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Laser Tissue Welding by Using Collagen Excitation at 1,720 nm Near-Infrared Optical Window III

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Abstract: Laser tissue welding (LTW) is a method of fusing incised tissues together. LTW has the potential to revolutionize plastic surgery and wound healing techniques by its ability to produce water-tight, scarless seals with minimal foreign body reaction. While using thermal mechanisms to achieve LTW, energy from the incident laser is absorbed by water in the tissue. As the water temperature increases, partial denaturing of the collagen triple helix briefly occurs, which is quickly followed by renaturation of collagen as the tissue cools, thus providing a watertight seal. This research study investigates the efficacy of direct collagen excitation at 1,720 nm to accomplish LTW. This wavelength falls within the near infrared (NIR) optical window III. The tensile strengths of pig skin that have been welded with NIR continuous-wave (CW) diode lasers at 1,455 nm, which promotes thermal mechanisms of tissue welding, and 1,720 nm wavelengths are compared. Near infrared lasers tuned to 1,455 nm and 1,720 nm were used to weld incised pieces of porcine skin together without extrinsic solders or dyes. The tensile force of the welded tissues was measured using a digital force gauge. The average tensile force of the welded pig skin using the 1,720 nm laser was approximately four times greater than that using the CW 1,455 nm laser, suggesting that LTW accomplished through direct collagen excitation in NIR Optical Window III provides greater tensile strengths.

1. Introduction

A major effort of modern surgeries is the development of new, minimally invasive methods of connecting organs and tissues that are simple and convenient in clinical practice [1]. Laser tissue welding (LTW) has long been investigated as a novel and promising wound healing technique that is accomplished by directing a laser beam at the edges of cut tissue [2-7]. Conventional methods to close surgical incisions by means of sutures, staples, or clips have many disadvantages, including inflammation, foreign body reaction, excessive scar tissue formation, and the lack of watertight seals [4,8]. The usage of laser radiation to bond tissue together has the potential to reduce or eliminate these problems, while also providing rapid, scarless wound healing and decreased surgical time [2-4,8-10]. Given these advantages, LTW can fuse segments of tissue from different organs and organ systems including blood vessels, skin, lung, heart, kidney, and eyes [7,10,11].

There are two primary native tissue constituents that contribute to welding: tissue hydration, and the presence of collagen. Previous studies have made consistent observations that the tensile strength of welded tissues is a function of the laser wavelength – tensile strength at the weld joint increases when the wavelength of the laser corresponds to wavelengths on the absorption curve of water [2,3,9,12]. Moreover, near infrared (NIR) continuous wave (CW) lasers tuned to 1,455 nm have performed quite well during LTW procedures as this wavelength falls within one of the main vibrational over-tones of water [2,4,5,12]; this allows for a more
even distribution of heat in the tissues and provides a full thickness weld for up to 2 mm thickness, closely matching the optical penetration depth of the laser [12]. Therefore, it is imperative for the tissue to be hydrated prior to welding. Collagen is one of the most abundant proteins in mammals, accounting for up to 30% of all proteins [13]. Collagen accumulates into fibers that form a triple helix, which is stabilized by bound water molecules that mediate hydrogen bonding between adjacent collagen molecules [5]; this cross linkage helps to maintain the distance between collagen fibers, which both provides the structural integrity to the overall helix and allows various tissues such as cornea, bone, skin, cartilage, and tendons to withstand stresses without damage [6,10].

The literature has extensively documented that the mechanism that drives LTW involves the denaturation and partial renaturation of collagen due to thermal dissipation of energy from laser light and tissue interactions [2,3,10-15]. During welding by irradiation, water in the tissue absorbs the laser energy by vibrational overtones and combinations of water modes [5,6]; water molecules then subsequently transfer this energy in the form of vibrational energy to the collagen triple helix [4-7,12,14]. When the core tissue temperature reaches 60-80°C [4,6,7,14], the bonds in the triple helical structure of collagen, namely hydrogen bonds, hydrophobic interactions, electrostatic interactions and some interchain covalent bonds, are disrupted, leading to partial reversible denaturation for a short duration of time [10-12]. This is followed by covalent and/or non-covalent bonding and cross linking of the proteins as the tissue cools, resulting in the formation of watertight full-thickness welds [4] that are indistinguishable from non-welded tissues under histopathologic or electron microscopic examination [11]. A full-thickness weld thus provides a measurable tensile strength of the tissue at the weld joint.

There are many laser parameters that can affect the welding procedure, including the wavelength, fluence, pulse duration, temperature, spot size, and the composition of the type of tissue [9,12]. In addition, successful welds require precise control of laser power and irradiation times to control tissue de-hydration and thermal damage [12]. To improve the tensile strength of weld-ed tissue and remove the accompanying thermal damage, some researchers have added dyes and protein solders, such as albumin and fibrin, during the welding procedure [3]. Other groups have used photoactive dyes to localize the light absorption to the desired tissue region, thereby eliminating unwanted collateral injury [3,11]; however, the major disadvantage is that these additional elements increase the likelihood of contamination and viral infection, pigmentation, and local cellular toxicities due to degradation products of these dyes and solders.

![Optical density (O.D) spectra of key NIR optical absorbers, including collagen. The collagen absorption peak at 1720 nm is due to the amino acid backbone of the protein, which was targeted for LTW in this study (Ref. [21]).](image)

Fig. 1. Optical density (O.D) spectra of key NIR optical absorbers, including collagen. The collagen absorption peak at 1720 nm is due to the amino acid backbone of the protein, which was targeted for LTW in this study (Ref. [21]).
NIR lasers have been employed as an effective, noninvasive optical technique for imaging tissues due to a reduction in scattering achieved at wavelengths between 650 nm - 950 nm [16-20]. This region on the NIR spectrum is often called the “first therapeutic window” [16,18,19]. In recent years, Lingyan Shi discovered the optimal optical window for deeper brain and tissue imaging from 1,600 nm – 1,870 nm [16-18], which has been coined the “Golden Window.” Due to greater imaging and sensing depth [17], Shi suggests that this NIR optical window may be ideal for examining different constituents of tissue, including collagen [16,17,20,21]. Moreover, as shown in Figure 1, collagen shows a peak in the optical density spectrum between 1,700 nm and 1,750 nm, which falls within the “Golden Window.” While many groups have attempted LTW by means of thermal mechanisms (i.e., water acts as a heat transfer agent) and non-thermal mechanisms (i.e. femtosecond / picosecond pulsed lasers) [1,10] with varying tensile force and tensile strength measurements, as shown in Table 1, few have made attempts by means of direct collagen excitation, which has the potential for better welding.

The focus of this study is to investigate the efficacy of tissue welding through direct collagen excitation at 1,720 nm – in the Golden Window – using ex vivo porcine skin tissue. The tensile strengths of welded tissue that have been welded with NIR CW diode lasers at 1,455 nm and 1,720 nm are compared.

2. Methods and Materials

2.1 Laser Systems

In this study, continuous-wave diode lasers at 1,455 nm and 1,720 nm wavelengths were used for tissue welding. The erbium fiber laser used was a B&WTek model BWF2 (B&W Tek, Inc., Newark, Delaware) that emits at a wavelength of 1,455 nm and had a maximum power output of 660 mW. The 1,720 nm laser (ALC-1720-03000-EM200,22-R, Akela Laser Corporation, Jamesburg, New Jersey) had a maximum power output of 1.25 W. Other pertinent parameters including laser spot size, scan speed, total number of scans, total irradiation time, and laser fluence are listed in Table 2. The fluence was measured in joules per square centimeter; it was calculated by taking the product between the dwell time and the laser power and dividing that number by the area of the laser beam. The dwell time was calculated by dividing the beam diameter by the scan speed of the laser. The energy density per unit volume was calculated by dividing the fluence by the tissue thickness, with the assumption that at a given power level and dwell time, the energy is equally distributed across the full thickness of the weld and totally absorbed by the tissue. For all the experiments, the beam diameter was kept constant at 80 µm.

2.2 Tissue Samples

The porcine skin tissue was purchased from a local butcher who had removed the excess subcutaneous fat. The skin was extracted from the dorsal region of the animal and was cleaned with 0.9% saline solution. The samples were cut into 10x20 mm² rectangular pieces, each having approximately 2 mm thickness. Prior to welding, the samples were left at room temperature in 0.9% saline solution to ensure adequate hydration of the tissues. A full-depth incision was then made using a scalpel in the center of the pig skin, yielding two separate 10x10 mm² pieces, as shown in Figure 2.

2.3 Tissue Welding Apparatus

The tissue welding system, as depicted in Figure 3, consisted of a computer-controlled translation stage for sample positioning that can be moved in a plane normal to the incident laser beam with micrometer resolution. The tissue holders connected to the translation stage and equipped with micrometer screws were used to keep the cut edges apposed in position during the welding process, as illustrated in Figure 2. The laser beam was focused on the middle
of the sample, between the two pieces along the incision line; the beam followed a simple zigzag pattern, as shown in Figure 4, along the length of the incision with an amplitude $A$ and a step size $\Delta x$ both equaling approximately 0.8 mm.

**Fig. 2.** Tissue sample extraction and preparation.

**Fig. 3.** Diagram of the laser system and the tissue welding apparatus [Ref. 10].

**Fig. 4.** Laser scan pattern.
Table 1. Previously recorded tensile force/tensile strength measurements using different lasers, wavelengths, and tissue samples, both in vivo and ex vivo.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tensile Strength (N/cm²)</th>
<th>Tensile Force (N)</th>
<th>Stress (N/cm²)</th>
<th>Strain (%)</th>
<th>Temperature (°C)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>2.60-3.10</td>
<td>2.60</td>
<td>1.00</td>
<td>3.00</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>Cartilage</td>
<td>1.80-2.20</td>
<td>1.80</td>
<td>0.60</td>
<td>2.00</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>3.40-3.90</td>
<td>3.40</td>
<td>1.40</td>
<td>3.00</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>1.50-1.90</td>
<td>1.50</td>
<td>0.60</td>
<td>2.00</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>2.60-3.10</td>
<td>2.60</td>
<td>1.00</td>
<td>3.00</td>
<td>2.00</td>
<td></td>
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<tr>
<td>Cartilage</td>
<td>1.80-2.20</td>
<td>1.80</td>
<td>0.60</td>
<td>2.00</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>3.40-3.90</td>
<td>3.40</td>
<td>1.40</td>
<td>3.00</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>1.50-1.90</td>
<td>1.50</td>
<td>0.60</td>
<td>2.00</td>
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</table>
2.4 Measurement of Tensile Force

Immediately following the welding procedure, the tensile force for each skin sample, which was kept at room temperature and maintained at the level of dehydration that occurred during welding, was measured using a digital force gauge (model EF025, Mark-10, Hicksville, NY), as conducted in prior studies [2,11,12,14]. The clip attached to the digital force gauge gripped one end of the tissue sample, while the other end of the sample was gripped by another clip (Figure 5). The tension force was applied perpendicular to the weld joint until the welded site broke. This breaking force was recorded, as shown in Table 2, and then divided by the surface area of the weld, as calculated from the length and the thickness of the incision, to acquire a tensile strength value. Differences in the tensile force (n = 7 and n = 11 for the tissues welded with the 1,455 nm and 1,720 nm CW diode lasers, respectively) were compared by a two-sided Student’s t-test.

Fig. 5. Diagram of the digital force gauge used for the tensile force measurement (Ref. [14]).
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Tissue 1</td>
<td>1.69 ± 0.69</td>
<td>0.36 ± 0.23</td>
<td>1.69 ± 0.69</td>
<td>0.36 ± 0.23</td>
<td>1.69 ± 0.69</td>
<td>0.36 ± 0.23</td>
<td>1.69 ± 0.69</td>
<td>0.36 ± 0.23</td>
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<td>0.36 ± 0.23</td>
<td>1.69 ± 0.69</td>
<td>0.36 ± 0.23</td>
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</table>
3. Results

Table 3 displays a summary of the total number of welds as well as the success rate. A weld was considered successful when a measurable tensile breaking force could be measured using the digital force gauge. Figure 6 shows results of LTW performed with 1,455 nm and 1,720 nm. The tensile force measurements using both lasers are shown in Table 2. The average tensile force of the welded pig skin using the CW 1,455 nm laser was 0.457 N. The average tensile force of the welded pig skin using the 1,720 nm laser was 1.663 N, approximately four times greater. There was a statistical significance in mean tensile force between pig skin welded with the CW 1,455 nm laser and the CW 1,720 nm laser, with $P = 0.035$. Figure 7 and Figure 8 display the tensile strength plotted against the energy density for the 1455 nm and 1720 nm lasers, respectively. The interpolated data in Figure 8 was determined by using a cubic spline.

<table>
<thead>
<tr>
<th></th>
<th>CW 1455</th>
<th>CW 1720</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Samples Welded</td>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td># of Successful Tissue Welds</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td># of Failed Tissue Welds</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>% Success</td>
<td>46.67%</td>
<td>47.83%</td>
</tr>
</tbody>
</table>

Fig. 6. Welded tissue using the (A) 1,455 nm CW laser and (B) 1,720 nm CW laser.

4. Discussion

The average tensile force of the welded pig skin using the CW 1,455 nm laser was, on average, smaller than those determined from the values listed in Table 1. This may be attributed to the changes in laser power and the type of laser used in the given experiment, as well as changes to the scan frequency and total irradiation time. In the present study, the differences in the laser power levels between the 1,455 nm and 1,720 nm were due to limitations of the laser systems. Since the 1,455 nm laser had a lower achievable power, the scan rate, total number of scans, and the total irradiation time had to be adjusted to both produce adequate weld joints with a measurable tensile force and mitigate visible thermal damage, which is generally indicated by slight discoloration and translucence at the weld joint. Moreover, since the molecular mechanisms involved in tissue welding were inherently different between the two laser wavelengths, the two methods required different laser and weld parameters. Figures 7 and 8 indicate that a correlation exists between energy density and tensile strength; however, there
is a point where optimal energy density is reached and the overall tensile strength does not increase further. While comparing tensile forces of welds accomplished with the 1,455 nm and 1,720 nm lasers, our findings show that the welds completed with the 1,720 nm laser were approximately four times greater. Therefore, it seems reasonable that direct collagen excitation provides significant improvements and induces collagen crosslinking, yielding stronger welds than traditional thermal mechanisms.

**Tensile Strength vs. Energy Density for CW 1455 nm**

![Graph showing tensile strength vs. energy density for CW 1455 nm](image)

*Fig. 7. Plotting tensile strength against energy density for the CW 1455 nm laser*

**Tensile Strength vs. Energy Density for CW 1720 nm**

![Graph showing tensile strength vs. energy density for CW 1720 nm](image)

*Fig. 8. Plotting tensile strength against energy density for the CW 1720 nm laser*
While these results are promising, challenges still exist in LTW, including inconsistent weld strength, the risk of thermal damage, tissue dehydration, and tissue buckling – tissue separation along the weld line due to thermal expansion [11]. Earlier studies by our group [10] showed that LTW by femtosecond laser pulses in the NIR range resulted in better welds with minimal tissue damage; additionally, during \textit{in vivo} studies, better wound healing was also observed when compared to LTW using CW NIR lasers. It was noted that the molecular mechanisms of femtosecond LTW were due to nonthermal mechanisms as the result of short pulses of lasers with extremely high peak powers (8.3 kW) as compared to CW lasers with an average power of only 300 mW. The molecular mechanisms of LTW using CW lasers, as mentioned earlier in the introduction, is primarily a thermally-dependent phenomenon involving reversible denaturation of collagen in the tissue as a result of increased temperature of the water within the tissue; however femtosecond pulses less than 100 femtoseconds with a pulse-to-pulse duration of 13.33 nanoseconds [10] would result in the reversible breakdown of weaker bonds such as hydrogen bonds and hydrophobic interactions by nonthermal mechanisms, thus mitigating the issue of thermal damage and tissue dehydration. The results from the current study will help inform the selection and modification of welding parameters to be used in future \textit{in vivo} studies using CW lasers and ultrafast laser within the NIR optical window III. We anticipate that these future studies will help address the aforementioned issues in LTW as well as provide a more realistic \textit{in vivo} model that more accurately replicates clinical applications.

5. Conclusions

The tensile strengths of welded pig skin with NIR CW diode lasers at 1,455 nm and 1,720 nm are compared. 1,720 nm welds yield tensile strengths that are approximately four times greater than that accomplished at the 1,455 nm wavelength.

Acknowledgements

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Disclosures

The authors declare no conflicts of interest.

References


