Wound healing of 6.45-μm free electron laser skin incisions with heat-conducting templates

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1 Introduction

The benefits of using lasers for incisions include their ability to precisely ablate tissue and their unique hemostatic properties. Unfortunately, laser use has been limited by the thermal damage produced, which has been shown to delay and/or impair wound healing.1–9 The development of devices that reduce the wound healing delay after laser incisions is important for making laser incision surgery clinically practical. Various strategies, in addition to a multitude of studies using various wavelengths of light,10,11 have been tried to limit this delay in wound healing. They include computerized control of the beam delivery,7,12 reduction in the spot size,13 alterations in the pulse durations, repetition rate, or step size,14–18 and the utilization of heat-conducting templates or media.18–22

Our research focuses on the utilization of heat-conducting templates with the Vanderbilt University free electron laser (FEL).23 The FEL is an infrared laser tunable to wavelengths between 2 and 9 μm. The pulse structure consists of 1-ps micropulses separated by 350 ps. Approximately 12,000 to 17,000 micropulses make up the 4 to 6-μm macropulse that repeats at 30 Hz. Each macropulse delivers about 30 mJ of energy to the tissue. The FEL was utilized at 6.45 μm, the amide II protein absorption band,24 for maximal ablative effects.

Previous work with the FEL revealed that metal heat-conducting templates with a central aperture reduced the lateral thermal damage by conducting heat away from the plane of the incision.19 A hole or slot in the template is also important to let steam and hot gases escape from the incision site. The use of a solid window that is transparent to the laser light would result in increased thermal damage by trapping the hot gases below the window and in the tissue. The greatest reduction in superficial lateral thermal damage occurred as the aperture of the metal templates approximated the FEL’s focused beam diameter (about 600 μm).18 The initial experiments described in this paper examined the effects of a transparent sapphire heat-conducting template on the lateral thermal damage of FEL incisions in in vitro human skin samples. These experiments were conducted because the future use of heat-conducting templates by surgeons will likely require the devices to be transparent to allow the incisions to be seen.

The remainder of this study investigated the effects that heat-conducting templates have on wound healing of rat skin. Our experience with heat-conducting templates and the FEL has shown their ability to reduce lateral thermal damage,
which we hypothesize will lead to enhanced wound healing. To test this hypothesis, we compared wound healing after incisions made with the FEL alone and incisions made utilizing metal templates. In addition, we compared wound healing after incisions were made with the FEL alone and with a transparent sapphire template or a scalpel.

2 Materials and Methods

2.1 Laser

The free electron laser at Vanderbilt University was utilized at a wavelength of 6.45 μm and 11.5 ± 1.55 J/cm² fluence. The laser was operated at 30 Hz with 4 to 6-μs macropulses. The FEL beam was focused with a 400-mm-focal length BaF₂ lens to a 636-μm ± 100-μm beam diameter. The computer-assisted surgical techniques (CAST) device was programmed to precisely control the delivery of the beam.7,12 When using the metal templates, the CAST was programmed to control the FEL to make 2-cm incisions as a series of spot ablations with a step size (the distance between successive spots) of 60 μm. When the sapphire template was used, the CAST was programmed to control the FEL to make 1-cm incisions as a series of spot ablations with a step size of 1.25 mm. For both setups, the CAST introduced an offset to prevent irradiating the same spots on consecutive passes, thereby ensuring the generation of a complete incision after multiple passes.

2.2 Heat-Conducting Templates

A copper and an aluminum template, each with a central slit width of 700 μm, were utilized as metal heat-conducting templates. A sapphire template with a central slit width of 700 μm was utilized as a transparent heat-conducting template. The templates were manually placed on the skin with the slit parallel to the beam’s linear incision path.

2.3 Sapphire Template and Acute Lateral Thermal Damage

Human skin samples were obtained with the approval of Vanderbilt University’s institutional review board as excess tissue from reduction mammoplasties or abdominoplasties. The tissue was stored in saline-moistened gauze, wrapped in a double layer of aluminum foil, sealed in a ziplock bag, frozen at −20 °C, and utilized within three months. A scalpel was used to excise 1.5 × 1.5-cm² individual specimens for the experiments. The human skin samples were placed on a solid glass slide and thawed for 1 h. The samples and the sapphire heat-conducting template were at room temperature (23 °C) during the time the linear incisions were made. Incisions were made with and without the sapphire heat-conducting template. Each incision took approximately 47 s.

Tissue samples were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with Gomori’s trichrome stain for light microscopic evaluation (Zeiss Axiosplan 2 Microscope, Carl Zeiss Inc., Thornwood, New York). Histological slides were photographed with digital microscopy (digital video camera, model ZVS-3C75DE, Carl Zeiss Inc.) and analyzed for depth and lateral thermal damage using computerized morphometric analysis (Zeiss ImagePro v. 3.0, Media Cybernetics, Silver Spring, Maryland). The zone of thermal damage, as indicated by tincture changes in the Gomori’s trichrome-stained tissue, was outlined. Lateral thermal damage was defined as the average thickness from this border to the wound edge. The average lateral thermal damage was determined over the superficial 150 μm of the dermis and over the entire depth of the incision. Values were averaged for each incision type and compared using a Student’s t-test (Statview, Abacus Concepts, Berkeley, California).

2.4 Heat-Conducting Templates and Wound Healing

Vanderbilt University’s animal care committee and animal use subcommittee approved all of the animal studies. Thirty-three Sprague-Dawley rats, initially weighing 250 g, were divided into two groups: A (21 rats) and B (12 rats). Rats in group A had FEL surgery utilizing metal heat-conducting templates. They received six longitudinal incisions on their dorsal pelt: two incisions with the FEL alone, two incisions with the copper template in place, and two incisions with the aluminum template in place. Surgery on the rats in group B utilized a sapphire heat-conducting template. They also received six longitudinal incisions on their dorsal pelt: two incisions with the FEL alone, two incisions with the sapphire template in place, and two incisions with a scalpel. Figure 1 illustrates the experimental setup of the laser incisions made utilizing a heat-conducting template. Full-thickness incisions required between 80 and 220 s of exposure to the FEL.

Since the beam was scanned in a straight line using the CAST, it was relatively easy to align the aiming laser beam coincident with the template slot for surgery on a small mammal such as the rats used in these studies. For ideal clinical surgical use, we anticipate fixing the template to the arm delivering the laser beam, thus permanently establishing correct alignment.

The rats were divided into three survival groups: 7, 14, or 21 days. An equal number of rats were in each group. Each rat was anesthetized with intramuscular ketamine (90 mg/kg) and xylazine (10 mg/kg). The dorsal pelt was shaved with an electric razor and cleaned with Novlasan solution (Wyeth, Madison, New Jersey). The rats were then placed into the operating field, where two small transverse scalp incisions were made just below the neckline, each one 2 cm lateral to the midline. A six-inch scalpel guide (Pilling Weck, Fort Washington, Pennsylvania) was placed in each incision and inserted into the fascial plane between the skin and muscle layers to prevent laser ablation of the underlying muscle layer. One milliliter of 1:00,000 epinephrine was given subcutaneously along
the track of each scalpel guide for improved hemostasis. Three full-thickness longitudinal skin incisions were made above each scalpel guide, yielding a total of six incisions per animal. The location (superior, middle, or inferior) on the animal’s dorsal surface for the various incision types was rotated to eliminate anatomical position bias. After the incisions were completed, the scalpel guides were removed and the wounds cleaned with sterile saline. Each incision was closed primarily with three (group B) or five (group A) interrupted 4-0 monofilament sutures (Ethilon; Ethicon, Somerville, New Jersey). Antibiotic ointment (polymyxin B sulfate, bacitracin zinc, neomycin sulfate; Altaire Pharmaceuticals, Inc. Holbrook, New York) was applied to the wounds postoperatively. The animals were housed in individual cages with food and water until their designated sacrifice time.

The animals were sacrificed at 7, 14, or 21 days using a CO₂ chamber and wound tissue was collected. Each wound was transversely cut into two separate samples. The first sample was tested for tensile strength with an Instron 5542 tensiometer (Instron, Canton, Massachusetts). The tensile strength (kgf/cm²) was calculated from the peak breaking force assuming a constant skin thickness.

The second part of the sample (a 2–3-mm strip of skin) was fixed in 10% formalin, embedded in paraffin, sectioned, and stained with either hematoxylin and eosin or Gomori’s trichrome stain for light microscopic evaluation. Qualitative histological grading of wound healing was performed in a blinded fashion by one investigator. The slides from each time point (days 7, 14, and 21) were evaluated in separate groups. All the slides were evaluated for the following categories in their respective groups using a scale of 1 through 4 where 1 is the least amount of optimal change for that category and 4 is the most amount for that category: reepithelialization, granulation tissue, and collagen formation. In addition, slides from the day 7 group were evaluated for acute inflammation, with 1 being the most and 4 the least amount of acute inflammation present. The final histological score was designated as the sum of the scores on all evaluated categories for that individual slide.

To help assign a score of 1 to 4, the slides were viewed for one characteristic (e.g., reepithelialization) and the best slides were put together in a group. The worst slides were put into another group. The remaining slides were placed into two groups if they were slightly better than average or slightly worse than average. The slides were then assigned numbers of 1 to 4 from the worst to the best. A score of 4 represents optimal wound healing.

The statistical significance was calculated using Statview (Abacus Concepts, Berkeley, California). When two groups were compared, an unpaired two-tailed Student’s t-test was used. When the data were normalized to the laser incisions made without a template, a single-group, two-tailed Student’s t-test was used. The mean value was compared with a normal value of 1.0. A p value of less than 0.05 was considered a significant change. Means are expressed ± the standard error of the mean (SEM).

### 3 Results

#### 3.1 Sapphire Template Acute Lateral Thermal Damage

Microscopic examination of the FEL incisions, both with and without a heat-conducting sapphire template, revealed a narrow neck of lateral thermal damage over the superficial 150 μm of dermis, increasing to wider lateral thermal damage at the deeper aspects of the incisions. This was consistent with our previous work with the Vanderbilt FEL and a stainless steel heat-conducting template. The mean incision depth with the heat-conducting sapphire template was significantly less than that seen without the use of this template (13 ± 103 μm versus 1919±70 μm, p = 0.0001). The lateral thermal damage over the superficial 150 μm of dermis with the heat-conducting sapphire template was significantly less than that seen without the use of this template (13.1±0.50 μm versus 17.0±0.56 μm, p = 0.0001). Thus the incision depth as well as the lateral thermal damage over the superficial 150 μm of dermis and over the entire depth of the incision was significantly reduced when the transparent sapphire heat-conducting template was used. Figure 2 shows representative micrographs illustrating the reduction in lateral thermal damage with the use of the sapphire template.

#### 3.2 Heat-Conducting Templates and Wound Healing

As indicated in Sec. 2.4, there were slight differences in the treatment of rats in groups A and B. To make certain that both groups were similar and could be compared, we first examined the laser incisions made without any templates. If the two groups are the same, these incisions should heal similarly. Figure 3(I) shows a comparison of the mean histology scores at days 7, 14, and 21 for both groups. The differences are not statistically significant. The p values for the differences are 7 days, p = 0.07; 14 days, p = 0.06; and 21 days, p = 0.89. In Fig. 3(II) we show a comparison of the mean tensiometry scores for the laser incisions made without templates for both groups. The differences are slight and not statistically significant. The p values are: 7 days, p = 0.64; 14 days, p = 0.91; and 21 days, p = 0.09.

After demonstrating that the laser incisions made without a template were the same between groups, we used these values in a paired-data analysis to reduce animal-to-animal variability in the subsequent analysis. On each animal, the measured tensile strengths and the histological score were ratioed to the average value of the no-template laser incisions for that animal.

Figure 4 plots the mean ratio for the histology score and mean ratio for the tensile strength from the incisions made with the sapphire, aluminum, and copper templates and the scalpel on days 7, 14, and 21. The major differences are seen at day 7. Slightly less difference is seen at day 14, and by day 21 all the incisions are similar. The values cluster near 1.0 on day 21, meaning all the wounds looked similar to the laser incisions made without using a template. The differences seen in Fig. 4 are not statistically significant.
Fig. 2 Representative histology of in vitro human skin showing the lateral thermal damage after FEL incisions. The arrows mark the zone of lateral thermal damage. (a) FEL incision without utilizing a heat-conducting template. (b) FEL incision utilizing a sapphire template. Gomori’s trichrome stain. Original magnification 100×. Scale bar=150 μm.

Fig. 6 Representative histology of rat dermis showing the state of wound healing 7 days after the various incision methods. (a) FEL incision with a 60-μm step size and without utilizing a heat-conducting template. (b) FEL incision using a sapphire template. (c) Scalpel incision. (d) FEL incision with a 1.25-mm step size and without a heat-conducting template. (e) FEL incision with an aluminum template. (f) FEL incision with a copper template. Gomori’s trichrome stain. Original magnification 40×. Scale bar=300 μm.
We combined the data from days 7 and 14 and plotted the mean values for each of the templates and the scalpel in Fig. 5. All of these values are statistically significant, except the aluminum template with the histological evaluation. The p values are shown in Table 1. Figure 6 shows representative micrographs of wound healing 7 days after laser incisions without templates, laser incisions with templates, and scalpel incisions.

4 Discussion

Our previous studies using \textit{in vitro} human skin indicated that metal templates reduce the lateral thermal damage of laser incision.\cite{18-21} However, these same studies showed that glass templates do not have sufficient thermal conductivity to be effective. Because it is clinically important for the surgeon to be able to view the laser–tissue interaction, we designed a template made of sapphire. The sapphire window is a crystal and would have a better thermal conductivity than glass. When the sapphire template was tested using \textit{in vitro} human skin, we did observe reduced thermal damage, and it was slightly less than what we observed with a copper or aluminum template. This reduction in thermal damage provided

![Fig. 3](image-url)  
**Fig. 3** Comparison of groups A and B laser incisions on the rat model made without any heat-conducting templates. Part I shows a comparison of mean histology scores at days 7, 14, and 21. Part II shows a comparison of mean tensiometry (N/mm²). Error bars show the standard error of the mean.

![Fig. 4](image-url)  
**Fig. 4** Comparison of the mean normalized histology scores (part I) and mean normalized tensiometry strength (part II) for laser incisions made on rats with sapphire, aluminum, and copper templates and scalpel incisions at days 7, 14, and 21. The histology score and the tensile strength for each animal were normalized by ratioing to the average values of no-template laser incisions for that animal. Error bars show standard error of the mean.

![Fig. 5](image-url)  
**Fig. 5** Comparison of the combined 7- and 14-day normalized histology scores and tensiometry strength of incisions made on the rat model. Error bars show standard error of the mean. An asterisk indicates values significantly different from the normal value of 1.0 (no-template laser incisions).
grounds for us to use the sapphire template in the second part of this study—the wound-healing studies with the rat model.

Two possible modifications could be made to obtain a further reduction in thermal damage with an optically transparent template. The sapphire could be cooled to increase its thermal conductivity. This would also provide a local anesthetic effect for the patient. Alternatively, one could use a synthetic diamond window as a template. Diamond has a thermal conductivity greater than copper, making it an ideal although more expensive template. The diamond could also be cooled for local anesthesia.

Our studies with FEL incisions demonstrate that heat-conducting templates improve both the tensile strength and histological maturation of wound healing 7 and 14 days after incisions. The increased tensile strength ratio was statistically significant with the combined data for days 7 and 14 with all of the templates. The improved histological maturation was statistically significant with the combined data for days 7 and 14 for all of the templates except the aluminum template. These results suggest that the heat-conducting templates minimize the delay in early wound healing. Figure 6 illustrates the differences in histological scoring. Here, with no template, delayed wound healing, with a 25 to 50% reduction in histological score [Fig. 6(a)] compared with the sapphire template [Fig. 6(b)] and the scalpel [Fig. 6(c)] is evident. Lesser but similar effects are seen with the larger step size (1.25 mm) with no template [Fig. 6(d)] compared with the aluminum template [Fig. 6(e)] and the copper template [Fig. 6(f)]. Minimizing this early delay may be clinically important because sutures usually are removed during the first week or early in the second week. Any early impairment of wound healing may lead to problems such as dehiscence or scar enlargement.

Although differences among the templates and the scalpel could be seen in Fig. 5, there are not enough data to find any statistical differences among these groups. Consistent with previous studies, scalpel incisions are superior, with statistically significant increased tensile strength and histological maturation compared with laser incisions made without the use of any template.1-5 With utilization of heat-conducting templates, wound healing after laser incisions more closely approximates scalpel incisions. The sapphire template approximated the metal templates in efficacy, but was slightly less effective than the scalpel incisions.

The use of heat-conducting templates with laser incisions did not significantly affect our wound-healing parameters at 21 days postoperatively. There may be no differences at the later time points, or our techniques may be unable to detect small differences. Whether the long-term results of wound healing are different in humans for laser surgery with heat-conducting templates needs to be studied.

Wound healing is a complex process and is influenced by many factors. In these experiments, we used the FEL, at what many people consider the optimal wavelength for incisions, creating minimal thermal and physical damage to the surrounding tissue. Thus, it is difficult to improve upon this optimal wavelength. In addition, we used rats as our wound-healing model. Again, this model is probably close to optimal for wound healing. It is difficult to create wounds that heal at different rates with this model. Thus, even though the templates demonstrate a statistically significant reduction in the lateral thermal damage, it would not be expected to translate into major changes in the wound healing of the rats.

We did observe some changes in the wound healing rates in Fig. 4, especially for the earlier healing times. However, these changes were small and not statistically significant. Combining the data from days 7 and 14 resulted in a large enough number of samples to measure statistically significant differences. Although the observed changes are small, a small difference in wound healing made with the best laser on a healthy and robust model could translate into large differences in wound healing when conventional lasers are used on older patients with underlying disease, such as diabetes.

Although these studies have been performed with the FEL, the results found here should translate to many other lasers, including those in common clinical use. Using the FEL allows us to select wavelengths that are preferentially absorbed by proteins or water. Thus, we can test if the laser target is important to the template. Thus far, we have not observed a strong wavelength effect.19,20 Therefore, this technology should be applicable to many other lasers, such as carbon dioxide or neodymium-doped yttrium aluminum garnet (Nd:YAG) lasers.

In conclusion, both metal (copper and aluminum) and transparent sapphire heat-conducting templates improve wound healing after FEL incisions. In this study, their benefit is evident 7 and 14 days postoperatively. Minimizing the wound healing delay seen after laser incisions may make laser incisions a more appealing option. This may be especially important in preventing dehiscence in diabetics, older patients, and sites under increased tension. Further studies are needed to more clearly characterize the effects of heat-conducting devices on lateral thermal damage and wound healing after laser incisions in animal and human skin. The ideal heat-conducting device, which would be transparent to the surgeon and completely eliminate the laser incision-induced delay in wound healing, should increase the utility of lasers for conventional surgery.

Acknowledgments

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References

3. B. R. Buell and D. E. Schuller, “Comparison of tensile strength in

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<th>p-Values for normalized histology and tensiometry data when days 7 and 14 are combined (data plotted in Fig. 5).</th>
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