Closure of skin incisions by laser-welding with a combination of two near-infrared diode lasers: preliminary study for determination of optimal parameters

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Abstract. Laser welding has the potential to become an effective method for wound closure and healing without sutures. Closure of skin incisions by laser welding with a combination of two near-infrared lasers (980 and 1064 nm), was performed for the first time in this study. One centimeter long, full-thickness incisions were made on the Wistar rat's dorsal skin. The efficiencies of laser-welding with different parameters were investigated. Incision-healing, histology examination, and a tensile strength test of incisions were recorded. Laser welding with the irradiance level of 15.9 W/cm² for both 980 and 1064-nm lasers and exposure time of 5 s per spot in continuous wave mode yielded a more effective closure and healing with minimal thermal damage, faster recovery, and stronger apposition in comparison with a suturing technique. The conclusion is that skin welding with a combination of two near-infrared diode lasers can be a good candidate for incision closure, and further investigations are in progress for clinical use. © 2011 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.3552648]

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1 Introduction

As a potential alternative technique for closure of tissue-incisions, laser welding has shown promises in clinical applications.^{1–3} The major advantages of the laser tissue welding are immediate water-tight closure of wounds, less scar formation, and no foreign-body reactions against the suture materials. The success of laser tissue welding depends on several factors, such as the wavelength of laser, optical and thermal properties of tissues, spot size, laser power, exposure time, pulse duration and repetition rate.^{4,5}

Infrared lasers have been used for tissue welding for decades because of their distinct capability to bring about the thermal apposition of biological tissues.^{6–9} CO₂ and Ho:yttrium-aluminum-garnet (YAG) lasers are absorbed within 2 to 20 μ m at the tissue surface and may cause unwanted thermal damage at long exposure times as well as high-power applications. For these reasons, these infrared lasers can only be used for extremely thin tissues. Diode lasers (800 to 830 nm) have been preferred for tissue soldering with nanoshells or indo-cyanine green (ICG) as an exogenous absorber, due to the good local-

ization of the thermal effect and enhancement of the low fusion strength.^{10–12} Nd:YAG laser was chosen for skin welding to achieve the deep heating at the weld site, which is necessary to produce strong, full-thickness skin welds.¹³ The skinwelding ability of a 980-nm diode laser was investigated because of its relatively higher absorption by water than other near infrared lasers, and good closure ability was reported in the early days of the healing period.^{14–16} In all of these previous works, however, a good deal of attention was paid to the skinwelding ability of a single laser, and little was paid to skin welding with more than one laser, as far as we know.

The pros and cons of skinwelding with near-infrared lasers were investigated by Tabakoglu and Gulsoy in their recent work.¹⁶ Their results showed that 980-nm diode laser was suitable for superficial welding, while a 1070-nm laser was suitable for deep welding because of its relatively deeper optical penetration. Since the tissue optical properties of a 1064-nm laser are similar to those of a 1070 nm laser, we expect to achieve more effective welding with full-thickness closure and stronger tissue-bonding with 980 and 1064-nm diode lasers simultaneously. In this study, the efficiency of skin welding with the combination of both near-infrared lasers was investigated and was compared with a suturing technique to determine the optimal parameters for further study.

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2 Experiment Design and Methods

2.1 Laser Systems

A 980-nm diode laser with a maximum output power of 2 W and a 1064-nm medical laser system with a maximum output power of 30 W were designed and manufactured by our group in the Laboratory of Excited State Processes, Changchun Institute of Optics, Fine Mechanics and Physics. Both laser beams were delivered with 200- μ m optical fibers. The parameters of the two lasers, such as power (0 to 2 W for 980 nm laser and 0 to 30 W for a 1064 nm laser), exposure time (0 to arbitrary-long, step 0.1 s), on–off duration of pulses (10 ms to 10 s, step 1 ms), etc., could be adjusted easily.

2.2 Animal Preparation

A total of 58 male Wistar rats from the School of Basic Medical Sciences, Jilin University, 270 to 320 g weight, were used in the experiments. They were housed in plastic cages and maintained on a 12 h-light/12 h-dark cycle in a temperature-controlled culture center ($25 \pm 2^\circ\text{C}$). Food and water were available. All studies were conducted under a directive suggestion of caring for laboratory animals approved by the Ministry of Science and Technology of China. Rats were anesthetized with ketamine (2 ml: 0.1 g, Hengrui Pharma, Jiangsu, China) by intraperitoneal injection (1.50 ml/kg). The hair at the site of laser application was shaved. One centimeter long, full-thickness incisions (over muscular layer) bilateral and parallel to the spine were made on the back of each rat with a no. 11 surgical blade. Iodophors (0.58% Iodophors, Manufacturing Laboratory of the First Hospital of Jilin University, Changchun, China) was applied topically before each operation to prevent infection and desiccation of the wound.

2.3 Laser Welding and Suture

Laser welding was performed as spots on the incisions, and the output power of each laser was calibrated using a power meter before each application. The tips of both optical fibers were located in parallel approximately 4.5 mm above and both laser spots overlapped on the incision during laser skin welding. The spot-size of each laser beam was 2.0 mm in diameter. The laser parameters selected here refer to previous literatures.^{13,15,16}

First, 18 rats were divided equally into six groups, and each rat had three pairs of incisions on the dorsal side parallel with the spine. Then we welded skin incisions with combined lasers in different irradiation doses and combination-modes. 980 and 1064-nm diode lasers were used simultaneously (Table 1).

Second, 40 rats were divided equally into two groups for comparison of the performance between laser welding and the suturing technique. For welding, six wounds on the dorsal side of each rat were welded using 980 and 1064-nm diode lasers simultaneously with parameters decided according to the results of the first experiment. For suturing, a single suture usp-3/0 metric silk was placed in the middle of each incision in order to prevent additional needle trauma. Piperacillin (Shijiazhuang Pharmaceuticals Company, Shijiazhuang, China) was applied topically to the wounds immediately after either laser weld or

Table 1 Laser parameters applied in the first experiment.

Group	Wavelength (nm)	Power density (W/cm ²)	Exposure time/spot ^a (s)	Total energy/spot ^b (J)
1	980	15.9	3	3.0
	1064	15.9	3	
2	980	15.9	5	5.0
	1064	15.9	5	
3	980	15.9	5	7.5
	1064	79.6	2	
4	980	15.9	5	10.0
	1064	79.6	3	
5	980	15.9	5	5.0
	1064	79.6	1	
6	980	28.7	3	5.0
	1064	73.2	1	

^aExposure time/spot means the irradiation times of both 980 and 1064-nm diode lasers on each irradiation spot.

^bTotal energy/spot means the total energy of combination of 980 and 1064-nm diode lasers on each irradiation spot.

suture in order to inhibit any kind of microbial reactions on the wounds.

2.4 Postsurgery Examinations

Postsurgery, rupture, infection, and scar-size of the incisions were recorded everyday. Histological examinations and skin tensile strength measurements for the incisions were performed on the 1st, 4th, 7th, 14th and 21st postoperative day. For histological examinations, 20 rats (10 from each group: laser welding and suture) were anesthetized with ketamine, and 1 cm $\times$ 1 cm pieces of skin containing the incision in the center were excised with a scalpel. The samples, including epidermis and dermis, were kept in cassettes containing a 10% formalin solution. Then, histological examinations were performed as described in previous works.^{14–16}

For tensile strength measurements, 20 rats (10 from each group: laser welding and suture) were anesthetized with ketamine, and 1 cm $\times$ 3 cm strips with the incisions in the center were cut off. Then, the measurements were performed immediately (within 10 min after the cut) in order to minimize drying of the tissues. One side of the skin sample was fixed, the other side was pulled steadily (vertical to the incision) with a tensiometer. At the moment when the incision ruptured, the reading of tensiometer in Newton was recorded as the breaking strength.

3 Results

3.1 Results of the First Experiment

The appearance of the incisions, which were welded with different irradiation doses, on the postoperative control days are

Fig. 1 Photographs of incisions welded with different laser parameters during the recovery period. (a) Comparison of closure and healing among incisions welded with different irradiation doses. No immediate closure was observed on incisions of the 1st group, and obvious epidermis necrosis appeared on incisions of the 3rd and 4th groups and then scab formation was observed on the 7th day. On the 14th day, visible scars could be found in the 3rd and 4th groups, whereas flat incision sites were presented in the 2nd group. (b) Comparison of healing processes among incisions welded with the same irradiation dose (5 J/spot) but with different combination modes of lasers. No or negligible necrosis and faster recovery was observed on incisions of the 2nd group in comparison with those of the 5th and 6th groups during the early healing-period. All incisions recovered completely and no significant difference could be found macroscopically after 14 days' healing. Thin arrows indicate the end points of the incision.

shown in Fig. 1(a). On the 0th day, all wounds were closed immediately except those welded with a total energy of 3 J per spot (1st group). On the 4th day, obvious epidermis necrosis was observed around incisions of the 3rd and 4th groups, and no necrosis was observed in the 2nd group which was welded with a total energy of 5 J per spot. On the 7th day, necrosis and gross scabs were still present on wounds of the 3rd and 4th groups but none in the 2nd group. The incisions of the 2nd group were seen as clear thin lines. On the 14th day, incisions of all groups were recovered without any scabs or necrosis. Visible scars could be observed in the 3rd and 4th groups, whereas flat incision-sites were presented in the 2nd group. As a result, a total energy of 5 J per spot was considered as a more appropriate irradiation dose for laser welding with 980 and 1064-nm diode lasers simultaneously.

At the same time, we investigated the influence of a different combination mode on the incision healing processes of the 2nd, 5th, and 6th groups, which were welded with the same irradiation doses but with different combination modes of lasers [Fig. 1(b)]. All wounds presented immediate water-tight closure after irradiation, whereas necrosis on the wound edge leading to scab formation could be observed in some samples of the 5th and 6th groups. On the 7th postoperative day, incisions of the 2nd group presented good recovery with fine linear scars, whereas some scabs still could be observed in incisions of the 5th and 6th groups. On the 14th day, all incisions recovered completely. In the 2nd group, especially, incisions scarcely discriminated with around natural skin. So we determined that skin welding with 980 and 1064-nm lasers simultaneously in continuous wave mode with irradiance of 15.92 W/cm^2 and exposure time of

5 s per spot yielded the most effective closure and healing with minimal thermal damage.

3.2 Results of the Second Experiment

3.2.1 Macroscopic examinations

An efficacy comparison between laser welding (15.9 W/cm^2 for 5 s per spot for both 980 and 1064-nm lasers) and suturing technique was carried out, and the results of macroscopic examinations are shown in Fig. 2.

It is shown in Fig. 2 that laser-welded incisions closed immediately without necrosis. Some mild scabs were observed in some samples on the 4th day. Most of the scabs sloughed on the 7th day, and the appearance of incisions were fine lines. However, the sutured incisions remained open 24 h after surgery. Until the 4th postsurgery day, most of the sutured incisions were closed, but uneven and irregular incision sites were observed. Infections and foreign body reactions can be observed in some sutured incisions although we have taken out the stitches. A scar line perpendicular to the incision site caused by the entrance of the needle and silk thread was detected on the 14th day. From the 21st postoperative day on, all the skin incisions were closed and recovered successfully, and no significant difference can be identified macroscopically.

3.2.2 Microscopic examinations

Skin samples were excised for histological examination on the 1st, 4th, 7th, 14th, and 21st postoperative control days (Fig. 3). Epidermal thickness (Fig. 4) and granulation area (Fig. 5) are considered as semiquantitative indicators for incision healing.

On the 1st postoperative day, welded incisions appeared tight closure on the surface of skin, but thermal denaturation could not be seen in the deep dermis, where laser heating was minimal, and full-thickness welds were not achieved. Negligible thermal damage was observed in the laser welded skin samples [Fig. 3(a)]. On the other hand, no closure was observed in the sutured incisions, and neutrophil leukocyte accumulation was presented at the wound site [Fig. 3(b)].

Fig. 2 Photographs of incisions closed either by laser-welding or suture during the recovery period. On the 0th day, sutured incisions remained open. Until the 4th postsurgery day, most of the sutured incisions were closed, but uneven and irregular incision-sites were observed. On the 7th day, tissue reaction was noted in some sutured incisions, and scar line perpendicular to the incision site was observed on the 14th day. On the other hand, mild scab formation was noted on the 4th day on some laser-welded incisions. Thin arrows indicate the end points of incisions.

Fig. 3 Microscopic observations of the incisions closed either by laser-welding or suture on postoperative control days. No-closure of the sutured incisions on the 1st and 4th days was observed more clearly on micro-inspection. The incisions are marked on the photographs with a face-to-face thin arrow during the healing period. The course of healing after the 14th postoperative day seemed to be similar in both groups. H&E, $\times 40$ for all wound samples, and the scale bar was $100\ \mu\text{m}$.

On the 4th postoperative day, for the welded wounds, the re-epithelization process was in its early stages as the epidermis began to bridge under the necrotic tissue, and inflammation with neutrophil infiltration appeared at the wound site [Fig. 3(c)]. Tight closure was observed in the epidermal layer and almost full-thickness was achieved. Necrotic detachment was observed between the stratum germinativum and the papillary dermis in some samples. The average epidermal thickness was $54.5 \pm 9.66\ \mu\text{m}$. On the other hand, the sutured incision sites were closed only at the epidermal layer and papillary dermis, and remained open at the deeper part [Fig. 3(d)]. Epidermal regeneration and migration of the epidermis beneath the clot were observed, and the average epidermal thickness of the sutured wounds was $112.13 \pm 18.87\ \mu\text{m}$, which was statistically thicker compared with the laser welded wounds ($p < 0.05$) [Fig. 4].

On the 7th postoperative day, re-epithelization was completed and full thickness closure was recorded for all incisions [Fig. 3(e) and Fig. 3(f)]. No significant difference between the laser welded and sutured wounds was observed in terms

of epidermal thickness (98.47 ± 14.3 and $86.88 \pm 20.34\ \mu\text{m}$, respectively, $p > 0.1$) [Fig. 4]. For the laser weld wounds, a complete epithelial bridge was formed, and noticeable fibroblast cells and collagen formation were observed in the zone nearby epidermis, and no inflammation existed. For the sutured wounds, newly deposited collagen could be found within a thin layer of granulation tissue that extended full-thickness through the skin, and inflammatory cells were no longer observed around the wound site. The granulation area of the laser-welded wounds ($28.1 \times 10^4 \pm 7.2 \times 10^4\ \mu\text{m}^2$) was statistically greater than that of the sutured wounds ($19.8 \times 10^4 \pm 7.0 \times 10^4\ \mu\text{m}^2$, $p < 0.05$) [Fig. 5].

On the 14th postoperative day, a complete and continuous layer of epidermis had formed across the surface of the laser-welded wound site, and neo-epidermis had sloughed all the necrotic tissue above it. Epidermal hypertrophy began to decrease, and the average epidermal thickness decreased to $48.19 \pm 9.04\ \mu\text{m}$. Wounds narrowed to a fine scar in the papillary and reticular dermis [Fig. 3(g)]. For the sutured wounds,

Fig. 4 Epidermal thickness measurements for incisions closed either by laser-welding or suture. Epidermal thickness of the laser welded incisions increased until the 7th day and then started to decrease to a normal value. However, epidermal thickness of the sutured incisions started to decrease on the 4th day. On the 4th and 14th days, the ET of the laser welded incisions was thinner (*) ($p < 0.05$). Bars (means + SD, $n = 8$).

Fig. 5 Granulation areas for incisions closed either by laser-welding or suture. On the 7th and 14th days, the granulation areas of the laser welded incisions were significant greater than those of the sutured incisions ($p < 0.05$). By day 21, there was no significant difference. Bars (means + SD, $n=8$).

the epidermal hypertrophy also began to decrease. The average epidermal thickness decreased to $61.75 \pm 6.78 \mu\text{m}$, which was statistically thicker than that of the welded wounds ($p < 0.05$) [Fig. 4]. Tissue volume was partially replaced with granulation tissue in wounds closed either by laser-welding or suture, and the granulation area of the laser-welded wounds ($37.6 \times 10^4 \pm 7.1 \times 10^4 \mu\text{m}^2$) was statistically greater than that of the sutured wounds ($25.7 \times 10^4 \pm 5.9 \times 10^4 \mu\text{m}^2$, $p < 0.05$) [Fig. 5].

From the 21st day on, no difference was observed between both groups. A full-thickness layer of granulation tissue was observed, and the epidermal thickness decreased to normal levels, around 30 to 40 μm , for all wounds. The visibility of the scar tissue had decreased, and there was no statistical difference between the laser welded wounds and sutured wounds in terms of granulation area ($26.4 \times 10^4 \pm 6.7 \times 10^4 \mu\text{m}^2$ and $22.5 \times 10^4 \pm 7.0 \times 10^4 \mu\text{m}^2$, respectively, $p > 0.5$) [Fig. 5].

3.2.3 Tensile-strength measurement

Tensile strengths were measured for both laser-welded and sutured wounds on the 1st, 4th, 7th, 14th and 21st postoperative control day [Fig. 6]. On the 1st postoperative day, the average tensile strength of the laser-welded incisions was $5.21 \pm 1.02 \text{ N}$, which was found to be even greater than the strength of the sutured incisions on the 7th day. No measurement was performed for sutured incisions on the 1st day due to the non-closure of the incisions. On the 4th postoperative day, the average tensile strength of laser-welded wounds was $6.45 \pm 1.10 \text{ N}$, which was significantly greater than that of the sutured wounds ($1.65 \pm 0.31 \text{ N}$, $p < 0.05$). On the 7th day, tensile strengths of the laser-welded and sutured incisions were $7.52 \pm 1.24 \text{ N}$ and $3.04 \pm 0.55 \text{ N}$, respectively, and a statistical difference was observed ($p < 0.05$). On the 14th day, there was no statistical difference between laser-welded and sutured incisions in terms of the tensile strength. At the end of the recovery period, the 21st postoperative day, the welded incisions were found to be much stronger than those of the suture group ($13.54 \pm 2.42 \text{ N}$ and $9.15 \pm 1.92 \text{ N}$ respectively, $p < 0.05$).

Fig. 6 Tensile strengths of welded and sutured rat skin samples during the healing period. No measurement was performed for sutured incisions on the 1st day. On both the 4th and 7th days, tensile strengths of the laser-welded incisions were greater than those of the sutured incisions (*) ($p < 0.05$). On the 21st day, laser-welded incisions were much stronger than the sutured incisions (*) ($p < 0.05$). Bars (means + SD, $n=8$).

4 Discussion

Laser tissue welding uses laser energy to activate photothermal bonds between tissue surfaces to fuse them together. The major disadvantage is thermal damage to the surrounding tissue which should be minimized with the correct selection of laser wavelength, operation mode, and irradiation dose.^{17,18} In the present study, aiming to achieve a full-thickness closure of incision, the ability and efficiency of skin welding with a combination of two near-infrared lasers (980 and 1064-nm lasers) was investigated using rat skin models, which have been widely used to simulate human conditions,^{14-16,18} and compared with the suturing technique.

As everyone knows, in the nearinfrared spectral region, the main chromophores are the water of the skin dermis and the lipids of epidermis. The absorption coefficient of skin at 980-nm reaches 0.35 cm^{-1} due to its relatively higher absorption by water ($\text{H}_2\text{O}-\mu_a = 0.43 \text{ cm}^{-1}$) and is approximately 0.15 cm^{-1} at 1064-nm as well as at 1070-nm.¹⁹ Therefore, a 1064-nm diode laser (similar to 1070-nm fiber laser) is expected to be suitable for deep welding too, as described by Tabakoglu and M. Gulsoy.¹⁶ Thus it is expected that more efficient welding (both superficial and deep closure of tissue) with stronger tissue-bonding and faster recovery will be achieved by welding with a combination of 980 and 1064-nm lasers.

Tabakoglu and Gulsoy investigated the skin closure abilities of a 980-nm diode laser and 1070-nm fiber laser with an irradiance level of 15.92 W/cm^2 in continuous mode for 5 s per spot (0.5 W, 5 s), respectively, by histological examination and mechanical tests during a 21 day healing period.¹⁶ Their results showed that 980-nm laser welded incisions could create one-third-thick welds just at the beginning of the healing period with a tensile strength greater than that of sutured incisions after one week of recovery, and 1070-nm laser welding yielded noticeably stronger bonds with minimal scarring at the end of the healing period. Thus, the irradiance level and exposure time mentioned above were adopted in this study. In addition, Fried

and Walsh reported that laser skin welding using 1064-nm laser (power density of 79.6 W/cm^2) and India ink produced strong weld strength (acute value of $2.1 \pm 0.7 \text{ kg/cm}^2$) with limited thermal damage.¹³ Therefore, a high irradiance level of about 79.6 W/cm^2 was also chosen as a reference for 1064-nm laser (power of 2.3 to 2.5 W) in this study.

A critical problem in the design of the second experiment is the choice of the control group. Here, we divided the selected 40 rats equally into the laser welding group and the suture group, and half of each group was used for histology examination and the others for a mechanical test. Of course, some other choices may also be suitable for comparison of the closure and healing process of the wound closed either by laser welding or suture. For example, dividing the 40 rats equally into two groups (one group for histology examination and the other for mechanical test), and, on the left-side three incisions on the back of each rat were welded, while on the right-side three incisions were sutured as a control. There were two reasons for our classification. First, the 40 random selected rats were all 270 to 320 g weight and housed in the same plastic cages. The living environment, such as room temperature and feeding were the same, as well as the operative procedure. Thus the individual difference was minimized and was insufficient to affect the experiment results. Furthermore, two rats from each group were sacrificed for histology examination or mechanical test on the post operative control days. The number of incision-samples (8 to 12 pieces) was enough for the Student's t-test, and thus the results had the statistical significance. Thus the choice of the control group was reasonable.

In order to keep the wound walls aligned during the welding procedure *in vivo*, one person clamped the end points of the incision with two forceps to make an anatomical anastomosis, the other welded the incision spot-by-spot as described above. For the control group, we chose the closure method with a single suture in the middle of each incision for the following reasons. First, in the early experiments, we had found that incisions which were closed by 3M Steri-Strip S Surgical Skin Closures (3M Health Care, St. Paul, MN) were ruptured on the first postoperative day. We were unable to do further evaluation of the healing of incisions. Thus, we used an usp-3/0 metric silk to close the wound in order to avoid rupture of the wounds. Second, only one knot was made in the middle of each incision in order to reduce foreign body reaction. Furthermore, we found through microscope observation that the foreign body reaction focused mainly around the suture, which had no serious effect on the healing process of the whole incision.

In the first study, macro-inspection of a 14-day wound-healing period revealed that skin welding with power density of 15.9 W/cm^2 for both 980 and 1064-nm lasers in continuous wave mode yielded the best welding efficiency with minimal thermal damage, fastest recovery, and flat incision sites [Fig. 1(a)]. The exposure time per spot and the energy density for both lasers were 5 s and 79.6 J/cm^2 , respectively. However, exposure time of 3 s per spot (in group 1) yielded no or negligible immediate closure due to less laser heat deposition and thus lower tissue temperature rise. On the other hand, wounds welded with high irradiance (73.25 to 79.62 W/cm^2 for 1064-nm in groups 3 to 6) appeared moderate to serious lateral thermal damage and carbonization of blood leakage leading to gross scab formation and delayed recovery. This could be explained that high irradiance caused high heat deposition and

Fig. 7 Comparison of tensile strengths of incisions welded with combined lasers and single laser. Significantly stronger tissue-bonding is detected in the incisions welded with combined lasers until the 7th day ($p < 0.05$). From the 14th day on, no statistical difference was observed ($p > 0.05$). Bars (means + SD, $n=8$)

rapid increase in tissue temperature, so high that it exceeded the reported appropriate welding temperature (50°C to 80°C)^{17,20} at the target tissue where irreversible tissue damage was caused.

In the second study, macro-inspection of a 21-day wound-healing period revealed that no anomalies were observed in health conditions, dietary habits, or behaviors of rats for both groups. A more uniform seal of the entire 1-cm long welded incision was detected during the early recovery period. However, the needle entrances on both sides were still open for most of the sutured samples, and were possible entrance points for microbial pathogens (Fig. 2). According to the results of histological examination, no significant difference was observed in wound healing of laser-welded and sutured incisions after 21 days (Fig. 3). The epidermal thickness, which was measured by microscopic observation, was considered as a semiquantitative indicator for wound healing. On the 14th wound healing day, the average epidermal thickness of welded incisions was statistically thinner than that of sutured ones (Fig. 4), indicating that faster recovery of epidermal thickness appeared in welded wounds. Furthermore, the incisions closed by both laser welding and the suturing technique were also compared in terms of tensile strength (Fig. 6). The mechanical tests showed that the tensile strengths of laser-welded samples continued to increase during the full recovery period, and were greater than those of sutured incisions. It was seen that the tensile strength of welded incisions on the 7th day had no statistical difference to sutured incisions after a 21 days' recovery ($p > 0.05$).

Furthermore, the welding effect with a single laser is also tested and compared with the case of combined lasers (Fig. 7). It was found that tissue welding using a single 1064-nm diode laser with irradiance level of 15.9 W/cm^2 (5 s per spot) obtained no immediate closure, which was not similar to a 1070-nm laser,¹³ and thus the welding effect was not tested. This could be explained that irradiance of 15.9 W/cm^2 was too low for skin-welding with 1064-nm laser without India ink to produce enough heat to fuse the separate tissue. As was shown in Fig. 7, acute tensile strength of $4.16 \pm 0.78 \text{ N}$ for the single 980-nm laser group was statistically weaker than that of

the combined lasers group (5.21 ± 1.02 N, $p < 0.05$). Tensile strengths of both groups continued to increase, and significantly stronger tissue-bonding was detected in the wounds welded with combined lasers until the 7th day ($p < 0.05$). From the 14th day on, no statistical difference was observed ($p > 0.05$). These results are consistent with the closure of wounds we detected. On the 1st postoperative day, the closure index (CI) of the wounds welded with a single 980-nm laser was 0.29 ± 0.09 , which was less than that of the combined lasers group ($CI = 0.49 \pm 0.1$, $p < 0.05$). It increased to 0.38 ± 0.07 after four days, but was still significantly less than that of the combined lasers group ($CI = 0.53 \pm 0.17$, $p < 0.05$). From the 7th day on, closure indexes of both groups reached about 1.0. As a result, tissue welding with combined lasers achieved deeper closure and stronger tissue-bonding, which supported the idea of wavelength selection in this study.

In conclusion, laser welding with a combination of two near-infrared diode lasers (980 and 1064-nm) yielded better results than suture in terms of faster recovery, better apposition of tissue and stronger closure, and was an important candidate for skin welding. Investigations in this study showed that irradiance of 15.92 W/cm² and exposure time of 5 s per spot in continuous wave mode for both 980 and 1064-nm diode lasers seemed to be an appropriate choice for laser skin welding, and minimal thermal damage and stronger appositions were achieved. Nevertheless, some limitations of this study, such as the number of animals and laser output from two individual fibers are necessary to be improved. In future work, further optimization of the laser parameters may be necessary and different modes of operation could be tested. Furthermore, the effective control of tissue temperature is a crucial factor in tissue welding, thus monitoring the temperature at the target tissue to investigate the photothermal interaction between laser and tissue also needs to be done.

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