# DYE-ASSISTED LASER SKIN CLOSURE WITH PULSED RADIATION: AN *IN VITRO* STUDY OF WELD STRENGTH AND THERMAL DAMAGE

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## ABSTRACT

Previous laser skin welding studies have used continuous wave delivery of radiation. However, heat diffusion during irradiation prevents strong welds from being achieved without creating large zones of thermal damage. Previously published results indicate that a thermal damage zone in skin greater than 200 $\mu$m may prevent normal wound healing. We propose that both strong welds and minimal thermal damage can be achieved by introducing a dye and delivering the radiation in a series of sufficiently short pulses. Two-cm-long incisions were made in guinea pig skin, *in vitro*. India ink and egg white (albumin) were applied to the wound edges to enhance radiation absorption and to close the wound, respectively. Continuous wave (cw), 1.06 $\mu$m, Nd:yttrium–aluminum–garnet laser radiation was scanned over the weld producing ~100 ms pulses. The cooling time between scans and the number of scans was varied. The thermal damage zone at the weld edges was measured using a transmission polarizing light microscope. The tensile strength of the welds was measured using a tensiometer. For pulsed welding and long cooling times between pulses (8 s), weld strengths of $2.4\pm0.9$ kg/cm$^2$ were measured, and lateral thermal damage at the epidermis was limited to $500\pm150$ $\mu$m. With cw welding, comparable weld strengths produced $2700\pm300$ $\mu$m of lateral thermal damage. The cw weld strengths were only $0.6\pm0.3$ kg/cm$^2$ for thermal damage zones comparable to pulsed welding. © *1998 Society of Photo-Optical Instrumentation Engineers.* [S1083-3668(98)00404-3]

**Keywords** skin closure; infrared radiation; collagen denaturation.

## 1 INTRODUCTION

Laser tissue welding has been investigated as an alternative means of skin closure over the past decade. Potential advantages of skin welding over conventional mechanical methods of tissue closure (e.g., sutures, staples) include increased immediate tensile strength, decreased operative repair time, accelerated wound healing, fluid-tight closure, reduced probability of infection, and improved cosmetic results.[1] Progress in the field of laser skin closure has been slow due, in part, to the large number of parameters that need to be optimized in the welding process. These parameters include wavelength, fluence or irradiance, pulse duration, repetition rate, irradiation time, spot size, dye selection, and adhesive selection. Past skin welding studies have used a wide range of values for these parameters making the interpretation of results and comparison between studies difficult. Table 1 outlines selected work in this field.[2–8]

The majority of previous skin welding studies have used a laser operated in either continuous wave (cw) mode[2–6] or quasi-cw mode with constant surface temperature control.[8] Laser welding of other tissues has been performed using either single pulses or multiple pulses of radiation with minimal cooling between successive pulses.[7,9–12] During cw welding, heat diffuses from the weld site into the surrounding healthy tissue, resulting in a large zone of thermal damage. Previous work by Walsh[13] and Green et al.[14] indicates that a zone of thermal damage greater than ~200 $\mu$m extending laterally from the weld site may inhibit wound healing and result in scarring. For skin welding, excessive scarring may be clinically unacceptable even if strong welds can be achieved. Pulsed delivery of radiation, with sufficient cooling time between pulses, may produce strong welds and limit the thermal damage zone to the immediate area of the weld site. Healthy tissue surrounding the weld site may be saved from unnecessary thermal damage. The effect of using multiple pulses of radiation to damage

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**Table 1** Selected publications on laser skin closure.

| Author | Garden (Refs. 2, 3) | Abergel (Ref. 4) | Dew (Ref. 5) | Wider (Ref. 6) | DeCoste (Ref. 7) | Poppas (Ref. 8) |
|---|---|---|---|---|---|---|
| Publication date | 1986 | 1986 | 1991 | 1991 | 1992 | 1996 |
| Animal model | minipig | mouse | pig | rat | guinea pig | pig |
| Laser | $CO_2$ | Nd:YAG argon, $CO_2$ | Nd:YAG | GaAlAs diode/ argon | Alexandrite | Nd:YAG |
| Wavelength ($\mu$m) | 10.6 | 1.06, 1.32, 0.488/0.515, 10.6 | 1.32 | 0.808,0.488/0.515 | 0.780 | 1.32 |
| Mode | cw | cw | cw | cw | Pulsed | cw |
| Pulse duration | | | | | 250 $\mu$s | |
| Repetition rate | | | | | 8 Hz | |
| Power (W) | 0.5 | 1.0 | <2 | 0.3/0.15 | 0.13–2.50 | ≤3 |
| Spot diameter (mm) | 0.8–1.0 | 0.8 | NA | 2.0 | 4.0 | 1.0 |
| Irradiance (W/cm$^2$) | 64–100 | 200 | NA | 10/5 | 1–20 | ≤375 |
| Operation time (s) | 95 | NA | NA | NA | 60 | 20 |
| Incision length (cm) | 2.5 | 5 | 9 | 3 | 1.5 | 2 |
| Dye | None | None | None | ICG/Fluorescein | ICG | None |
| Dye conc. (mg/ml) | | | | 0.02/1.0 | 0.03–3 | |
| Adhesive | Sutures staples | Forceps | Sutures tape | Fibrinogen | Forceps | Albumin suture |
| Immediate weld strength ($kg/cm^2$) | 1.1–1.5 | NA | 1.0 | NA 4.7 (5 days) | 0.25–0.50 (<70 g) | 0.15–0.30 |
| Thermal damage ($\mu$m) | >600 $\mu$m | NA | NA | NA | 500 $\mu$m | 700 $\mu$m–2.3 mm |
| Wound healing (days) | 90 | 56 | 600 | 28 | Immediate | 14 |

tissue selectively has been discussed previously for ophthalmic applications.[9]

The purpose of our scientific study was to use pulsed delivery of radiation and a dye to produce strong welds with minimal thermal damage to healthy tissue surrounding the weld site. Pulsed welding was compared to quasi-cw welding and sutures. The total irradiation time and the cooling period between pulses were varied and correlated with the measured weld strength and thermal damage at the weld site. Pulsed welding not only produced welds of strength comparable to cw welding, but also a much smaller zone of thermal damage at the weld site. Weld strengths were comparable to suture apposition strengths, but not breaking strengths.

## 2 MATERIALS AND METHODS

All experiments were performed *in vitro* using guinea pig skin. Adult female albino guinea pigs (Harlan, age 7–8 weeks, weight 400–500 g) were anesthetized with halothane and then euthanized with an intravenous overdose of sodium pentobarbitol (Nembutal). Animals were shaved and then epilated with a chemical depilator (Nair). The dorsal skin, including epidermis and dermis, was excised with a scalpel and sectioned into squares of approximately $3 \times 3$ cm. Tissue samples were then enclosed in a petri dish and preserved on a damp saline-soaked towel until used. All experiments were completed within 12 h of tissue preparation.

A 2-cm-long full-thickness incision was made in each skin sample with a No. 15 scalpel. All incisions were made parallel to the spine. Approximately 2–5 $\mu$l of India ink (Koh-I-Noor, ~100 nm particle diameter) was then applied to the wound edges with a micropipette. Excess dye was removed with a paper towel. Histological analysis revealed that the dye penetrated 20–50 $\mu$m into the skin from the incision edge. After the India ink dried, a thin layer of egg white (10% albumin) was applied to the wound edges as a temporary adhesive.

Welding was performed with a continuous wave Nd:yttrium–aluminum–garnet (YAG) laser (Lee Laser, Model 703T) emitting radiation at a wavelength of 1.06 $\mu$m. The radiation was coupled into 600 $\mu$m core diameter silica optical fiber (3M) for flexible delivery. A 5-mm-diameter [full width at half maximum (FWHM)] laser spot size was maintained during the experiments. The beam profile was approximately Gaussian. The power delivered to the tissue was kept constant at 10 W$\pm$0.2 W for all experiments. The output end of the fiber was scanned along the axis of the weld site using a stepper-motor-driven translation stage (Klinger Scientific Corp.) to simulate pulsed delivery of the radiation. The stepper motor was controlled by a personal computer (Hewlitt Packard 386) that allowed programming of the scan velocity, cooling time between scans, and total number of scans. During welding, the velocity of the translator was kept constant at 47.6 mm/s, resulting in a pulse duration of ∼100 ms, for the fixed 5-mm-diameter laser spot size.

The cooling time between scans was varied to study the difference between cw and pulsed welding. Three cooling times were selected: 1.6, 4.0, and 8.0 s. The cooling time is defined as the average time the laser beam takes to return to a particular 5 mm spot at the weld site during scanning. Between scans, the laser beam was incident on high-reflecting metal plates placed on either side of the tissue sample. These cooling times represent quasi-cw welding with minimal cooling, intermediate cooling, and long-duration cooling, respectively. Quasi-cw cooling was chosen to provide an estimation of the thermal damage achieved during cw welding, in which tissue temperatures are maintained above collagen denaturation thresholds for long periods of time. Intermediate cooling was studied in an attempt to allow the tissue to cool below denaturation temperatures between pulses, and to limit thermal damage to surrounding healthy tissue. Long-duration cooling was used to allow the temperature of the tissue to fall to approximately room temperature between pulses, and avoid buildup of the baseline temperature with successive pulses.

The total energy delivered to the weld site was varied between ∼60, 90, 150, and 300 J by changing the number of scans across the tissue between 14, 21, 35, and 70 passes. Each scan delivered 4.2 J of total energy to the weld site. The fluence at any particular point along the incision was 5.1 J/cm$^2$. The total energy, $E(J)$, incident on the surface of the 2-cm-length weld is

$$E = \frac{p \times l \times s}{v}, \qquad (1)$$

where $p$ is power (10 W), $l$ is weld length (20 mm), $s$ is number of scans, and $v$ is velocity (47.6 mm/s). The total operation time ranged from 30 s to 10 min,

**Table 2** Summary of welding parameters.

| | |
|---|---|
| Animal model | albino guinea pig |
| Incision length | 2-cm-full thickness |
| Laser | cw 50 W Nd:YAG |
| Wavelength | 1.06 $\mu$m |
| Pulse duration | 100 ms |
| Cooling time | 1.6, 4.0, and 8.0 s |
| Repetition rate | 0.63, 0.25, 0.12 Hz |
| Power to tissue | 10.0$\pm$0.2 W |
| Spot diameter | 5 mm (FWHM) |
| Total energy | 60–300 J |
| Operative time | 30 s–10 min |
| Dye | India ink |
| Absorption coefficient | 3500 cm$^{-1}$ |
| Adhesive | egg white (10% albumin) |

depending on both interpulse cooling times and the total energy delivered to the weld site.

Upon completion of welding, the samples were placed in a covered petri dish onto a saline-soaked towel to preserve hydration of the tissue. Within 12 h of tissue preparation, the samples were processed for either tensile strength measurements or histology. Table 2 provides a summary of the laser parameters used in this study. Figure 1 shows a
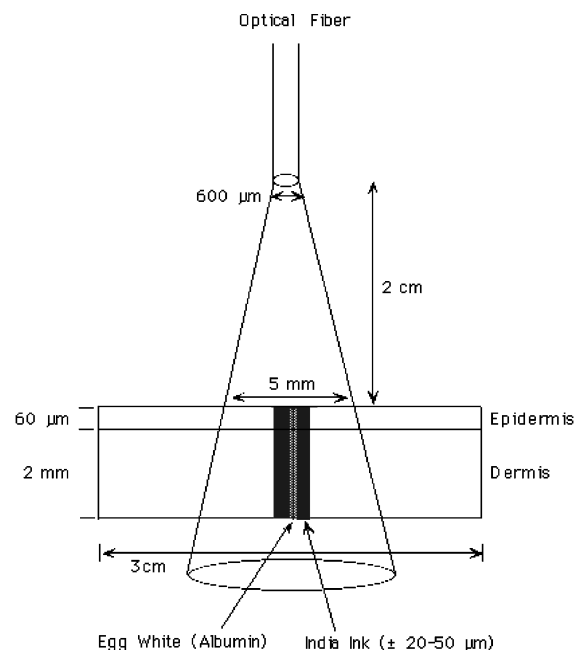


**Fig. 1** Experimental configuration for dye-assisted pulsed laser skin welding.

diagram of the configuration used for welding skin.

Tensile strength of welded incisions was quantified using a tensiometer (MTS Sintech 20/G), with a 500 lb. load cell ($\pm 50$ g accuracy). The length and thickness of each weld was measured before being tested. The tissue was gripped by clamps along the full width of the tissue on each side of the weld. The tensiometer then pulled transversely to the axis of the weld at a rate of 6.35 mm/min. A weld was judged as being broken as soon as a visible hole in the tissue could be seen at its weakest point. The breaking strength of the weld was divided by the weld length and thickness to arrive at a tensile strength (kg/cm$^2$). A minimum of seven samples were tested for each set of laser parameters.

Suture control studies were also prepared. The 5'0 Nylon sutures (Ethicon) were used to appose the 2-cm-long skin incisions. Either 1, 3, or 5 interrupted sutures were placed equidistant along the incision. The sutured wounds were also pulled apart using the tensiometer. Two suture strength values were recorded. The first value corresponded to when a hole could be seen in the tissue between the suture apposition points and represented the degree of tissue apposition. The second value was taken at the point at which the sutures failed. A minimum of four samples were tested for each set of parameters.

The thermal damage zone near the weld site was quantified following standard histologic preparation of tissue samples. A $4\times4\times2$ mm sample of tissue was sectioned with a scalpel from the center of each completed weld, and stored in 10% formalin. Samples were processed in graded alcohols and xylines, paraffin embedded, sliced with a microtome, and stained with Hemotoxylin and Eosin dyes. A light microscope (Nikon) fit with crossed linear polarizers (Prinz) was used to analyze the sections. The boundary separating laser-denatured collagen from native collagen could be delineated based on the degree of collagen birefringence: native collagen transmitted light, while domains of denatured collagen did not. Thermal damage measurements were recorded at three different depths in the tissue, the epidermis, the middle of the dermis, and the bottom of the dermis, and measured laterally from the center of the weld site. Measurements were consistently taken at the demarcation point where complete birefringence loss was seen.

It was sometimes difficult to determine a cutoff point separating native from denatured tissue. Typically, there would be a small, but significant region of tissue in which a gradual change from native to denatured tissue occurred. This region of partially denatured collagen made quantitative measurements of thermal damage difficult to perform. It should also be noted that birefringence measurements of thermal damage to tissue *in vitro* will not indicate delayed damage to tissue that could occur in the first few days following *in vivo* welding.

Statistical analyses were conducted on selected groups of data for weld and suture strengths and thermal damage measurements. ANOVA was used to determine statistical significance within and between data sets.

## 3 THEORY

A pulse duration of $\sim100$ ms was selected in this study based on heat transfer theory.[15,16] An approximate solution for the thermal relaxation time, $\tau_{Th}$, from the heat diffusion equation is given as follows:

$$\tau_{Th} = \frac{d^2}{4\kappa}. \qquad (2)$$

To limit heat diffusion from the weld site during the laser pulse, the pulse duration, $\tau_p$, must be on the order of, or less than, the thermal relaxation time, where $d$ is the desired width of thermal damage, and $\kappa$ is the thermal diffusivity ($\kappa\sim1.3\times10^{-3}$ cm$^2$/s for skin).[17] For our application, the $\sim100$ ms pulse duration used in these experiments was expected to yield an acceptable zone of thermal confinement of $\sim230$ $\mu$m. It is important to note that Eq. (2) represents an approximate, order-of-magnitude estimate of the thermal relaxation time, rather than an exact solution to the heat diffusion equation.

The tissue also needs to cool down to room temperature between scans to prevent baseline temperature rises with successive pulses. The rate of thermal diffusion is determined by the mass density, heat capacity, and thermal conductivity of the tissue being studied, as well as the geometry of the heat transfer problem. A conservative estimate for the time of temperature decay during the cooling phase, $\tau_c$, is on the order of 50–100 times the thermal relaxation time.[18,19] By using suitably short pulses ($\tau_p<\tau_{Th}$) with sufficient cooling between pulses ($\tau_c>100\tau_{Th}$), selective thermal damage to the weld site can be achieved without irreversibly damaging the tissue adjacent to the weld.

Welding was performed with near-infrared radiation that penetrates deeply into skin; thus uniform, full-thickness welds could be obtained. At near-infrared (IR) wavelengths, scattering of radiation dominates absorption. Scattering of photons within the tissue can result in a significant decrease in penetration of the radiation and uniformity of the heating at deeper tissue depths. By using a large diameter beam, the radiation penetrates to the deeper layers of the tissue;[20] thus, the temperature distribution is more uniform, and the welds should be stronger. Further, a large spot size allows easy alignment of the beam during welding. It is also probable that a high degree of scattering in the tissue aids the welding process by scattering a signifi-

cant number of photons towards the dye layer at the weld site, where the photons are then absorbed.

India ink was used in this study for several reasons. First, an external absorber of radiation is necessary to selectively confine absorption of radiation to the immediate area of the weld site. Second, India ink is a much stronger absorber than other dyes used in welding. For example, Indocyanine Green (ICG), previously used with a diode laser for skin welding, has an extinction coefficient of $2 \times 10^5$ $M^{-1}$ $cm^{-1}$ at 805 nm.[5,6] If a concentration of 1 mg/ml of ICG is applied to skin, the absorption coefficient is $\sim 600$ $cm^{-1}$. Fluorescein Isothiocyanate, previously used with an argon laser for skin welding, has an extinction coefficient of $6 \times 10^4$ $M^{-1}$ $cm^{-1}$ at 495 nm.[5] If a concentration of 1 mg/ml is applied to skin, the absorption coefficient is $\sim 350$ $cm^{-1}$. The absorption coefficient of 100% India ink at 1.06 $\mu$m was measured to be approximately 3500 $cm^{-1}$. For comparison, the absorption coefficient of skin is 0.3 $cm^{-1}$ at 1.06 $\mu$m. Thus, India ink serves as a very effective absorber of radiation that, when combined with pulsed delivery of radiation, can limit heating of the tissue to the immediate area of the weld site. Third, unlike other welding dyes, India ink absorbs strongly over a wide range of visible and IR wavelengths.[21] It is not necessary to search for a laser with a wavelength that matches the absorption peak of the dye. Instead, one simply chooses a laser with a wavelength that has a deep penetration depth in tissue and allows for uniform, full-thickness heating of the weld site. Finally, India ink has a low toxicity when applied to skin, and is routinely used in the tattoo industry.[22] The obvious disadvantage of using India ink is that the dye permanently tattoos the skin. Thus, the cosmetic result is not desirable. The use of India ink must, therefore, be limited to scientific rather than clinical studies of skin welding, or to laser welding involving internal organs.

## 4 RESULTS

### 4.1 TENSILE STRENGTH MEASUREMENTS

Weld strengths were recorded for three sets of cooling times and four sets of total energy, as shown in Figure 2. The cooling durations, $\tau_c = 1.6$, 4.0, and 8.0 s, also stated as $\tau_c = 16$, 40, and 80 $\tau_p$, represent short, medium, and long-duration cooling between successive laser pulses, respectively. The total energy represents the total amount of energy supplied to the tissue along the 2-cm-long incision. For a fixed cooling time, the tensile strength of the weld increased with total energy delivered to the weld site. There is a clear difference in weld strengths when comparing the values for 60 J of energy and 300 J of energy, regardless of cooling time ($p < 0.001$). The maximum weld strength was approximately 2.4 $kg/cm^2$, obtained with a cooling time of $80\tau_p$ and a total energy of 150 J. A delivery
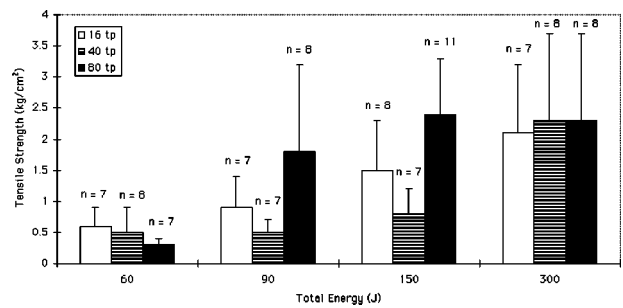


**Fig. 2** Tensile strength of laser welds plotted as a function of total energy and cooling time between pulses. The bars represent mean values ± the S.D., calculated from a minimum of seven samples per a set.

of 300 J of energy for all three cooling times produced similar strengths. It is not clear whether weld strengths continue to increase for energy levels greater than 300 J, or plateau at a certain level. We terminated the study at 300 J, due to the large amount of thermal damage being seen at the weld site.

The tensile strength results show large standard deviations, sometimes as high as 60% of the weld strength. Such deviations can be attributed to errors in measurement, differences in tissue properties across samples, and perhaps most importantly, a technical inability to produce consistent apposition of tissue during closure prior to welding.

A control study was also completed to compare suture strengths with strengths obtained with our best laser welding parameters. The results are shown in Figure 3. Tensile strength is plotted as a function of the number of interrupted sutures used per 2-cm-long incision. The left bar in each column represents the tensile strength of the sutures at their breaking point. This tensile strength is very large, approximately 8–11 $kg/cm^2$, and is statistically independent of the number of sutures used
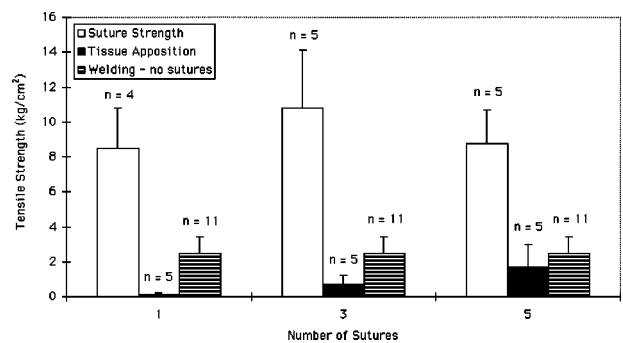


**Fig. 3** Tensile strength of sutured wounds as a function of the number of sutures used to close the wounds. Left bar in each column represents value at which sutures fail. Middle bar represents value at which apposed tissue edges separate. Right bar is the maximum strength achieved with laser welding and no sutures (150 J, 8 s cooling), included in this graph for comparison. The bars signify mean values ± the S.D., calculated from a minimum of four samples per a set.
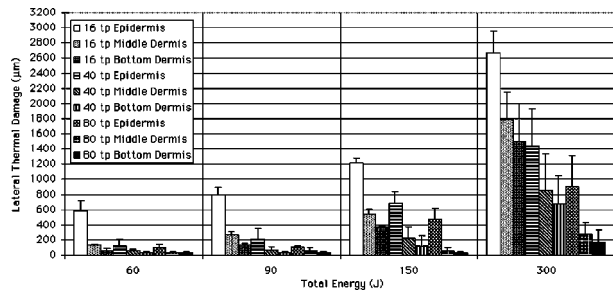
**Fig. 4** Width of thermal damage zone as a function of total energy delivered to the weld site, interpulse cooling times, and location in skin. The bars signify mean values ± the S.D., calculated from a set of three samples.

($p>0.25$). The middle bar in each column represents the tissue apposition strength of the sutures, or the point at which the tissue opens up between sutures. Statistical analysis shows that the suture apposition strength increases with the number of sutures used to close the wound ($p<0.05$): as more sutures are applied to a wound of a fixed length, the distance between sutures decreases, and tissue apposition strength between suture points increases. All of the suture apposition strengths are below 2.0 kg/cm$^2$. The right bar in each column represents the best weld strength achieved with laser welding in the absence of sutures (150 J total energy and 8 s cooling between scans). It is present for comparison between laser weld strengths and suture strengths. Figure 3 clearly indicates that while *in vitro* laser welded incisions are not as strong as sutured incisions, laser welded incisions have strength comparable to that at which tissue tears apart between suture points when five sutures are used ($p>0.25$).

## 4.2 HISTOLOGICAL STUDY OF THERMAL DAMAGE

Previously published results suggest that if the zone of thermal damage lateral to the incision exceeds $\sim 200\ \mu$m, then the wound healing process becomes delayed and excessive scarring occurs.[13,14] A study of thermal damage zones produced during quasi-cw wave and pulsed welding was performed. The results are shown in Figure 4. Lateral thermal damage extending out from the weld site is plotted as a function of both total energy delivered and the cooling time between pulses. Four sets of total energy and three sets of interpulse cooling times were studied.

Figure 4 demonstrates several points. First, a statistical analysis shows that within each data set, thermal damage at the epidermis is significantly greater than in the underlying dermal tissue ($p<0.05$). This is most likely due to a gradient in temperature from the top surface of the skin to the bottom surface. Both absorption and scattering of radiation contribute to this phenomenon.

Second, increasing the cooling time between laser pulses greatly reduced the thermal damage at the weld site. There is a statistically significant difference between epidermal thermal damage for welding with interpulse cooling times of 1.6 and 8 s, for comparable energies ($p<0.01$). For comparable weld strengths of 2.0–2.4 kg/cm$^2$, quasi-cw welding (1.6 s of cooling between pulses) produced a zone of thermal damage of $2700\pm 300\ \mu$m at the epidermis, while pulsed welding with a long cooling duration (8 s of cooling between pulses) produced only $500\pm 150\ \mu$m of thermal damage. It is important to note that the thermal damage produced during true cw welding with no cooling would probably be greater than the 2700 $\mu$m measured for quasi-cw welding.

Figure 5 shows three representative images of weld sites as viewed through a transmission polarizing microscope. Only two parameters were varied across these samples; total operation time and cooling time between pulses. In each image, the weld site is centered in the middle of the picture. The collageneous tissue around the weld site is either native, undamaged collagen, which is birefringent and thus appears white, or thermally denatured collagen, which is not birefringent and thus appears dark. Note the layer of India ink running through the full thickness of the weld, from the epidermis to the bottom of the dermis.

In Figure 5(a), the total energy delivered was 300 J, with 8 s of cooling between scans. The thermal damage zones at the epidermis, mid-dermis, and bottom dermis measured $\sim$500, $\sim$200, and $\sim$50 $\mu$m, respectively. In Figure 5(b), 300 J of energy was delivered to the tissue, but with only 4 s of cooling between scans. The thermal damage zones had widths of $\sim$1750, $\sim$500, and $\sim$250 $\mu$m. In Figure 5(c), 150 J of energy was delivered with only 1.6 s of cooling between scans. The damage zones measured $\sim$1250, $\sim$500, and $\sim$200 $\mu$m.

Although the total energy delivered to the weld site in Figure 5(c) is only half of that in Figures 5(a) and 5(b), the thermal damage is roughly comparable to Figure 5(b), due to the short cooling time between pulses. The weld in Figure 5(a), completed with long interpulse cooling times, shows much less thermal damage than in Figures 5(b) and 5(c), where shorter cooling durations were allowed between pulses. In all three photographs, it appears that full-thickness laser welds were not achieved. Instead, laser fusion of tissue occurred to approximately 50%–70% depth in the tissue. Egg white albumin fills the wound gap in the deepest layers of the tissue. We believe that incomplete apposition of the tissue prior to welding is probably the cause of the less-than-full-thickness welds, since thermal damage at the bottom of the dermis indicates sufficient collagen denaturation at that depth.
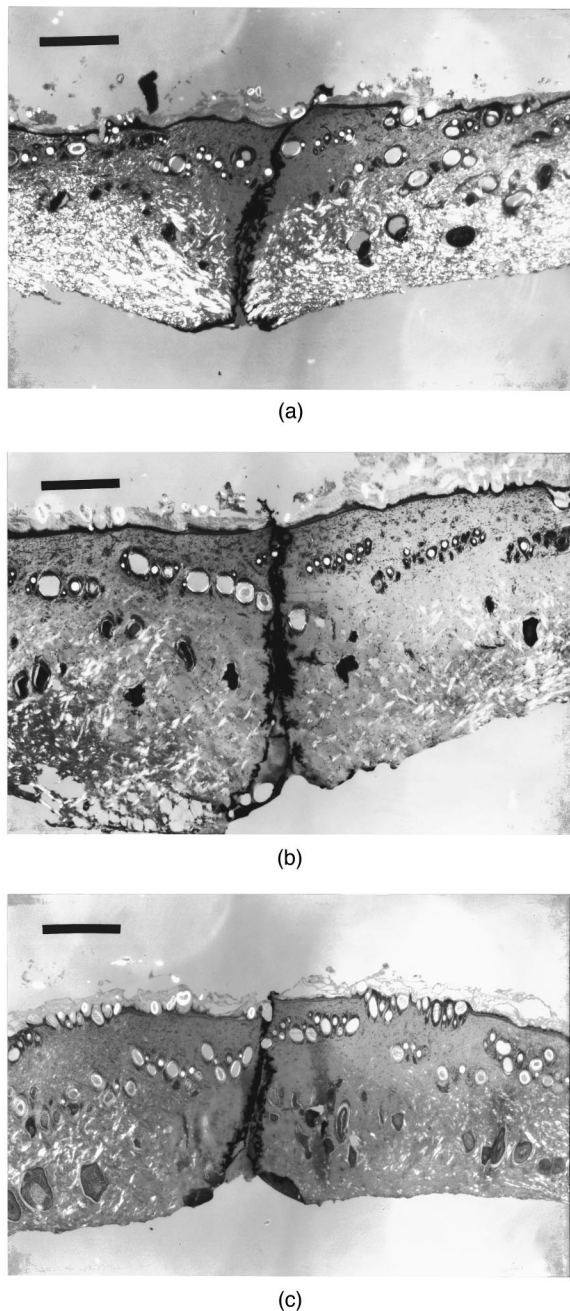
**Fig. 5** Birefringent images of laser welds photographed using a transmission polarizing light microscope (bar=400 $\mu$m). The laser parameters were kept constant except for total energy and cooling time between pulses. (a) 300 J, 8 s between pulses, (b) 300 J, 4 s between pulses, (c) 150 J, 1.6 s between pulses. Note the increase in the zone of thermal damage as the cooling time is decreased between pulses. Egg white albumin is present at the bottom of the weld site in each image.

## 5 Discussion

Weld strengths of 2.4 kg/cm$^2$ were measured using pulsed delivery of radiation. The immediate *in vivo* weld strength for skin is typically in the range of 0.15–0.50 kg/cm$^2$.[1,7,8] Higher tensile strengths on the order of 1 to 2 kg/cm$^2$ have been published in the literature.[2,5] In one of these studies, however, a

CO$_2$ laser was used to weld skin, creating excessive thermal damage and poor wound healing.[2,23] In the other study, both tape and sutures were used to facilitate wound apposition and strength.[5]

In our experiments, it was not possible to use long-duration cooling to limit lateral thermal damage at the epidermis to a 200-$\mu$m zone. However, the middle and deeper layers of the dermis showed thermal damage zones less than 200 $\mu$m in width. The thermal damage zone at the epidermis was also reduced significantly from 2700 to 500 $\mu$m by replacing cw welding with pulsed welding and long-duration cooling. There are several possibilities for reducing thermal damage even further. Cooling times longer than $80\tau_p$ and/or pulse durations shorter than 100 ms may be necessary to provide for complete thermal relaxation in the tissue. It is also possible that thermal damage will be reduced in progressing from *in vitro* to *in vivo* welding studies due to increased hydration and blood flow in the tissue, thus eliminating any need to refine the laser parameters.

In general, the typical thermal damage profile seen in the histology was funnel shaped, with a large zone of thermal damage in the upper layers of the dermis and less damage deeper within the dermis. This thermal damage profile demonstrates that even for deeply penetrating radiation at the 1.06 $\mu$m wavelength, there is a substantial temperature gradient in the tissue. Both absorption and scattering of radiation control light penetration. The dermis is a highly scattering medium that causes an attenuation in the amount of radiation reaching the deeper layers of the skin. This attenuation with depth results in a temperature gradient, which in turn translates into a thermal damage gradient.

In evaluating the clinical value of laser welding, it is important to compare weld strengths with tissue apposition strengths as well as suture strengths. Although sutures provide strong closure of tissue, the wound actually opens up long before the sutures fail. The tissue gap between suture apposition points is an important indicator of wound healing because the wound is no longer fluid tight, and therefore serves as a potential site of infection. A perforation in the skin, if not properly sealed, will also result in the generation of scar tissue during the wound healing process. Our data indicate that *in vitro* weld strengths are comparable with the tissue apposition strengths achieved using five sutures to close a 2-cm-long incision.

One of the goals of welding is to limit or eliminate the use of sutures in closing tissue. Therefore, alternative means need to be developed to appose the wound edges at the weld site prior to welding. An adhesive, egg white albumin, was needed in this study to create strong, reproducible welds. The issue of tissue apposition is an important problem that needs to be addressed. Previous work by Fenner et al. suggests that weld strengths may depend

strongly on the degree of apposition.[24] Therefore, the type of apposition system used to aid in laser skin closure will probably be just as important as the choice of laser parameters in obtaining a successful weld.

In summary, it is possible to obtain strong welds and limit thermal damage by delivering radiation in a pulsed mode and allowing sufficient cooling time between pulses. A dye is also necessary to confine absorption and the ensuing thermal damage to only the immediate area of the weld site. The present *in vitro* studies suggest that pulsed welding may provide better wound healing and cosmetic results than welding with a continuous wave laser. Although *in vivo* studies will be necessary to study the wound healing process after pulsed welding, our present *in vitro* results have allowed us to determine a range of laser parameters that produces strong welds while limiting thermal damage.

## Acknowledgments

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