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In vivo assessment of the effect of a cream containing *Avena Rhealba*® extract and hyaluronic acid on the restoration of the skin barrier in de-epidermised skin produced with an erbium-YAG laser

Background: Wound healing studies require standardised methods for evaluating wounding and skin repair. *Objectives:* Our study aimed to demonstrate the suitability of the erbium-YAG (Er-YAG) laser method to produce reliable epidermal lesions for evaluation of different skin repair creams. *Materials and methods:* Skin de-epidermised by Er-YAG laser (four uniform epidermal ablations, area 8 × 8mm, in 21 healthy subjects) was treated with a product (A) containing *Avena Rhealba*® extract and hyaluronic acid and assessed for epidermal regeneration and barrier restoration. This treatment was compared to two reference products (B) and (C) and an untreated control. Over 22 days of treatment, double-blind measurements of wound characteristics were made for instrumental (wound surface area, barrier restoration, 3D skin topography) and clinical evaluation (lesion quality and tolerance). *Results:* Tested product (A) resulted in a shorter time (9 days) and faster rate of wound closure than product C (12 days) and the untreated zone (16 days). Results for products (A) and (B) were similar. Clinical evaluation of lesion quality showed the same trends as the wound area/closure parameter. Barrier recovery assessments revealed that all three products showed a similar rate of decreasing Transepidermal Water Loss (TEWL), which was significantly faster than the rate for the control. *Conclusion:* In conclusion, the laser-induced epidermal wound model provided standardised lesions, enabling discrimination between different topical skin repair products.

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Key words: epidermis, erbium, laser, skin abrasion, wound healing

Wound healing is a dynamic process involving three phases: inflammation, tissue regeneration and tissue remodelling [1, 2]. Studies of wound healing can be performed in patients presenting with lesions or using standardised, well-tolerated and reproducible models of wound induction in human volunteers. Several methods have been used to generate partial thickness wounds, such as induction of suction blisters [2, 3], mechanical or abrasive methods to remove all the epidermis (using a sandpaper block [4], a surgical brush [5] or a dermatome), or by burning using a brass block heated to 100 °C and left on the skin for short periods of time [6]. However, most of these techniques are laborious, generate varying degrees of pain and provide only limited control over the depth of skin tissue removed, so that comparison of experimental wounds is difficult. CO_2 and Erbium:Yttrium-Aluminium-Garnet (Er-YAG) lasers used in dermatology for skin resurfacing [7, 8] constitute an alternative and more attractive method for experimental wounding, allowing rapid and controlled skin ablation [9].

Er-YAG lasers in particular – with a 2940 nm wavelength specifically absorbed by water – enable progressive ablation of a few micrometres of tissue, while limiting residual thermal damage. Controlled abrasion of the epidermis is therefore possible, as demonstrated by Lee *et al*. [10, 11] in a mouse model and by Alster [12] on human skin.

A pilot study by Ferraq *et al.* [13], comparing wound induction by laser with that by suction blisters in 10 healthy volunteers over 7 days, demonstrated that Er-YAG laser was faster than the suction blister method and induced regular flat-bottomed wounds with more uniform depth than suction blisters, whilst showing comparable healing characteristics.

The objective of the present study was to demonstrate the suitability of the Er-YAG laser method to produce reliable epidermal lesions for the evaluation of different skin repair creams. With this aim, we applied a product containing *Avena Rhealba*® extract and hyaluronic acid or two reference products to skin de-epidermised by Er-YAG laser and

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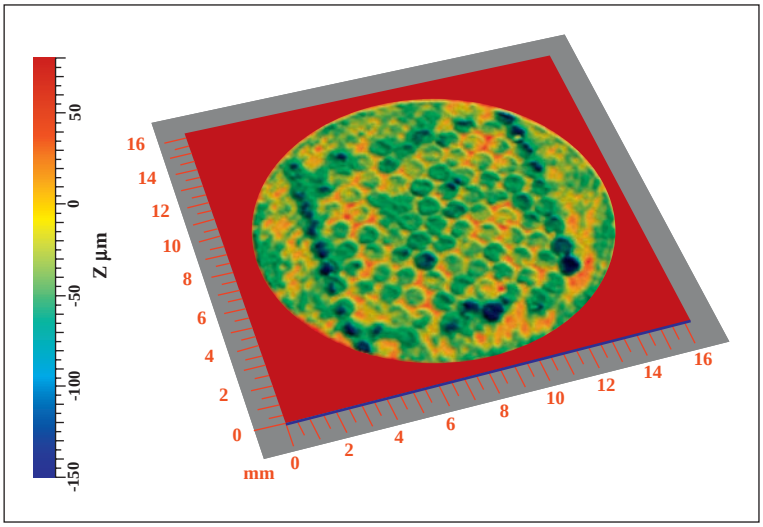

Figure 1. Erbium YAG laser induction.
Typical 3D reconstructed image of the wound obtained by scanning silicon rubber replicas, mean depth Z = 54.4 ± 14.2 µm (n = 10). Induction was performed with the following parameters: laser energy: 100mJ, frequency: 5Hz, pulse length: 450 µs, spot size: 1.5 mm, wound size: 0.6-0.7 cm^2 (Extracted from the article published by Y. Ferraq *et al.* in *Lasers Surg Med* 2012, reprint with permission).

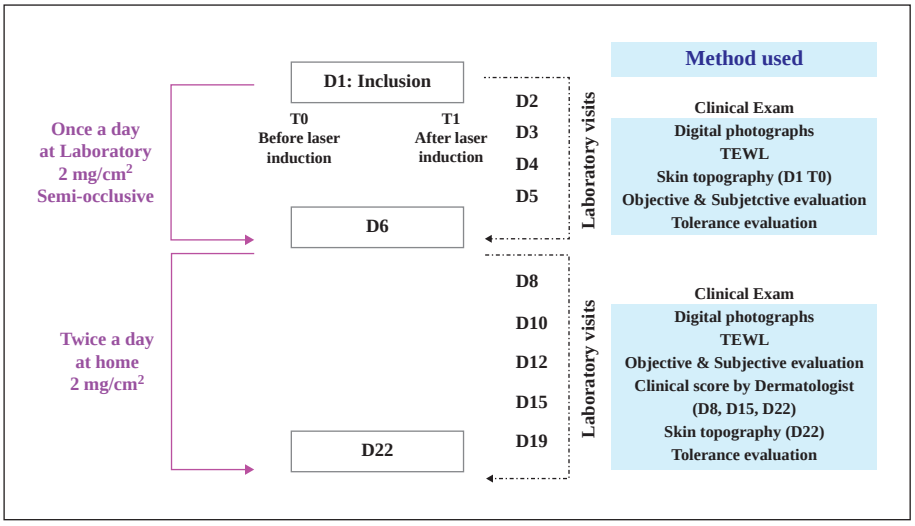

Figure 2. Study design.

assessed (both instrumentally and clinically) the epidermal regeneration and barrier restoration over 22 days. Results were compared to an untreated control.

Materials and methods

This monocentric, randomised clinical study was designed to compare the study product to two benchmark skin repair creams and a control. It was conducted during November 2012, at the PRODERM Institute for Applied Dermatological Research (Hamburg, Germany) according to the ethical principles of the declaration of Helsinki and the guidelines for Good Clinical Practice (CPMP/ICH/135/95). Treatments and measurements were randomised with a double-blind clinical evaluation for the three products and open evaluation for the untreated control.

Method of Er-YAG laser epidermal ablation

Epidermal ablations were made with the Er-YAG laser (lesion size 8 × 8 mm, surface area between 0.6-0.7 cm^2, fluence 15 $J.cm^{-2}$, laser energy 100 mJ, frequency 5 Hz, pulse length 450 µs, SMART System, Deka® Company, Italy), as described by Ferraq *et al.* [13], on the forearms of 21 healthy volunteers *(figures 1,2)*. On Day 1, two uniform square ablations of this type were made under local anaesthesia on each flexor forearm (four in total): one near the wrist and one near the elbow of each subject *(figure 1)*.

Clinical and instrumental evaluation

Subject recruitment and selection

Twenty-one volunteers (mean age 37.1 years (18-45 years), 17 females, 4 males), with a normal skin of phototype ≤III,

Table 1. Patient demographics

Gender	
Male (n)	4
Female (n)	17
Age range (mean), years	18-45 (37.1)
Phototype	≤III

were recruited to participate in the study *(table 1).* Subjects with skin lesions or wounds on the inner side of the forearms, pathological wound healing, a history of allergic reactions to one of the study products, or acute or chronic disease, as well as those who had undergone treatment with non-steroidal anti-inflammatory drugs, anticoagulants, diuretics or a treatment liable to induce methaemoglobinemia were excluded from the study. All subjects were provided in person with the detailed protocol of the whole study and signed an informed consent form before study enrolment.

Objectives and assessment

The re-epidermisation efficacy of the product was first evaluated by assessing the time to wound closure. This was monitored by measuring the wound surface area (WSA) on calibrated digital photographs of the inner forearm sites, with images being taken at all evaluation visits after laser induction (D1) up to D22 [13, 14]. The time to wound closure was the minimum time to obtain total re-epidermisation or disappearance of the wound *i.e.*, when WSA was equal to 0.

To confirm the efficacy of re-epidermisation, the following parameters were also evaluated and measured:

- Skin barrier function, evaluated by TEWL measured with the Aquaflux system (BIOX Systems Ltd, London) at each visit from D1 before laser induction up to D22, and the process of re-epidermisation of the wound, assessed by measuring WSA at all time points. The global rates of re-epidermisation and of decreasing TEWL were calculated by combining the value of the first regression slope (between D2 and D6) and the value of the second regression slope (between D2 and Dx = minimum time for wound closure).
- The quality of re-epidermisation on each treated area was assessed at D8, D15 and D22 by two methods (subjective and quantitative). The subjective method involved the scoring of lesion healing quality by a dermatologist, on a grade scale ranging from 0 (very poor) to 10 (very good). The quantitative method involved evaluating the skin surface by 3D topography (fringe projection), using silicon rubber (Silflo®, J&S Davis Ltd, UK) replicas taken immediately before laser induction and at D22. These were scanned as 3D images and analysed by Primos® Compact (GFMesstechnik GmbH, Germany), as previously described [13, 15], providing data on skin roughness (parameter Rt).
- Calibrated digital photographs of the inner forearm sites were taken at all visits.
- The global tolerance was assessed on each treated area by a trained investigator, using a 4-point scale ranging from 1: very good tolerance to 4: very poor tolerance leading to treatment discontinuation. Adverse events were evaluated at each visit during the study.

Wound treatment and protocol

The test product was a wound repair cream (product A) containing *Avena Rhealba*® extract and hyaluronic acid. The skin repair reference products contained panthenol and madecassoside (Product B) and resveratrol-copper complex (Product C).

The study took place over 22 days and included daily evaluation visits from D1 to D6, followed by further evaluations on D8, D10, D12, D15, D19 and D22 *(figure 2).*

After Er-YAG laser epidermal ablations, approximately 8 mg of the tested product (A) or one of the two reference products (B, C) were applied once daily by the investigator, according to a randomisation schedule, from D1 to D6 (under a semi-occlusive patch), then twice daily by the subject at home, between D6 and D22. All products were compared to an untreated control zone only submitted to laser wound induction. Epidermal regeneration (WSA and 3D skin topography) and barrier restoration were measured instrumentally and clinically, as described above.

Statistical analysis

Quantitative data are expressed as the mean ± standard deviation. Intra-group and inter-group comparisons (between product A *versus* control, and A *versus* B or C) were evaluated by analysis of variance, taking into account the product and site as fixed factors and the subject as a random variable. When the product effect was significant, a two by two comparison of the products was performed based on the differences between adjusted means. Statistical significance was defined as $p \leq 0.05$.

Results

Time to wound closure and TEWL measurement once re-epidermisation was complete

WSA measurements showed that the laser wound was completely healed after 9 days with products A and B, whereas healing required 12 days with product C and 16 days in the absence of treatment (control). Thus, product A resulted in a 7-day gain in wound re-epidermisation compared to the control *(figure 3).* Once the wound was totally re-epidermised and closed (Dx = 9 for products A and B), the measurement of TEWL provided an additional indication about wound healing *(table 2).* There was no significant difference in TEWL between the studied products. However, TEWL was significantly lower with all the tested products than in the untreated control area, showing that the restoration of the skin barrier was markedly faster with products A and B (68% decrease in TEWL at Dx = 9) than for the control (52%).

Rates of re-epidermisation

Two phases were observed in the rate of decrease in WSA: a first period of relatively slow healing (D2-D6) and a second period, from D6 until wound closure (Dx), when healing was faster *(table 3).* The global rates of wound closure between products A and B were not significantly

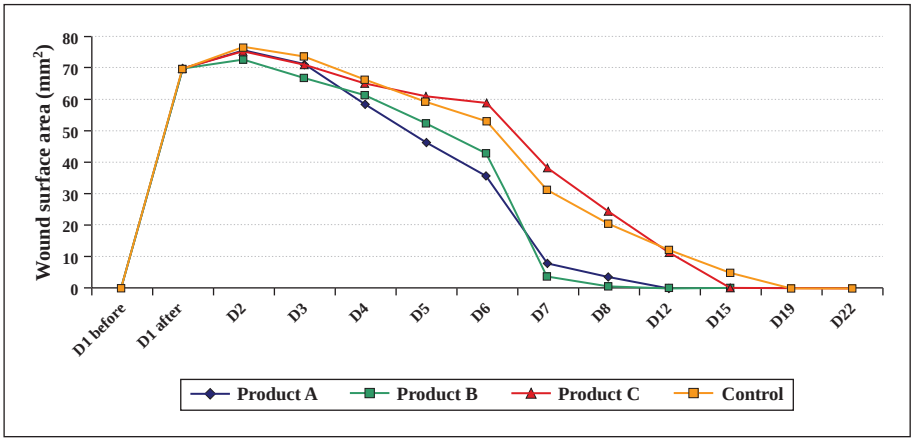

Figure 3. Change in wound surface area (WSA) with treatments.
Two regression slopes can be observed, corresponding to the D2-D6 and D6-Dx periods, with Dx = the day when wound closure is total (= healing). The global rate of wound closure is calculated on the basis of the combination of the two slopes. For products A and B, the day range for total wound closure was (D8-D12), it was (D12-D15) for Product C and (D15-D19) for the control.

Table 2. Time to wound closure and changes in TEWL with the different treatments at the time of closure

	Time for closure of wound surface area		TEWL Measurements at Dx = 9 days	
Product	Intragroup analysis (Mean ± SD), n = 21	Intergroup analysis *versus* Product A	Intragroup analysis (Mean ± SD), n = 21	Intergroup analysis *versus* Product A
A	9.1 ± 2.68		24.24 ± 5.64	
B	8.57 ± 2.58	**p = 0.5460 NS**	24.43 ± 10.33	**p = 0.898 NS**
C	12.14 ± 4.28	**p = 0.0004 S**	25.43 ± 8.63	**p = 0.578 NS**
Control	16.1 ± 3.37	**p<0.0001 S**	35.92 ± 11.01	**p<0.0001 S**

Dx = Minimal time for wound closure, S: Statistically significant, NS = Not Statistically significant

Table 3. Evaluation of the global rate of wound closure

	Rate of re-epidermisation (D2-D6) (Mean ± SD), n = 21	Rate of re-epidermisation (D6-Dx) (Mean ± SD), n = 21	Global rate of re-epidermisation (Mean ± SD), n = 21	Changes in global rate/Product A
Product A	-10.48 ± 5.15	-19.98 ± 20.09	15.23 ± 12.62	
Product B	-7.38 ± 5.31	-28.47 ± 21.95	17.9 ± 13.63	equivalent to Product A
Product C	-4.21 ± 1.28	-19.9 ± 18.93	12.06 ± 10.11	inferior to Product A
Control	-6.0 5 ± 4.85	-4.43 ± 1.50	10.48 ± 3.18	inferior to Product A

Dx = Minimal time for wound closure

different, whereas the rate of re-epidermisation was significantly faster with products A and B than with product C or with no treatment *(table 3)*.

Quality of healing

Both clinical scoring at D15 and D22 and quantitative roughness parameter Rt measurements at D22 *(figure 4)* showed that skin quality was not significantly different after treatment with the various tested products ($p>0.05$ at D15 and D22). However, a significant difference in the change in skin roughness between D22 and D1 was observed in favour of product A *versus* product C and the no treatment control ($p\leq0.05$ for both comparisons). Furthermore, intra-group analysis showed that the application of product A led to total restoration of the skin surface to the level observed before laser wounding ($p = 0.661$ at D22 *versus* D1), whereas the skin surface remained significantly different from baseline for the untreated wound ($p<0.002$ at D22 *versus* D1, *figure 4*). Intra-group analysis also demonstrated that skin surface quality was significantly better for the area treated with product A than for the untreated wound ($p<0.001$ at D6, D15 and D22). The digital pictures of the wounds *(figure 4C)* enabled good visualisation of the re-epidermisation process, progressing until the state of the skin at D22 was similar to that at D1 before laser induction.

Tolerance

Globally, the tolerance of the three products was good to very good (grades 1-2). Only one adverse event was

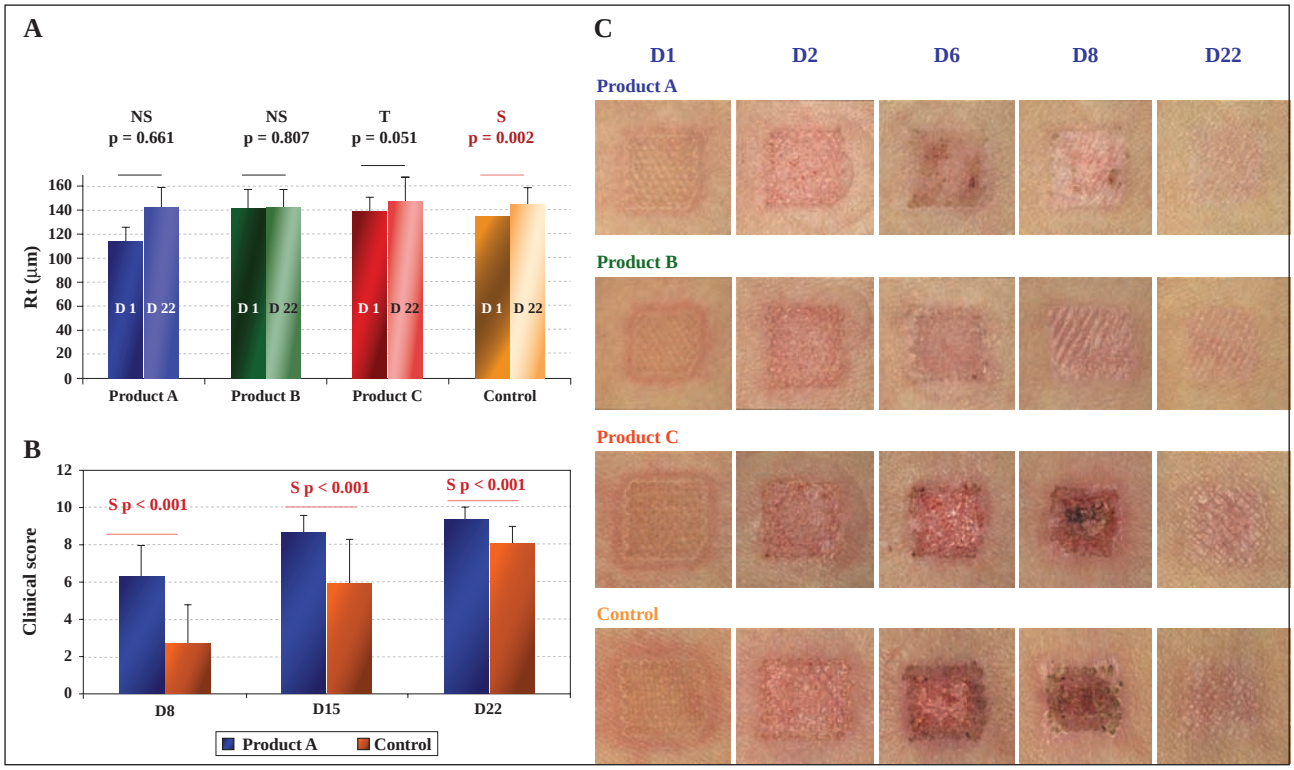

Figure 4. Evaluation of healing quality.
A) Quantitative evaluation of skin roughness (Rt, µm) at D1 before laser induction and at D22 for each tested product (A, B, C) and for the untreated control. Intra-group comparison was performed by variance analysis, S: Statistically significant if $p \leq 0.05$, NS: Not Statistically significant, T: Tendency if $0.05 < p \leq 0.1$. **B)** Qualitative evaluation by dermatologists of wound quality at D8, D15, and D22 for the untreated control and for the wounds treated with product A. Inter-group comparison was performed by variance analysis of the studied parameters, taking into account the product and site as fixed factors and the subject as random variable. S: Statistically significant if $p \leq 0.05$. **C)** Illustrative pictures, showing the changes in wound appearance at D1 (after induction), D2, D6, D8 and D22 for the untreated control and for wounds treated with product A, B or C.

reported during the study: one subject experienced itching and swelling during 1 hour on all the areas where the study products had been applied, leading to a temporary interruption of product application. However, the effects were of short duration and did not persist over time.

Discussion

The method described in this study allowed the generation of standardised wounds and the qualitative and quantitative comparison of skin regeneration following the use of three different skin repair products. The efficacies of products A and B were similar in terms of the time and rate of lesion closure (9 days) and healing quality of the Er-YAG laser-induced wounds. Both of these products resulted in more rapid epidermal regeneration and better skin quality than product C (12 days) or the untreated control (16 days). This represents a 7-day gain between the wound treated by product A and the untreated control. All three wound repair products showed a similar rate of decreasing TEWL, and this rate was significantly faster than that measured for the untreated control. This demonstrates that use of repair products results in a faster rate of barrier regeneration.

This study did not aim to elucidate the mechanism of action of the products tested, but rather to validate the wounding model and the evaluation methods used to quantify the effects of various products on epidermal regeneration and skin barrier restoration. In particular, the changes in TEWL showed similar trends to the changes in WSA between the wounds treated by the tested products and the untreated area. This was to be expected as evaporative water loss is directly dependent on the surface area of damaged skin.

In the study by Ferraq *et al.* [13], (Er-YAG, 50 µm ablation depth corresponding to the thickness of the epidermis, 15 J/cm^2, area 1 cm^2), the laser wound surface area decreased steadily to only 50% closure at day 7 and TEWL had not reached baseline values on day 7. These results are in line with those obtained in our study using smaller laser-induced wounds, in which re-epithelialisation time was 16 days for the untreated wounds, as assessed by measuring the WSA on calibrated digital photographs.

In the study by Trookman *et al.* [16] (Erbium/carbon dioxide laser, penetrating to the epidermis, four uniform circular 5mm diameter wounds performed on the volar forearm of 20 subjects), the average TEWL decreased rapidly after D4, but did not reach baseline values until day 18. Digital photographs of the untreated wounds showed scabbing on days 4 and 7, as also observed in our study on

days 6 and 8 *(figure 4C)*. Trookman *et al.* [16] graded the general untreated wound appearance as 'good' at day 11 and almost 'very good' at D18 (on a scale ranging from 'poor', to 'fair', 'good', 'very good' and 'excellent').

Experimental studies of wound healing lack methods for standardised wounding and *in situ* depth assessment. One of the strengths of our study was the use of an Er-YAG laser for wound induction, generating wounds more uniform in depth and surface than the suction blister method [13]. The wounds produced by Er-YAG laser are also reproducible, as re-epidermisation time in our study was comparable to that described in the study by Ferraq *et al.* [13]. Furthermore, we coupled this reliable method of wound induction to a 3D imaging technique for non-invasive monitoring of the depth of experimentally induced wounds [15]. The standardised lesions produced by this laser wounding model enabled detection of small differences between treatments that could otherwise go unnoticed. A potential limitation of this study was its relatively small sample size; however, each subject was its own control. Finally, the results obtained with the various skin repair products may also be achieved on clean superficial wounds, such as those created in dermatological practice. Further studies would be needed to evaluate the skin repair properties of these products on other types of wounds.

In conclusion, standardised lesions were obtained using this laser-induced epidermal lesion model, enabling different topical skin repair products to be evaluated. The product containing *Avena Rhealba*® extract and hyaluronic acid resulted in more rapid epidermal regeneration (a 7-day reduction in the time needed for re-epidermisation compared with the untreated control) and better skin quality compared with a reference product and an untreated area. Thus, this epidermal lesion model can be used to discriminate between various skin repair products and to quantify the effects of products on epidermal regeneration and skin barrier restoration. Moreover, this type of wounding method resembles the types of procedure performed in laser-assisted interventional dermatology, suggesting that this model could have a relatively wide range of applications and, in particular, could be used to evaluate skin repair products used after laser procedures. ■

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