

Laser-Assisted Penetration of Allergens for Patch Testing

Mary Veremis-Ley, Haydee Ramirez, Elma Baron, Kaija Hanneman, Lela Lankerani, Heather Scull, Kevin D. Cooper, and Susan T. Nedorost

Background: Patch-test patients often complain of itching and inconvenience.

Objective: To demonstrate (1) the usefulness of laser-assisted alteration of the stratum corneum to enhance allergen delivery and (2) patient satisfaction with this procedure.

Methods: The LAD-01 (erbium:yttrium-aluminum-garnet) laser unit was used to alter stratum corneum from patients with known sensitivity to nickel or Kathon CG. These allergens were then applied to the laser-pretreated sites for 60 minutes. Results were observed at 24, 48, and 96 hours and at 1 week. One patient who refused conventional patch testing was tested with an entire modified North American standard series tray with the laser patch-test technique. An additional patient with previously demonstrated positive atopy patch-test reactions to environmental organisms was retested with laser pretreatment to the same antigens.

Results: Three of three patients known to be sensitive to Kathon CG and eight of eleven known nickel-sensitive patients had positive reactions at the laser-pretreated sites. The patient who was tested with the entire standard series demonstrated relevant positive reactions to formaldehyde and to a textile resin. One subject with known reactions to three environmental organisms reproduced patch-test responses with laser pretreatment. No irritant reactions were noted. Patients reported no pain.

Conclusion: With further modification, laser pretreatment may improve patient convenience and decrease irritant test reactions owing to occlusion.

CONVENTIONAL PATCH TESTING involves applying suspected allergens to the intact skin of a patient's back under occlusion with tape for 48 hours as recommended by the International Contact Dermatitis Research Group (ICDRG)¹ and then observing the back at intervals for 1 week after application. Patients must keep the test site, usually the skin of the back, dry during the testing procedure, for two reasons²: (1) to prevent loosening of the adhesive holding the patches during the first 48 hours and (2) to prevent the ink grids that mark the position of application of the various allergens tested from washing away.

Patch testing has good sensitivity and specificity³ and is a sound and relatively safe method for identifying allergens in patients with allergic contact dermatitis.⁴ There are, however, a number of difficulties with this testing modality. Many patients find it inconvenient and at times impossible to keep their backs dry for the week-long

testing procedure. This is especially difficult for patients whose occupation involves manual labor or for those who live in a humid climate. Some patients have intractable dermatitis that covers the skin of the back, which necessitates the use of an alternative test site. These sites are limited as flexing of many body areas results in non-occlusion of the adhesive tape. Even patients who do not have these contraindications to standard patch testing are inconvenienced by the current methodology and often wish there were some other test available so that they could have their daily shower.

There are numerous factors that influence the elicitation of contact allergy in individuals. These factors include the vehicle, the degree of percutaneous penetration, the effect of diseased or traumatized skin, the effect of occlusion, the total dose, surface concentration, and the anatomic site of exposure.⁵ Our objective is to modify some of these variables to more quickly elicit diagnostic test reactions of allergic contact dermatitis in patients, with fewer restrictions for activity and bathing and without increasing the risk of sensitization of previously nonallergic patients.

Components of the stratum corneum form a permeability barrier against external agents.⁶ Disruption of this barrier results in ultrastructural change that allows an influx of substances from the surface into the deeper layers of the skin.⁷ The erbium:yttrium-aluminum-garnet (Er:

From University Hospitals of Cleveland/Case Western Reserve University, Cleveland, OH, and the VA Medical Center, Cleveland, OH.

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YAG) laser has a wavelength of 2,940 nm and water as its chromophore, which allows good penetration into the epidermis.⁸ The Er:YAG laser used in this study was the LAD-01 (Norwood Abbey, Victoria, Australia). Each time the laser is activated, it emits a single pulse with a full-width half-maximum (FWHM) pulse width of approximately 600 μ s. The energy density is 3.5 J/cm². The temporal shape of the pulse can be approximated to a gaussian curve, but the spatial distribution is approximated to an even distribution across the beam (ie, a flat top beam shape). The ablative properties of the Er:YAG laser have been compared to those of a carbon dioxide laser.⁹ A single pulse of the Er:YAG laser can remove an area of the stratum corneum¹⁰ up to 20 μ m in depth, which could allow substances to enter through a perturbed barrier. More recently, a study by Baron and colleagues showed that treatment with an Er:YAG laser followed by lidocaine cream is a more efficient means of inducing skin anesthesia than the application of topical lidocaine to intact skin.¹¹ Technology that would allow allergens to move through the stratum corneum more efficiently has been studied in a guinea pig model.¹² Removal of the stratum corneum with the LAD-01 laser prior to the application of test allergens could preclude the need for tape occlusion of the test sites, thus avoiding many of the logistical problems with the current testing methods.

The aim of our investigation was to demonstrate the usefulness of laser-assisted allergen delivery in the evaluation of allergic contact dermatitis and to assess patient satisfaction with laser-assisted allergen delivery. We hypothesize that laser-assisted penetration of allergens through the stratum corneum can facilitate patch testing in patients with allergic contact dermatitis in a safe, efficient, and effective manner.

Methods

All procedures were approved by the institutional review board at University Hospitals of Cleveland/Case Western Reserve University, Cleveland, Ohio.

Laser Treatment and Patch Testing

Healthy adult patients with all skin types known to be sensitive to nickel or to 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (Kathon CG, Rohm and Haas, Philadelphia, PA) were recruited and were tested with their known allergen by the conventional technique using Finn Chambers (Epitest Ltd Oy, Tuusula, Finland) and Scanpor tape (Alpharma A/S, Vennele, Norway) on one side of the back; laser-treated skin was tested

with standard and reduced concentrations of the non-occluded allergens on the other side of the back (Fig 1). Patients were recruited from a database of approximately 650 patients maintained at the Contact Dermatitis Clinic at Case Western Reserve University or from fliers advertising the study. Patients were eligible if they had a patch-test reaction of + or greater according to the standard ICDRG reading scale at days 3, 4, or 7 after patch-test placement and had no evidence of angry-back syndrome (ie, numerous positive reactions suspected of being false positives). Patients recruited by advertising were tested with their suspected allergen via the conventional technique to confirm a + or greater patch-test reaction. Eligible patients had to meet the standard criteria for patch testing, which include the following: no recent use of systemic immunosuppressives (allowance of < 10 mg of prednisone per day), no usage of topical steroids or nonsteroid immunosuppressives on the test sites for 2 weeks before testing, no current phototherapy, no use of a tanning salon, and no current pregnancy.

Standard 48-hour patch testing with the patient's known allergen was carried out on the right side of the back with Finn Chambers and Scanpor tape; this was considered to be the positive control for test results. Laser treatment was performed on the left side of the back. The LAD-01 unit, a lightweight portable Er:YAG unit, was used to treat the test sites on the left side of the back prior to allergen application. The following were the laser parameters



Figure 1. Response to the conventional technique that uses Finn Chambers and Scanpor tape can be seen on the right side of the patient's back. Responses with standard and reduced concentrations of non-occluded allergens on laser-pretreated skin can be seen on the left side of the back.

on the LAD-01 unit: 2,940 nm wavelength, 3 mm diameter spot size, fluence of 250 mJ per pulse, and 300 ms pulse duration. A single pulse was used at nine test sites on the left side of the back. Test sites were at least 1.5 cm apart. One laser-treated test site served as a laser control with no allergen added. On four of the laser-treated test sites on the left side of the back, allergens at standard and reduced concentrations were applied for 5 minutes and then wiped off with cotton swabs moistened with water or mineral oil. On the other four laser-treated test sites, allergens at standard and reduced concentrations were applied for 60 minutes and then wiped off. A standard concentration of allergen was applied for 5 minutes and for 60 minutes on nonlaser-treated skin on the left side of the back to determine if there was any reaction on non-occluded nonlaser-treated skin at 5 and 60 minutes. Allergens were removed with individual cotton swabs after the specified times. Test site locations were marked with a surgical pen and a fluorescent highlighter to allow the location to be read with either visible or black light. Patients were asked to keep their backs dry and to return for readings at 24, 48, and 96 hours and at 1 week after the initial application of allergens; standard ICDRG criteria were used to record the strength of the observed allergic reaction. At the end of study, patients were asked to report pain, using a visual analogue scale previously established and used for a topical anesthesia delivery study.¹¹ Patient satisfaction was assessed with a self-administered questionnaire.

The standard concentration of nickel used was 2.5% in petrolatum (pet). Reduced concentrations of nickel used were 0.025% pet, 0.1% pet, and 0.25% pet. Combined 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one, also known as Kathon CG, was tested at a standard concentration of 0.01% aqueous (aq) and at reduced concentrations of 0.00001% aq, 0.0005% aq, and 0.001% aq. (Antigens tested near their sensitization threshold by conventional technique were tested at reduced concentrations with laser pretreatment to reduce the risk of sensitization in naïve subjects by the test allergens.) All allergens were obtained from Chemotechnique Diagnostics (Malmö, Sweden).

One patient who refused conventional testing was tested with a modified standard screening tray of diluted allergens (Chemotechnique's North American standard screening tray with the addition of some Chemotechnique allergens from the vehicle series) applied in a non-occluded fashion to laser-pretreated skin. Concentrations of allergens that are usually used at lower concentrations to prevent irritancy were increased (Table 1). Concentrations of allergens that were used close to their sensitization thresh-

Table 1. Standard Allergens Tested at Higher-Than-Usual Concentrations

Substance	Concentration Tested (%) [*]	Standard Concentration (%) [*]
para-Phenylenediamine	2.0	1.0
Cinnamic aldehyde	2.0	1.0
Carba mix	12.0	3.0
Thiuram mix	4.0	1.0
Potassium dichromate	1.0	0.25
Nickel sulfate	20.0	5.0 aq
Diazolidinylurea	2.0	1.0
Paraben mix	15.0	12.0
Euxyl K400 [†]	5.0	2.5
Fragrance mix	16.0	8.0
Amerchol L-101 [†]	70.0	50.0
Cocamidopropyl betaine	2.0 aq	1.0 aq

aq = aqueous.

^{*}Petrolatum vehicle unless otherwise noted.

[†]Chemotechnique, Malmö, Sweden.

old were not increased. The patient returned for readings at day 2 and at 1 and 2 weeks. The 2-week reading was performed to detect sensitization by the test allergens, which is a theoretic risk with all patch tests and which may be a greater risk with laser pretreatment.

One patient with known allergies to dust mite, *Candida albicans*, and *Malassezia sympodialis* was tested with dust mite 10% (Chemotechnique's 20% mix of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, diluted at half strength in petrolatum), *Candida albicans* 4%, *Malassezia sympodialis* 40%, and a proprietary *Candida albicans* skin test antigen (Candin, Allermid Laboratories, Inc., San Diego, CA). The *Candida albicans* and *Malassezia sympodialis* were both grown at University Hospitals of Cleveland and were then killed by ultraviolet radiation. The precipitated crude extract was suspended in petrolatum, weight per volume. The patient returned for readings at 6 hours and at day 2.

For each part of the study, patients were observed for adverse events and were surveyed for satisfaction with the technique.

Histologic Analyses

Laser-Treated Skin

A healthy volunteer was recruited to donate punch biopsy specimens from buttock skin after treatment with the laser. Four 6 mm punch biopsy specimens were taken immediately after delivery of one, two, and three pulses of the laser and from untreated control skin. Each biopsy

specimen was split in two: one-half was embedded in paraffin for routine hematoxylin and eosin (H & E) staining, and the other half was embedded in optimal cutting temperature (OCT) compound embedding medium (Sakura Technical Co., Tokyo Japan) and frozen (for possible future immunohistochemical analysis).

Laser-Treated Skin versus Tape-Stripped Skin

A second healthy volunteer was recruited to donate punch biopsy specimens from buttock skin after treatment with laser and D-Squame tape strips (CuDerm Corporation, Dallas, TX). This subject received one pulse of the laser and tape stripping to separate sites on buttock skin. Five D-squame strips were applied sequentially to the designated site, and each was gently rubbed on the buttock skin for approximately 1 minute before being stripped off. Each tape strip showed stratum corneum components. The entire procedure took approximately 5 minutes in comparison to the laser treatment, which took only a few seconds. Immediately after laser treatment and tape stripping, a 6 mm punch biopsy specimen was taken from each site. The specimens were split in half, one half to be sent for H & E staining and the other half to be embedded in OCT and frozen.

Analysis of In Vivo Transepidermal Water Loss

In three additional healthy volunteers, the DermaLab TEWL Probe (Cortex Technology, Hadsund, Denmark) was used to measure transepidermal water loss (TEWL) either immediately after one pulse of the laser or immediately after the application of five D-Squame tape strips to nonsun-exposed skin on the subject's buttock or volar arm. Similarly, to determine changes in TEWL in laser-treated skin over time, an additional four healthy volunteers were recruited to receive one laser pulse on the buttock skin or volar forearm skin. TEWL measurements of the laser-treated site were taken at 0, 24, and 48 hours. TEWL was also measured on untreated skin (control).

Microsoft Excel (Microsoft Corporation, Redmond, WA) was used to generate the statistical analysis of the data.

Results

Laser-Pretreated Patch-Test Sites in Known Nickel-Sensitive and Kathon CG-Sensitive Patients

A total of 14 patients completed part one of the study. Eleven volunteers with nickel sensitivities were enrolled (all women, 19–61 years of age). All were tested with nickel 2.5% pet. Of the 11 nickel-sensitive volunteers, 8 had a positive reaction at the laser-pretreated site where allergen

had been applied for 60 minutes. Two of these patients reacted to nickel on normal nonlaser-treated unoccluded skin. Three of the known nickel-sensitive patients did not react after up to 60 minutes of contact on laser-treated skin.

Three patients with Kathon CG sensitivities were enrolled (2 males, aged 52 and 58 years; 1 female, aged 68 years). All three were tested with Kathon CG (0.01% aq), and all had positive reactions at the laser-pretreated site where allergen had been applied for only 60 minutes. Two patients reacted to Kathon CG on normal nonlaser-treated unoccluded skin.

The two Kathon CG-sensitive volunteers and the two nickel-sensitive volunteers who had reactions on normal skin had demonstrated sensitivity without occlusion and did not need laser or conventional patch testing to elicit a response. The three known nickel-sensitive patients who failed to react after 60 minutes of allergen exposure on laser-pretreated skin were insensitive and needed conventional testing with occlusion to obtain positive results (Table 2).

Laser Pretreatment with an Entire Standard Series

The one subject who was tested with the extended standard screening series presented with a pruritic dermatitis in the anterior and posterior axillary lines, on his back, around his umbilicus and posterior waistline. There was relative sparing of his legs and complete sparing of his scalp and hands. This pattern suggested a possible textile allergy. His dorsal feet were severely affected although interdigital web spaces, soles, and toenails were normal. He wears leather work boots that are lined with Gore-Tex waterproof fabric (W.L. Gore, Newark, DE). He currently does cement work. Sixty-one allergens were applied on laser-pretreated test sites. On his day 2 reading, he had a ? reaction to formaldehyde 1% aq, a + reaction to nickel 20% pet (one of several allergens tested at higher-than-standard concentrations), a ?/+ reaction to Fixapret CPN (Chemotechnique Diagnostics, Malmö, Sweden), and a + reaction to nickel 5% aq. At 1 week, his ? reaction to formaldehyde 1% aq became a + reaction; all other reactions remained the same. All sites were negative at 2 weeks. There was no

Table 2. Nickel and Kathon CG Results in Known Responders

<i>Substance</i>	<i>N</i>	<i>B</i>	<i>L</i>	<i>F</i>
Kathon CG	3	2	1	0
Nickel	11	2	6	3

N = number of known responders; B = number of known responders who had reactions on both laser-pretreated skin and normal skin; L = number of known responders who had a reaction on laser-pretreated skin; F = number of known responders who did not have a reaction on laser-pretreated skin.

evidence of sensitization. The patient was counseled on textile avoidance. At follow-up, he reported that his torso completely cleared after he switched to wearing white cotton T-shirts. His feet were unchanged, but he was still wearing the same work boots.

Laser Pretreatment with Environmental Organisms in a Subject with Known Positive Reactions to Environmental Organisms

A subject with previously demonstrated reproducible atopy patch tests to dust mite, *Candida albicans*, and *Malassezia sympodialis* was retested with these antigens using Finn chambers on intact skin. He was also tested with these antigens on laser pre-treated skin both open (without Finn Chambers) and occluded with Finn chambers for 60 minutes, after which the antigen was wiped off of the skin. At 6 hours, his positive reaction to dust mite, *Candida albicans*, and *Malassezia sympodialis* was produced at the laser-pretreated occluded and unoccluded sites. At 6 hours, there was no reaction at the conventional patch-test site nor at the laser-pretreated commercial *Candida* skin test sites concordant with his previous testing. By day 2, there was evidence of sensitivity at the conventional patch-test site.

Other Observations

Irritant Reactions and Laser Pretreatment

No patient had a reaction to the laser-pretreated skin to which no antigen had been applied (these skin sites served as a negative control).

Tolerance of Laser Pretreatment

With the exception of pruritus at positive reaction sites, no side effects were reported by patients. None of the patients reported any pain with the laser-treated sites. All patients reported that laser pretreatment was more convenient than conventional patch testing. There was, however, an immediate epidermal color change at the laser-treated sites, which consisted of a white color change in skin types II and III and a dark color change in skin types IV and V. The color change lasted for approximately 7 to 10 days, at which time the skin returned to its normal color. Clinically, this appeared as an adherent crust.

Histologic Changes Induced by Laser Treatment (Not Observed in Tape-Stripped Skin)

With one pulse of the laser, there is disruption of the orderly architecture of the upper epidermis to the granular layer, with focal areas of necrosis. With two pulses of the laser, there is a higher degree of disruption and necrosis.

With three pulses, there is frank necrosis of the epidermis. Three superimposed pulses would be expected to ablate up to 60 μ in depth, which would result in necrosis of most of the epidermis in areas of average thickness. We believe the color change in the skin that we described as an “adherent crust” is the result of the intercellular edema that is observed in the upper epidermis (Fig 2).

Specimens stained with H & E and prepared after five D-Squame tape strippings look essentially normal when compared to normal skin and skin exposed to one pulse of the laser (Fig 3).

Transepidermal Water Loss Increased by Laser Treatment

There was a significant difference in the TEWL of laser-treated skin ($p < .05$) and no significant difference in that of tape-stripped skin ($p > 0.1$) (Fig 4).

There was a rapid increase in TEWL (approximately three times the baseline value) immediately after laser treatment. TEWL fell back to normal by 48 hours (Fig 5).

Discussion

For many years, it has been recognized that the vehicle in which a chemical allergen is encountered on the skin can have a profound effect on the response elicited.⁵ To induce a response in a previously sensitized individual, the allergen must be able to penetrate the outer lipophilic layer of the skin, the stratum corneum.¹³ Removal or disruption of the lipophilic stratum corneum may make an aqueous vehicle even more advantageous for allergen penetration through laser-pretreated skin. We speculate that aqueous vehicles may be preferred for testing on laser-pretreated skin. There are, however, drawbacks to using water as a vehicle, such as the following: (1) the expected instability of aqueous solutions¹⁴; (2) the evaporation of aqueous solutions, which makes the dissolved allergens irritant in nature¹⁵ by changing their concentration; (3) the poor solubility of some allergens in water; and (4) microbial contamination. The correct selection of vehicles for patch testing is crucial in the evaluation of allergic contact dermatitis.¹⁶

Although petrolatum is regarded as the most suitable base for most allergens, we propose that antigens for laser-assisted patch testing might be better tested in aqueous vehicles. We also propose that antigens for laser-assisted patch testing could be tested at higher-than-usual concentrations if they are considered to be irritants, because irritation is less of a problem when there is no occlusion.¹⁷

Skin type (but neither race nor gender) can influence epidermal permeability barrier function, according to a

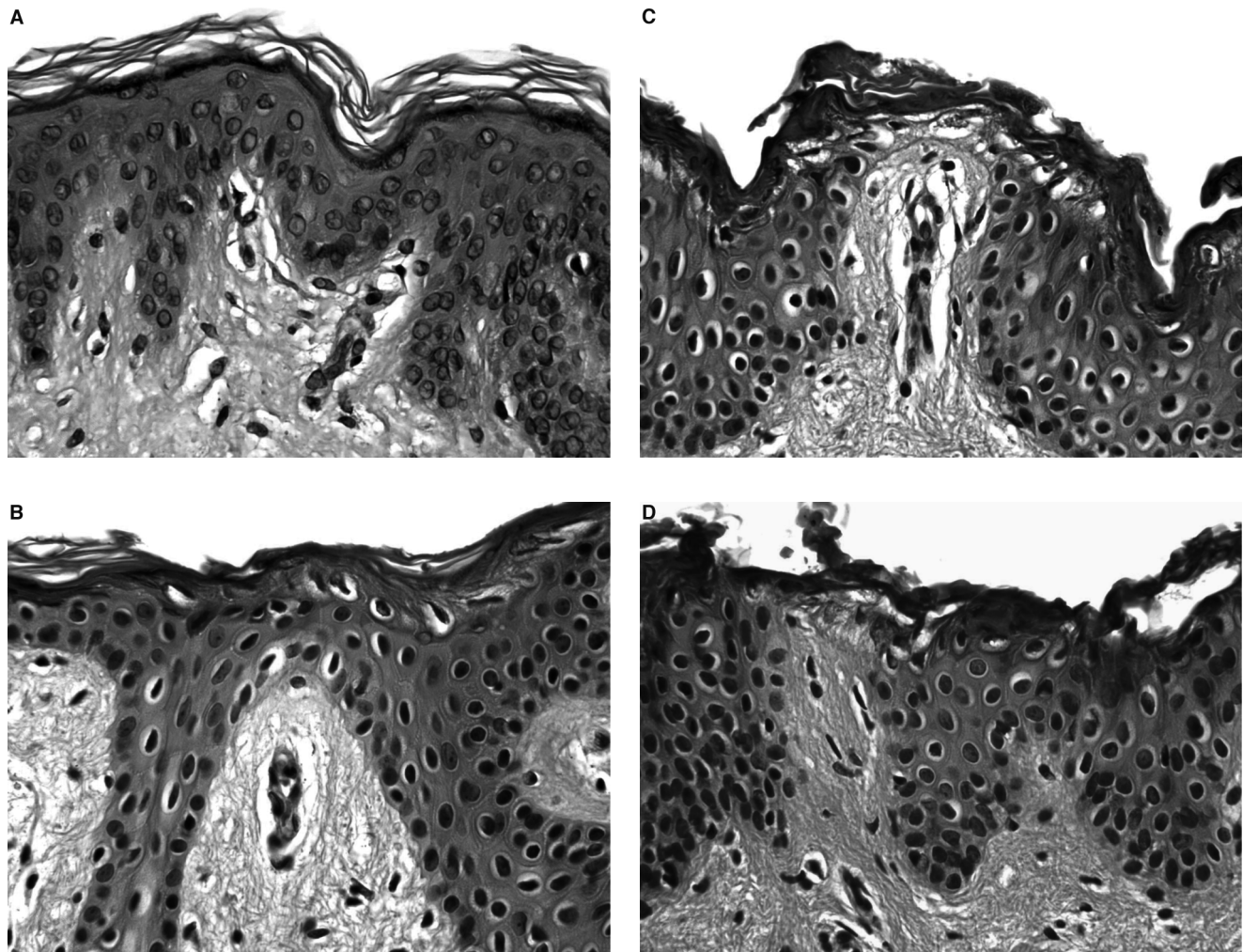


Figure 2. A, Biopsy specimen taken from normal skin. B, Biopsy specimen taken from normal skin after one laser pulse; there is disruption of the epidermis and granular layer, with focal areas of necrosis. C, Biopsy specimen taken from normal skin after two consecutive laser pulses; there is a higher degree of disruption and necrosis. D, Biopsy specimen taken from normal skin after three consecutive laser pulses; there is frank necrosis of the epidermis and granular layer. (All stained with hematoxylin and eosin, $\times 40$ magnification)

study by Reed and colleagues.¹⁸ Although the stratum corneum is of equal thickness in African Americans and white Americans,¹⁹ the stratum corneum of African Americans displays more cell layers than does the stratum corneum of white persons.²⁰ In our study, 3 of the 11 nickel-sensitive patients did not have a positive reaction on laser-pretreated skin; 2 of these 3 patients were African Americans.

The volunteers reported laser-assisted patch testing to be painless and more convenient as they did not have to wear tape or keep the back dry. The visible white color change (adherent crust) in the epidermis made by the single pulse of the LAD-01 laser resolved in 7 to 10 days. This epidermal change served as a marker for where the pulse was applied on the skin. The color change was extremely important because it marked the laser-treated test sites so

that the volunteers did not have to keep their backs dry. Volunteers were very pleased with this convenience.

Why not use tape strips instead of the LAD-01 laser? Tape stripping is inconvenient, time consuming (5 minutes for five D-Squame tape strips as opposed to seconds for one laser pulse), and uncomfortable for the patient. In addition, and perhaps most importantly, tape stripping does not mark the test site.

Previous modifications of patch-test methodology have been intended to improve convenience for the provider. This methodology improves patient satisfaction and may encourage more patients to undergo this important diagnostic procedure. Irritant reactions would be expected to be less common when occlusion is not used, and in fact, none were observed in this study. This may allow changes in allergen

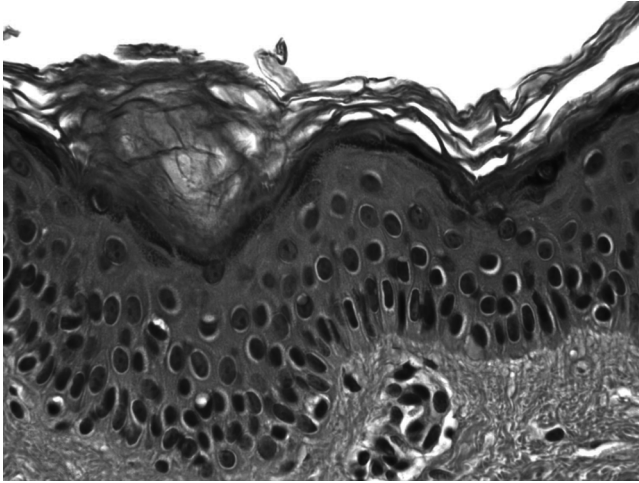


Figure 3. Biopsy specimen from normal skin after five D-Squame tape strippings. (Hematoxylin and eosin staining, $\times 40$ original magnification)

concentration for substances for which irritancy (rather than sensitization risk) limits patch-test concentrations.

In summary, volunteers were extremely happy with this new technique. There was no need for volunteers to wear patches or to keep their backs dry, thus avoiding many of the logistic problems with current testing methods. There was no scarring or pain reported in this study. Validation of this technique for all of the antigens on the standard tray could require the reformulation of many antigens and extensive restandardization testing. Variables to be ad-

TEWL values of control vs Time 0, 24, and 48

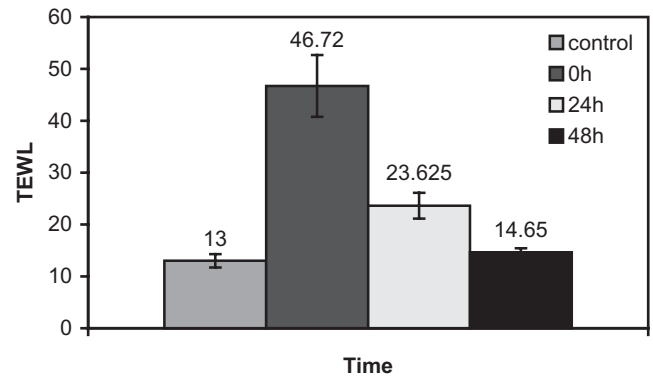


Figure 5. There was a rapid increase in transepidermal water loss (TEWL) immediately after laser treatment. TEWL returned to normal by 48 hours.

dress would include (but are not limited to) the physico-chemical properties of allergen and vehicle, the effect of the laser on the ability of keratinocytes to metabolize some antigens,²¹ and the effect of various exposure times and skin types on results.

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Comparison of TEWL after treatment with laser vs D-Squame (time 0)

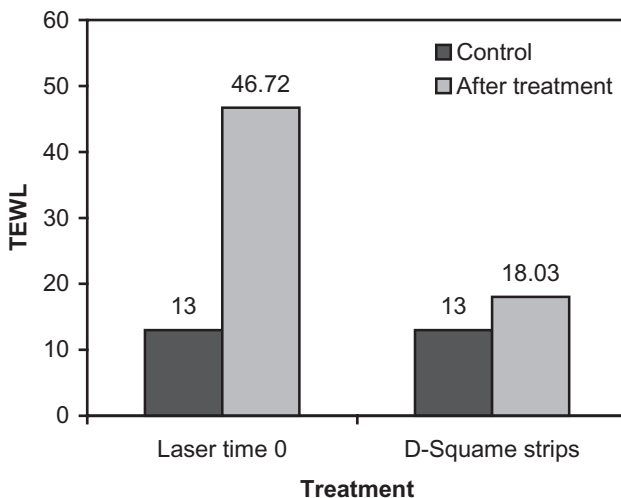


Figure 4. There is a significant difference in the transepidermal water loss of laser-treated skin and no significant difference in that of tape-stripped skin.

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