Skin Pre-Ablation and Laser Assisted Microjet Injection for Deep Tissue Penetration

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Background and Aims: For conventional needless injection, there still remain many unresolved issues such as the potential for cross-contamination, poor reliability of targeted delivery dose, and significantly painstaking procedures. As an alternative, the use of microjets generated with Er:YAG laser for delivering small doses with controlled penetration depths has been reported. In this study, a new system with two stages is evaluated for effective transdermal drug delivery. First, the skin is pre-ablated to eliminate the hard outer layer and second, laser-driven microjet penetrates the relatively weaker and freshly exposed epidermis. Each stage of operation shares a single Er:YAG laser that is suitable for skin ablation as well as for the generation of a microjet.

Methods: In this study, pig skin is selected for quantification of the injection depth based on the two-stage procedure, namely pre-ablation and microjet injection. The three types of pre-ablation devised here consists of bulk ablation, fractional ablation, and fractional–rotational ablation. The number of laser pulses are 12, 18, and 24 for each ablation type. For fractional–rotational ablation, the fractional beams are rotated by 11.25° at each pulse. The drug permeation in the skin is evaluated using tissue marking dyes. The depth of penetration is quantified by a cross sectional view of the single spot injections. Multi-spot injections are also carried out to control the dose and spread of the drug.

Results: The benefits of a pre-ablation procedure prior to the actual microjet injection to the penetration is verified. The four possible combinations of injection are (a) microjet only; (b) bulk ablation and microjet injection; (c) fractional ablation and microjet injection; and (d) fractional-rotational ablation and microjet injection. Accordingly, the total depth increases with injection time for all cases. In particular, the total depth of penetration attained via fractional pre-ablation increased by 8 ~ 11% and that of fractional–rotational pre-ablation increased by 13 ~ 33%, when compared with the no pre-ablation or microjet only cases. A noticeable point is that the fraction-rotational pre-ablation and microjet result is comparable to the bulk ablation and microjet result of 11 ~ 42%. The penetration depth underneath ablated stratum corneum (SC) is also measured in order to verify the pre-ablation effect. The penetration depths for each case are (a) 443 ± 104 μm; (b) 625 ± 98 μm; (c) 523 ± 95 μm; and (d) 595 ± 141 μm for microjet only, bulk ablation and microjet, fractional ablation and microjet, and fractional–rotational ablation and microjet, respectively. This is quite beneficial since any healing time associated with ablation is significantly reduced by avoiding hard-core bulk ablation. Thus the bulk pre-ablation and microjet may well be superseded by the less invasive fraction-rotational ablation followed by the microjet injection. The density of micro-holes is 1.27 number/mm² for fractional ablation and 4.84 number/mm² for fractional–rotational ablation. The penetration depths measured underneath the ablated SC are 581 μm (fractional ablation and microjet) and 691 μm (fractional–rotational ablation and microjet).

Conclusions: Fractional–rotational ablation increases number of micro-holes in a unit area, enabling fast reepithelialization and high drug delivery efficiency. Optimization of system parameters such as ablation time, number of ablations, and injection time will eventually ensure a macromolecule delivery technique with the potential to include vaccines, insulins, and growth hormones, all of which require deeper penetration into the skin. Lasers Surg. Med. © 2016 Wiley Periodicals, Inc.

Key words: ablation; transdermal drug delivery; Er:YAG laser; fractional laser; microjet; needle free

INTRODUCTION

Needle-free injection offers significant advantages when applied to macromolecule delivery including vaccines, insulin, local anesthetics, and growth hormones [1,2]. The system is also known to overcome already acknowledged drawbacks to needle use such as site lesions, pain

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associated with the invasive procedure, disposal of medical waste, and safety issues associated with reuse.

Previous efforts to develop needle-free techniques include the application of a needless injector for insulin delivery through subcutaneous injections [3–6] requiring drug penetration depths of a few millimeters. Rapid insulin absorption is ensured with such non-invasive procedures, leading to efficient control of glucose level. However, there still remain many unresolved issues such as the potential for cross-contamination from the jet splash back, poor reliability regarding the delivered dose, and significantly painstaking procedures associated with the injection [7–9]. As an alternative, the use of microjets generated with Er:YAG lasers to deliver small doses per pulse at 10 Hz with controlled penetration depths have been reported to be quite competitive with previously attempted methods [10–12].

Meanwhile, the SC or the topmost hard layer of the skin is the main obstacle to cutaneous injection. Especially, high-molecular-weight substances show low permeation into the skin. One possible way of overcoming the SC layer is skin ablation with mid-infrared range lasers such as Er:YAG [13–16]. For example, drug permeation can be enhanced by utilizing the laser, and the drug can be topically applied to the ablated skin surface [17–22].

Among the available options, fractional lasers allow efficient drug insertion through grid-patterned micro-holes. High-molecular-weight substances can be delivered into the deeper layer while skin damage is minimized by controlling the laser fluence, pulse number and exposure time. With fractional lasers, ablated holes surrounded by tissue are created, and full epidermal wounds are avoided with a suggested minimum healing time. The efficiency of drug permeation can be increased further by using a rotation of the fractional laser because the density of micro-holes per unit area is significantly increased [22].

In this study, we successfully enhanced the injection depth via the combination of pre-ablation and microjet injection. First, skin is ablated with the Er:YAG laser and then, the microjet is injected through the pre-ablation site. In this manner, energetic microjets easily pierce the relatively weak strength layer. Here, three kinds of pre-ablation types are considered: bulk, fractional, and fractional–rotation. The pros and cons of each pre-ablation type are investigated to determine the optimal conditions for injection. Meanwhile, the parameters relative to microjet generation are fixed with reference to our previous optimal results: 150 μs pulse duration, 1 J energy, 10 Hz operated Er:YAG laser, 30° nozzle angle, and 150 μm nozzle diameter.

MATERIALS AND METHODS

The detailed mechanism is given in Figure 1 a. The outermost layer of the skin is subjected to elimination using the three types of pre-ablation. The mechanism for fast microjet generation is based on the laser-driven bubble expansion within a driving chamber separated by an elastic membrane between the drug and water. Two separate processes of pre-ablation and microjet generation are integrated into a single unit as shown in Figure 1 b. Each process shares a single laser source. In stage one, the laser beam passes through the ablation unit to pre-ablate the skin, and then the microjet injector is connected to the laser. In the second stage, after eliminating the SC layer via pre-ablation, the ejected microjet penetrates the target skin with ease. A more elaborate discussion on the interaction between bubble and microjet can be found in the authors’ earlier works [10–12]. The specification of the laser source used in the present study is as follows: Er:YAG (2940 nm, 150 μs pulse duration, 1 J energy, and 10 Hz operation frequency).

The ablation parameters of the mid-infrared-range laser are shown in Table 1. The optimal fluence of pre-ablation is
determined to be 8 J/cm², based on previous studies. The goal of pre-ablation is the elimination of the SC, which is a barrier against substance permeation from the outside of the skin. This process can enhance microjet permeation into the skin. In this study, pig skin is selected for quantification of the injection depth based on the two-stage procedure, namely pre-ablation of, and microjet injection into the treatment site.

Three kinds of pre-ablation are devised, consisting of (a) bulk ablation; (b) fractional ablation; and (c) fractional–rotational ablation. A top view image of ablation patterns is shown in Figure 2. The number of laser pulses are 12, 18, and 24, and this value is the same for all of the three ablation types compared. The idea behind the (c) fractional–rotational case is a subtle enhancement of the concept suggested by Lee et al. [22],

![Table 1](image)

**TABLE 1. Ablation Data for the Mid-Infrared-Range Beam: Pulse Duration, Energy, Ablation Rate and Ablated Diameter**

<table>
<thead>
<tr>
<th>Laser</th>
<th>Pulse duration</th>
<th>Target</th>
<th>Energy</th>
<th>Ablation rate</th>
<th>Ablated diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our system (bulk)</td>
<td>EnYAG</td>
<td>150 μs</td>
<td>Pig skin</td>
<td>8 J/cm²</td>
<td>13–15.6 nm (per pulse)</td>
</tr>
<tr>
<td>Our system (fractional)</td>
<td>ErYAG</td>
<td>150 μs</td>
<td>Pig skin</td>
<td>8 J/cm²</td>
<td>6.7–11.4 pm (per pulse)</td>
</tr>
<tr>
<td>Hibst et al.[13]</td>
<td>EnYAG</td>
<td>250 μs</td>
<td>Pig skin</td>
<td>10–73 J/cm²</td>
<td>18–414 pm (per pulse)</td>
</tr>
<tr>
<td>Walsh et al.[14]</td>
<td>ErYAG</td>
<td>250 μs</td>
<td>Guinea Pig skin</td>
<td>5.4–72.5 J/cm²</td>
<td>8.4–400 nm (per pulse)</td>
</tr>
<tr>
<td>Hohenleutner et al.[15]</td>
<td>EnYAG</td>
<td>250 μs</td>
<td>Human</td>
<td>1.6–7 J/cm²</td>
<td>1.75–15.3 pm (per pulse)</td>
</tr>
<tr>
<td>Khatri [16]</td>
<td>EnYAG</td>
<td>250, 350, 700 μs</td>
<td>Human Skin</td>
<td>5 J/cm²</td>
<td>3.3–6.7 pm (per pulse)</td>
</tr>
<tr>
<td>Lee et al.[17]</td>
<td>EnYAG</td>
<td>100 μs</td>
<td>Nude mice</td>
<td>2.5–5 J/cm²</td>
<td>10–20 pm (per pulse)</td>
</tr>
<tr>
<td>Nelson et al. [18]</td>
<td>ErYSGG</td>
<td>250 μs</td>
<td>Pig skin</td>
<td>1 J/cm²</td>
<td>2.2 pm (per pulse)</td>
</tr>
<tr>
<td>Hsederdal et al. [19]</td>
<td>Fractional CO₂</td>
<td>3 ms</td>
<td>Pig skin</td>
<td>130 J/cm²</td>
<td>1850 pm</td>
</tr>
<tr>
<td>Genina et al. [20]</td>
<td>Fractional EnYAG</td>
<td>200 μs</td>
<td>Human Skin</td>
<td>0.5–3 J</td>
<td>150–300 nm (total)</td>
</tr>
<tr>
<td>Dierckx et al.[21]</td>
<td>Fractional EnYAG</td>
<td>250 μs</td>
<td>Pig skin</td>
<td>60 J/cm²</td>
<td>170 nm (per pulse)</td>
</tr>
</tbody>
</table>

![Fig. 2](image)

Fig. 2. Top view of burn pattern for each ablation type: (a) Type-1 bulk ablation, (b) Type-2 fractional ablation, and (c) Type-3 fractional–rotational ablation.
and here, the fractional laser is rotated by 11.25° at each pulse for optimal elimination of the topmost layer. The ablation efficiency is quantified by the ablation depth and the mass removal. The ablation depth is measured from the cross-sectional images of pre-ablation skin, while the mass removal is quantified by the weight change of the sampled skin due to a pre-ablation procedure.

Meanwhile, the microjet dispersion pattern of the inner skin can be confirmed by using a tissue marking dye (blue color, Polysciences, Inc.). The injector liquid is mixed with dye and ejected in the form of a microjet. Here, the average jet velocity is about 130 m/s, which is enough to penetrate the toughest outermost layer of porcine skin. Then the stained skin is carefully sectioned with a razor blade. The cross-sectional view of a skin is imaged with a Nikon camera.

**RESULTS AND DISCUSSION**

**Pre-Ablation**

The benefits of a pre-ablation procedure prior to actual microjet injection to the penetration outcome is examined by comparing the depth of ablation via three types of pre-ablation and the depth of penetration via microjet injections. There are three types of pre-ablation schemes that are considered, namely bulk ablation, fractional ablation, and combined fractional–rotational ablation, all of which are commonly practiced by dermatologists. A typical cross-sectional view of a pre-ablated pig skin is shown in Figure 3 with 12, 18, and 24 laser pulses.

For bulk ablation, the ablated area is relatively large in comparison to the nozzle diameter. This suggests that there is no alignment issue associated with missing a
target, as high drug delivery efficiency is achievable for every single injection administered. However, recovery will be quite slow since the damaged area is extended for the bulk target. Using a different style, the outermost skin layer can be eliminated with minimal skin damage with the application of fractional ablation. Here, numerous micro-holes can efficiently assist drug transport, but they pose an alignment issue regarding hitting the ablated hole with the microjet from a nozzle. Nevertheless, healing time is minimized because full epidermal wounds are avoided by the generation of many micro-holes as opposed to bulk ablation of the target site. As for the third type of pre-ablation, fractional–rotational ablation incorporates the benefits of these two methods, which include both short healing time and a highly effective procedure. As the number of micro-holes in the unit area for fractional–rotational ablation is higher than for fractional ablation, the penetration depth for the fractional–rotational type is also shallower due to the rotational effect of the beam. This is a favorable condition for increasing the precision to align the impact position of the microjet as well as for ensuring fast recovery of the ablative wounds in comparison to the bulk type method described above.

The efficiency of each ablation type is evaluated using the ablation depth and removed mass with the same laser fluence of 8 J/cm². Ablation depths are shown in Figures 4 and 5. The result from a type-1 ablation (bulk) is the highest among the types shown in Figure 5 a and b. The total ablation depth is, however, proportional to the laser pulse repetition for all types. In particular, ablation depth per pulse decreases with an increasing number of repetitions because of thermal coagulation. Although the three ablation types showed different ablation efficiency, all three procedures are effective in the elimination of the top most layer of a porcine skin.

Another parameter that can be used as a measure of pre-ablating the skin is the total mass of removal as shown in Figure 5 a and b. The mass removed per pulse decreased with the increase in the number of repetitions, showing a similar trend as the ablation depth. Interestingly, the mass removed from type-3 (fraction-rotational) ablation is larger than type-1 and type-2, due to the effect of thermal

![Fig. 5. Ablation efficiency compared for each pre-ablation type by (a) total mass removed and (b) mass removed per pulse. The fluence is the same at 8 J/cm² for all cases.](image)

![Fig. 6. Cross sectional view of a single spot with indicated number of injections (or time) for blue dye: (a) 100 injections, (b) 300 injections, and (c) 500 injections.](image)
coagulation. The Er:YAG laser is an efficient tool for skin ablation due to its absorptivity in water. Repetitive laser pulses in the same spot accelerate dehydration of the skin, which results in the reduction of ablation efficiency. However, in type-3 (fractional–rotational) ablation, the targeted spot is naturally varied due to rotation and the thermal coagulation effect is minimized, which results in the most effective skin ablation among all the skin ablation types studied.

**Subsequent Injection Results**

The injection time is one key factor in drug delivery. In Figure 6 showing the microjet only result, the penetration depth of injection is illustrated by tracing the tissue marking dye in blue. It is clear that the depth becomes deeper with increasing injection time, and the amount of delivered doses also increases with the number of injections.

The cross sectional view of the porcine skin test in Figure 6 is further analyzed as shown in Figure 7. First, pre-ablation is responsible for enhancing the penetration depth. The penetration depth of bulk ablation (12 pulses) and microjet is increased by $12 \sim 14\%$ and that of bulk ablation (18 pulses) and microjet is increased by $11 \sim 42\%$ when compared to the microjet only case. These results indicate that the number of ablation pulses does not have a huge impact on penetration depth once the toughest outermost skin layer is removed.

A comparison of all possible combinations of microjet injection is given in Figure 8. The four combinations are (a) microjet only; (b) bulk ablation and microjet injection; (c) fractional ablation and microjet injection; and (d) fractional–rotational ablation and microjet injection. The dispersion of marking dye (blue) as well as some ablative surface patterns are visible in the figure. The quantification of the total penetration depth is shown in Figure 9. Here, total penetration depth includes both SC ablation

![Fig. 7. Penetration depth per injection time compared for microjet only and pre-ablation and microjet.](image)

![Fig. 8. Cross sectional view of a single spot with 300 injections and 18 pre-ablation pulses for (a) microjet only, (b) bulk ablation and microjet, (c) fractional ablation and microjet, and (d) fractional–rotational ablation and microjet.](image)
and microjet injection depths. Accordingly, the depth increases with injection time for all cases. In particular, the depth of penetration attained via fractional pre-ablation increased by 8~11% and that of fractional–rotational pre-ablation increased by 13~33%, when compared with the no pre-ablation or microjet only cases.

The penetration depth underneath ablated SC is also measured in order to verify the pre-ablation effect. The penetration depths for each case are (a) 443 ± 104 μm; (b) 625 ± 98 μm; (c) 523 ± 95 μm; and (d) 595 ± 141 μm for (a) microjet only; (b) bulk ablation and microjet; (c) fractional ablation and microjet; and (d) fractional–rotational ablation and microjet, respectively. A noticeable point is that the fraction-rotational pre-ablation and microjet result is comparable to the bulk ablation and microjet result. This is quite beneficial since any healing time associated with ablation is significantly reduced by avoiding hard-core bulk ablation.

**TABLE 2. Relation Between Density of Micro-Holes and Drug Delivery Efficiency**

<table>
<thead>
<tr>
<th>Ablation type</th>
<th>Density of micro-holes(number/mm²)</th>
<th>% Ratio of ablated holes to target area</th>
<th>Penetration depths(μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractional</td>
<td>1.27</td>
<td>3.97</td>
<td>581</td>
</tr>
<tr>
<td>Fractional–rotational</td>
<td>4.84</td>
<td>19.35</td>
<td>691</td>
</tr>
</tbody>
</table>

Fig. 9. Penetration depth per injection time compared for microjet only, bulk ablation and microjet, fractional ablation and microjet, and fractional–rotational ablation and microjet. For all pre-ablation types, 18 reps of laser pulses and 8 J/cm² are used.

Fig. 10. Cross sectional view of the multi-spots (10 spots) at 500 injections per spot and 18 pre-ablation pulses for (a) microjet only, (b) bulk ablation and microjet, (c) fractional ablation and microjet, and (d) fractional–rotational ablation and microjet.
As for the non-single spot test, 500 microjet injections are administered at each spot and a total of 10 spots are considered to verify the possibility of controlling the dosage. The between-the-spot distance is set at 200 μm. Here, higher density of micro-holes can enhance the chance of microjet hitting the holes. Shown in Figure 10, the depth of penetration is deeper for cases with a pre-ablation scheme. Presumably, the most effective combination, bulk pre-ablation and microjet, may well be superseded by the less invasive fractional–rotational ablation followed by the microjet injection. Fractional–rotational ablation and microjet injection is more efficient since number of micro-holes in a unit area is increased in comparison to fractional ablation and microjet case. Here, the density of micro-holes is 1.27 number/mm² for fractional ablation and 4.84 number/mm² for fractional–rotational ablation. Consequently, the penetration depths measured underneath the ablated SC are 581 μm (fractional ablation and microjet case) and 691 μm (fractional–rotational ablation and microjet case). Table 2 shows the increased chance of microjet hitting the ablated micro-holes from approximately 4% to 20%.

CONCLUSION

A two-stage procedure for laser-assisted microjet injection for transdermal drug delivery is described. The Er:YAG laser is selected as a beam source for both pre-ablation of the treatment site and subsequent microjet ejection as it has the highest absorption coefficient in water for its wavelength. Because type-3 pre-ablation increases the number of micro-holes in a unit area, which is related to penetration efficiency. Type-3 pre-ablation also has advantage of fast recovery due to minimized tissue damage near micro-holes. Furthermore, the present system has the potential for use in macromolecule delivery systems, including vaccines, growth hormones, and insulin in the near future.

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