ORIGINAL ARTICLE

Journal of Drugs in Dermatology

In Vivo Histological Evaluation of Non-Insulated Microneedle Radiofrequency Applicator With Novel Fractionated Pulse Mode

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ABSTRACT

INTRODUCTION: Microneedle radiofrequency is a novel method that allows non-thermal penetration of the epidermis followed by RF coagulation in selected depth of the dermis surrounded by zone of non-coagulative volumetric heating. The first generation of Microneedle RF applicators used insulated needles. These treatments were limited by a few factors, including low volume of dermal heating, lack of effect in the papillary dermis and pinpoint bleeding during the treatment. The system tested in this study (EndyMed PRO, Intensif applicator, EndyMed Medical, Cesarea, Israel) utilizes special extra sharp tapered non-insulated microneedles and a special pulse mode, allowing full coagulation during treatment and higher effective volume of dermal heat.
METHODS: After Ethics Committee approval, one female pig (Type Large white X Landrace, 34 Kg) was chosen for the study. The animal was anesthetized using Ketamine, Xylazin and Isofluran. The EndyMed PRO, Intensif applicator (was used for treatment with different needle depth penetration (1mm-3.5 mm) and in multiple energy settings. Six mm punch biopsies were harvested for histological analysis at the following time points: immediately after the treatment, 4 days after the treatment and 14 days after the treatment. H&Eand Masson-Trichrome stains were processed.
RESULTS: Visual inspection of the treated skin, immediately after the treatment, revealed arrays of pinpoint erythematous papules surrounded by undamaged epidermal tissue. Treatment field showed no sign of bleeding. Mild to moderate Erythema and Edema developed a few minutes after the treatment, varying according to the total energy delivered. The histologies taken 4-day after therapy showed in all energy settings, dry micro crusts over the treatment zones, with full healing of epidermis. In the 14-day specimens there was a replacement of the crusts/debris by a normal looking stratum corneum with complete healing of epidermis and dermis.
DISCUSSION: The current in vivo study confirms that the EndyMed PRO Intensif applicator effective and predictable tool to create cylindrical micro zones of coagulation in the papillary and reticular dermis with minimal damage to the epidermis. The histologies taken 4 days and 14 days after treatment show rapid epidermal renewal with predictable volume of coagulation in dermis related to the length of the needle and the power used. Coagulation of capillaries during treatment allows a dry treatment field. The predictability of the effect and minimal downtime may offer a significant advantage over treatments with ablative fractional lasers of insulated RF microneedles.

J Drugs Dermatol. 2013;12(12):1430-1433.

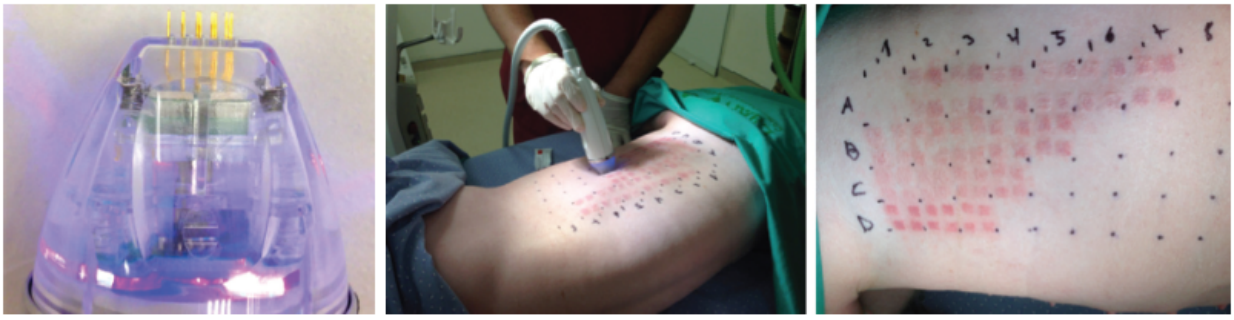
INTRODUCTION

Traditional skin resurfacing CO_2 laser devices, though highly effective in tissue coagulation, are associated

with prolonged recovery time, bleeding, oozing, and risk of post treatment hyper or hypopigmentation.[1-5] In addition, these lasers are very problematic in treating darker skin types or sensitive Asian skin. In “fractional” laser or radiofrequency skin resurfacing treatments, thermally ablated or coagulated microscopic zones of epidermis and dermis are spaced in a grid over the skin surface; the non ablated zones in the uninjured surrounding tissue serve as a reservoir of cells that accelerate and promote rapid healing[6]. The use of radiofrequency (RF) overcomes some of light based disadvantages offering enhanced tissue penetration independent of skin color. Fractional skin resurfacing using multisource RF generators and 112 flat micro electrodes, has been shown to ablate the epidermis to a depth of 100-150 micron with simultaneous volumetric heating of the dermis to a depth of 2.8 mm achieving good clinical results and safety profile.[7,8,9,10] Microneedle radiofrequency allows non-thermal penetration of the epidermis followed by RF coagulation in selected depth of the dermis. Hantash et al,[11,12] delivered RF energy for 4 seconds, through ten, partially insulated, 6 mm long, micro-needles inserted in an 20 degrees angle into the skin. To prevent pain and epidermal damage, the treatment required local infiltration with lidocain and active epidermal cooling. Histological examination showed, RF thermal zone (RFTZ) through day 28 post-treatment but were replaced by new dermal tissue by 10 weeks.

Another type of RF microneedles delivery system uses 3.5 mm long microneedles inserted vertically into the skin, allowing a significant reduction in pain. The first generation of vertically inserted RF microneedles were insulated allowing only a small volume of

FIGURE 1. Left: Sterilized treatment tip, showing 25 tapered, extra sharp, gold plated microneedles (Diameter 300 micron). Center and Right: During and immediately after, treatment the treatment field was completely dry and clean. Varying degree of Edema and Erythema developed a few minutes after treatments according to the different energy doses. No bleeding points were noted - proving effective hemostasis. Coagulation was confirmed by histological analysis of samples taken immediately, 4 days and 14 days after the procedure.



dermal heating around the tip of the needle. In addition due to the insulation of a significant part of the needles, coagulation efficacy was low and massive pinpoint bleeding was experienced during treatment.[13] Seo et al,[14] used a non insulated microneedle RF system to treat 25 Asian patients. Microscopic examination of hematoxylin-eosin stained sections showed dermal remodeling after microneedle fractional RF. A significant increase in dermal collagen content was observed at 4 weeks after three sessions of fractional RF compared to the baseline. This matched more than 50% improvement in Fifty-six percent of treated patients.

The system tested in the current study (EndyMed PRO, Intensif applicator) utilizes special extra sharp tapered non insulated microneedles and a special pulse mode allowing full coagulation during treatment and higher effective volume of dermal heat. This prospective study describes for the first time the histological effects of this type of RF microneedle applicator, on in vivo Porcine skin immediately, 4 days and 14 days after a single treatment.

"The use of radiofrequency (RF) overcomes some of light based disadvantages offering enhanced tissue penetration independent of skin color."

METHODS

Device Description

The EndyMed PRO, Intensif applicator (EndyMed Medical, Cesarea, Israel) was used in this study. The system platform (1MHZ, Power up to 65W), incorporates six phase controlled independent radiofrequency generators that allow multiple applications including non ablative face/body skin tightening, micro ablative

fractional skin resurfacing and radiofrequency microneedles skin remodeling for controlled dermal coagulation. Software control of RF delivery assures constant power emission, independent of tissue impedance. In the current study, the system was used with the Intensif microneedle RF applicator. This specific applicator is equipped with a sterilized tip, of 25 extra sharp gold plated microneedles. The needles (300 diameter) can be inserted to a customized depth of 0 to 3.5 mm with a 0.1 mm step resolution. The handpiece incorporates a special step motor allowing smooth insertion - minimizing epidermal trauma and pain. Power can be adjusted from 0 to 25 W with increments of 1 W and pulse duration can be adjusted in 30 ms increments. In this in vivo study we examined different needle depth penetration (1mm-3.5 mm) and multiple energy settings (400 mJ/pin, 112mJ/pin. 56mJ/pin, 40mJ/pin).

Treatment Procedure

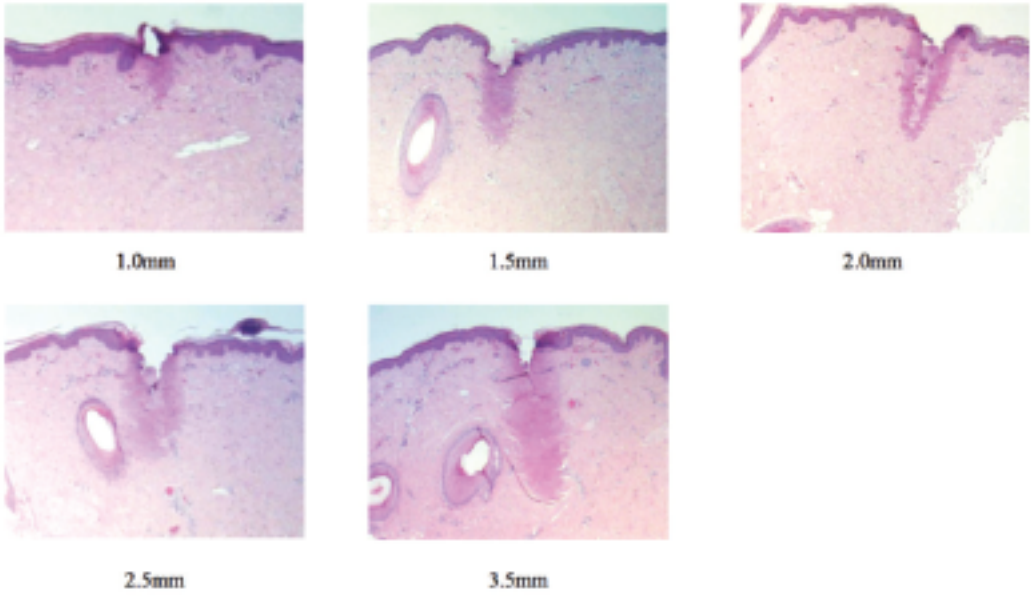
After Ethics Committee approval, one female pig (Type Large white X Landrace, 34 Kg) was chosen for the study. The animal was anesthetized using Ketamine, Xylazin and Isofluran. To simulate human treatment protocol Emla cream was applied to animal skin for 30 minutes and wiped with a moistened gauze pad. Six mm punch biopsies were harvested for histological analysis at the following time points: immediately after the treatment, 4 days after the treatment and 14 days after the treatment. Skin samples were fixed in formalin solution (10%), processed into biopsies, stained with H&Aand Masson-Trichrome and examined microscopically by a board-certified Dermatologist.

The treatment took place in a certified veterinary clinical testing facility (the institute of animal research, kibbutz Lahav, Israel). Treatment was performed after full anesthesia was verified. Treatments included different power parameters and needle penetration depths.

RESULTS

Visual inspection of the treated skin, immediately after the treatment, revealed arrays of small coagulative thermal lesions surrounded by undamaged epidermal tissue. Treatment field showed no sign of bleeding. Examining the H&Eprocessed slides it was clear that tissue coagulation was relative to needle length (=penetration depth), as shown in Figure 2.

FIGURE 2. Histology of in vivo pig skin immediately after treatment with different needle penetration depth. H&E stain coagulation depth matching needle penetration depth.



Histology of in vivo pig skin immediately after treatment after treatment - H&Estain reveals epidermal and dermal coagulation matching needle penetration depth. Coagulation volume is proportional to the energy

applied. 4 days after treatment - H&E stain reveals complete epidermal healing with crusting = debris of coagulated tissue naturally excreted upwards to the surface of the epidermis. 14 days after treatment - H&Estain reveals complete epidermal and dermal healing. (Figure 3,4).

The Effect of Energy Density on the Level of Coagulated Tissue

Another aspect that was evaluated in this work was the effect of the needles depths on the level of ablation. When performing a clinical treatment there is a great significance to the physician ability to keep the clinical effect constant or with minimal on the entire treatment area, regardless of the differences in treatment location, parameters and other conditions.

The hypothesis that was studies was that reducing the needle depth while keeping the rest of the parameters unchanged, will result in higher energy density due to the decrease in needles surface area, what will lead to an increase in the diameter of coagulated tissue.

This hypothesis was proved to be right as seen in Figure 5.

DISCUSSION

Fractional lasers systems have been shown in the past to ablate and coagulate micro zones of skin. Fractional skin resurfacing applicators using multiple flat epidermal microelectrodes, allow a simpler yet effective epidermal and dermal effect suitable to all skin types with less risk for Post Inflammatory Hyperpigmentation (PIH) or other adverse effects. Fractional RF systems using multiple generators of RF seem to have the ability to provide both a fractional epidermal coagulation and non-coagulative volumetric heating of the dermis.[7,8,9,10] Microneedle RF technology is newly developed technique meant to coagulate dermal micro zones with full control of coagulation depth without epidermal ablation and in a more predicable way than fractional lasers. Treatment with non-insulated needles, based on impedance differences between the epidermis (high impedance) and the dermis (low impedance) allows better coagulation and heating of higher volumes of dermal tissue as compared to insulated needles. The current study examined for the first time to our knowledge, the effects of microneedle RF needles on live animal skin, immediately, 4 days and 14 days after treatment.

FIGURE 3. Histology of in vivo pig skin immediately after treatment (needle depth 2.0mm). Left column: Immediately after treatment - H&E stain reveals coagulation matching needle penetration depth. Coagulation volume is proportional to the energy applied. Center column: 4 days after treatment - H&E stain reveals complete epidermal healing with crusting = debris of coagulated tissue naturally excreted upwards to the surface of the epidermis. Right column: 14 days after treatment - H&E stain reveals complete epidermal and dermal healing.

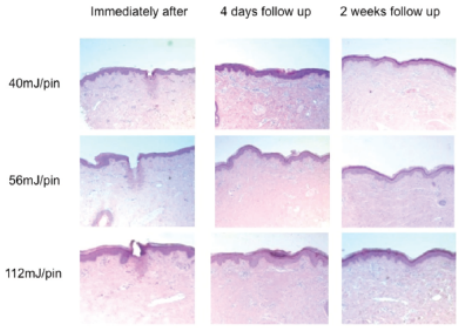
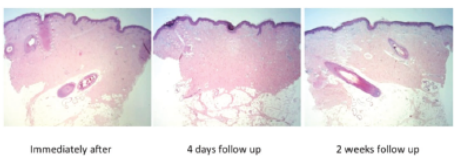
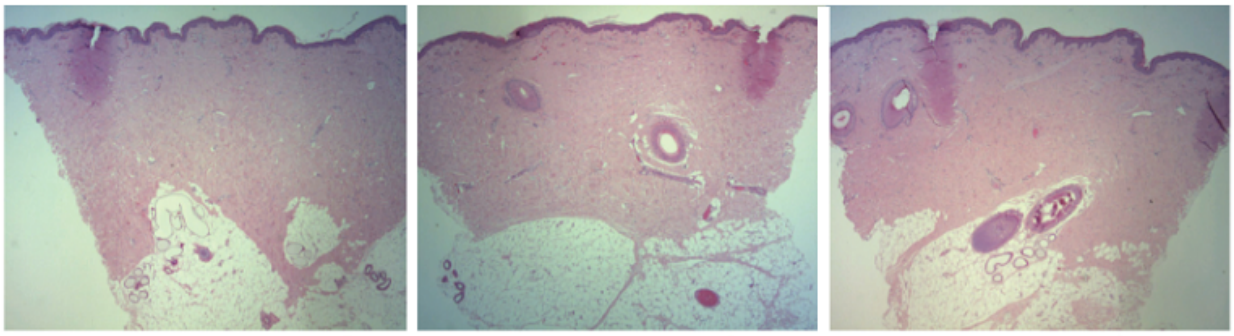


FIGURE 4. Histology of in vivo pig skin immediately, 4 days and 14 days after treatment with excessive power and needle depth (maximal needle length = 3.5mm, 400mJ/pin). Slides showing at 4 days, micro crusts on the epidermis, matching the debris of coagulated tissue excreted upwards and full epidermal healing at 14 days after treatment.



The current in vivo study confirms that the EndyMed PRO Intensif™ applicator effective and predictable tool to create cylindrical micro zones of coagulation in the papillary and reticular

FIGURE 5. Reduction in coagulated tissue diameter with the increase in needles depth. Left: wide coagulated tissue with 1.5 mm penetration. Center: middle size coagulated tissue at 2.5 mm penetration. Right: narrow coagulated tissue at 3.5 mm penetration. Other energy parameters were unchanged.



dermis with minimal damage to the epidermis. The histologies taken 4 days and 14 days after treatment show rapid epidermal renewal with predictable volume of coagulation in dermis related to the length of the needle and the power used. Coagulation of capillaries during treatment allows a dry treatment field. The predictability of the effect, the ability to heat both the papillary and reticular dermis and the minimal downtime, may offer a significant advantage over treatments with ablative fractional lasers or insulated RF microneedles.

DISCLOSUREs

System and consumables were supplied by Endymed Medical, Cesarea, Israel.

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