatment possibilities targeting specific areas as well as indications can be designed [11]. In our study the ablation zone was surrounded with a thin area of coagulation, which could have contributed to collagen remodeling and consequently adding extra value to improve the final results as shown in the study conducted by Trelles et al. [14]. Although we didn't study the long-term collagen remodeling process, other studies demonstrated that although re-epithelialization was completed 3 days after the fractional treatment, dermal remodeling can last more than 4 weeks [13,16]. After the treatment with CO_2 laser using higher energies (300 mJ) the mixed lymphocytic and granulomatous infiltrate was observed even after 4 weeks [16], which could explain the risk of scaring [21,22].

It should further be mentioned that the aforementioned changes were observed on abdominal skin, and it is expected that the healing process on facial skin is even faster.

V. CONCLUSIONS

Our clinical as well as histological findings clearly demonstrate that the wound healing process after Er:YAG fractional treatment is significantly shorter when compared to full-beam ablative treatment, and results also in milder side effects.

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