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Welding Artificial Biomaterial with a Pulsed Diode Laser and Indocyanine Green Dye

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ABSTRACT

Laser tissue welding is a sutureless method of wound closure that has been used successfully in nerve, skin, and arterial anastomoses. We welded an elastin-based biomaterial that elicits minimal foreign body reaction to the intimal surface of porcine aorta. The aorta was stained with indocyanine green dye to efficiently absorb the 808 nm diode laser light. Laser welding with a pulsed diode laser thermally confines heating to stained portion of tissue, minimizing adjacent tissue damage. Laser welds of stained aorta to biomaterial were attempted by sandwiching the samples between glass slides and applying pressures ranging from 4–20 $\mathrm{N/cm^2}$ for 5 ms pulse durations and 83 $\mathrm{mJ/mm^2}$ radiant exposure. Welds were successful for pressure above 5 $\mathrm{N/cm^2}$. Transmission measurements of stained aorta were made using radiant exposures of 6–129 $\mathrm{mJ/mm^2}$ using pulse durations of 0.5–5 ms. Transmission increases and reaches a maximum of 80–85% with successive pulses for radiant exposure greater than 26 $\mathrm{mJ/mm^2}$ for a spot size of 9 $\mathrm{mm^2}$ and 13 $\mathrm{mJ/mm^2}$ for a spot size of 36 $\mathrm{mm^2}$.

1. INTRODUCTION

Laser tissue welding is the process of uniting or fusing two pieces of tissue with a laser. Upon cooling, a bond is established between tissue edges. Tissues that have been successfully welded include gallbladder, intestine and artery.[1–3] The advantages over traditional suture and staple methods are no foreign body reaction, less scar formation, no leakage, and shorter operating times.[4] The disadvantage is that the mechanism is poorly understood, resulting in confusion over the ideal parameters for welding. It is not known if the mechanism varies for different types of tissue, laser wavelengths, and temperature at the weld site. Finally, the success of the weld is operator dependent — one surgeon may develop a technique that cannot be replicated by another surgeon.

Laser tissue welding is primarily a thermal process. Therefore, we are interested in heating of only the weld site. In studies of laser tissue interaction Jacques defined the optical zone as the depth at which the fluence has dropped to 35% of the original radiance.[5] At pulse durations below

$$t = d^2/\kappa$$

heat confinement is achieved, where κ is the thermal diffusivity of the tissue. Our laser source was an 808 nm pulsed diode laser with a pulse length of 1–5 ms.

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A light absorbing chromophore is applied to the weld site: this dye has strong absorption peaks at the laser wavelength used. The strong absorption localizes heating to the stained area. We used indocyanine green as our chromophore, which has a peak at 775 nm in water.

The elastin based biomaterial used in these welding studies has had preliminary success in cardiovascular, urological, and gastro-intestinal repair.[6] The biomaterial is an artificial connective matrix made of elastin, fibrin, and collagen. The proteolytic action of thrombin on fibrinogen produces soluble fibrin monomers. These soluble fibrin monomers form a stable adduct with elastin, and a connective matrix is then formed when these fibrin-elastin adducts are connected by insoluble fibrin.

2. MATERIALS AND METHODS

2.1. Biomaterial Recipe

To make the biomaterial, 280 mg of filtered insoluble elastin was swollen in an excess of phosphate buffer*. Only 40 μm or smaller particles were used. The mixture was vortexed, centrifuged, and the excess buffer discarded. Then, the swollen elastin was dissolved in 2 ml of phosphate buffer. To this was added 2 mg of collagen in 0.6 ml of phosphate buffer, 67 mg of fibrinogen (Sigma) in 1 ml of phosphate buffer, and 200 μL of 14 mg/ml thiorea solution. 33 units of thrombin in 0.2 ml of water was added to the elastin mixture. The mixture was vortexed and quickly poured into molds. The molds were incubated in a 37° water bath for 30 minutes.

The biomaterial was stored in 33% ethanol solution at 4°. One hour before use it was transferred to a 0.9% saline solution at room temperature, and 15 minutes before use the biomaterial was transferred to fresh saline solution.

2.2. Penetration Depth of Indocyanine Green

Indocyanine Green (ICG) is the most widely used colorimetric indicator for circulatory studies.[7] It has a peak absorption of light at 805 nm when bound to albumin, and a peak at 775 nm when dissolved in water. ICG degrades over time when exposed to light, so proper storage in a light tight environment is important for insuring the same quality with each staining. We used ICG as the chromophore in all our experiments. It is important to know how far ICG is penetrating into the aorta and if it is penetrating evenly so we have an accurate depth for the optical zone. We measured the penetration depth of ICG in water, albumin and healon solder on fresh and previously frozen aorta. A saturated solution of 5 mg/ml of ICG was used in water and in 25% human serum albumin. The healon solder was made from 0.85 ml of 5 mg/ml ICG in 25% albumin and 0.85 ml of sodium hyaluronate. After each ICG solution was applied with a disposable pipette the ICG was allowed to soak into the aorta for 1, 2, 3, 4, 5, 10, 15, and 20 minutes before being blotted away using cheese cloth. The samples were stored in the freezer for easier observation on its side under the microscope. Measurements were taken by observing cross sections, and by measuring the depth of penetration with a reticule in the eyepiece.

*The buffer contained in one liter of water, 0.103 g of $NaH_2 \cdot 4H_2O$, 0.035 g of Na_2PO_4, 0.203 g of $MgCl_2 \cdot 1H_2O$, 0.147 g of $CaCl_2 \cdot 1H_2O$, and 8.76 g of NaCl.

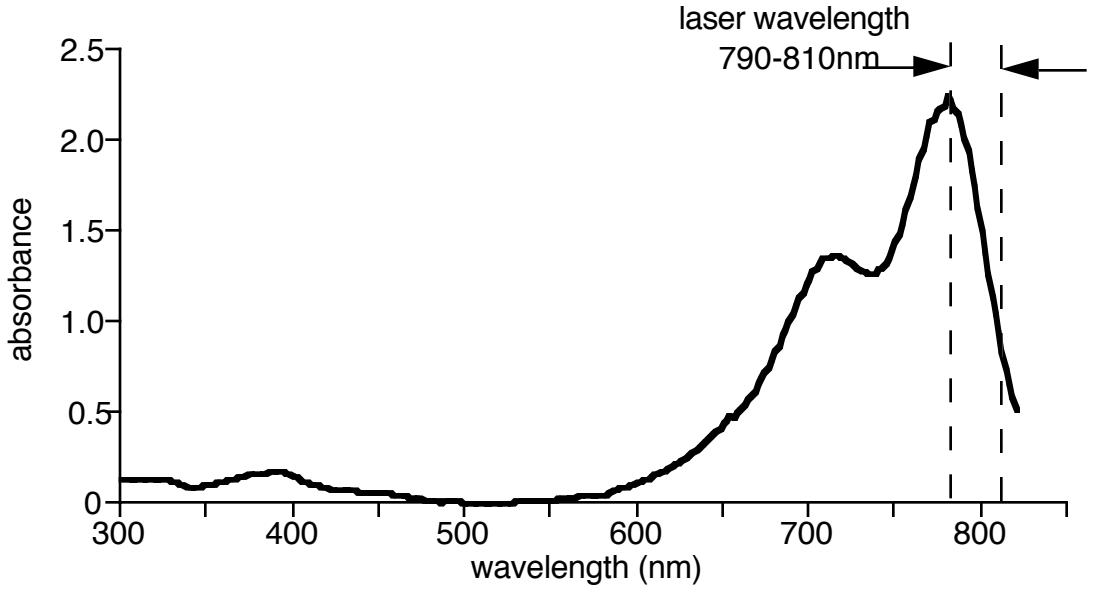

Figure 1. Absorption spectra of indocyanine green in water.

2.3. ICG Transmission Measurements

We noted that ICG bleaches from green to orange after exposure to a series of diode laser light pulses. Postulating that bleaching could be a factor in the mechanism of welding, bleaching of ICG was assessed on aorta that was previously frozen and then immediately stained with 5 mg/ml ICG solution in water. The intima of thawed aorta was stained with ICG solution by using a disposable pipette to spread the ICG evenly. The excess was blotted away using cheesecloth. The aorta was kept under cheesecloth moistened with saline to prevent dehydration. Pieces of aorta were trimmed to approximately 2×3 cm to fit between two glass slides, and the slides were held together using tape.

Our laser source was a pulsed diode laser at wavelength between 790–810 nm from Star Medical Technologies. A condenser was fitted to the handpiece of the laser, yielding a uniform square 3×3 mm or 6×6 mm spot. The condenser was placed flush against the glass slide so that the laser light passed through the ICG layer first. Pulse length and laser current could be selected over a range of energies with an output range of 0.15–3.25 J and 0.5–5 ms. Optimum power was maintained by good condition of the highly reflective condenser.

An integrating sphere six inches in diameter coated with 98% reflecting Spectralon from Labsphere was used to make transmission measurements of the stained aorta. The sphere had four ports, each at 90° around the circumference of the sphere. We mounted a sample of stained aorta in front of a port so that the sample completely covered the port hole. The diode laser handpiece was mounted so that the condenser was flush against the sample. A silicon photodiode detector was mounted 90° from the sample. The other two ports were plugged. An internal baffle between the entrance port and the detector port prevented light from the sample reaching the detector before being diffusely scattered.

The pulse length was held constant and the radiant exposure was varied. The laser delivered 0.5, 1, 3, and 5 ms pulses. Transmission measurements were done using the 6×6 mm and 3×3 mm condensers. Transmission was measured after each pulse. Transmission measurement of an unstained piece of aorta was used as a reference for maximum transmission. Transmission was calculated as the ratio of voltage change for stained aorta and voltage change for unstained aorta.

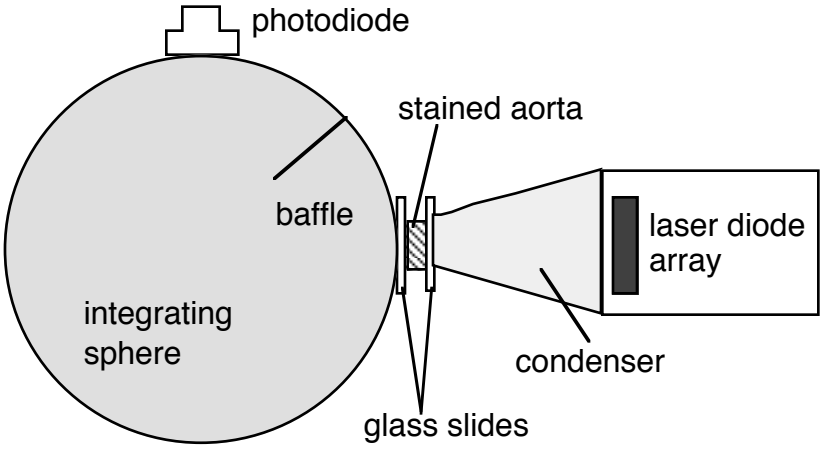

Figure 2. Transmission measurements set up

2.4. Welding with Pressure

To determine the pressure needed to make a strong weld of biomaterial to aorta we made a glass slide, biomaterial, aorta, slide sandwich. We used a scale that measured up to 2.5 kg, and a brass ring to exert the pressure. The condenser fit within the ring. The sample size of the aorta/biomaterial sample varied from 1 cm^2 to 1.5 cm^2.

3. RESULTS

We measured penetration depth of the ICG into stained aorta, transmission measurements of the stained layer of aorta, and pressure needed to achieve a successful weld between biomaterial and stained aorta.

3.1. Penetration Depth of Indocyanine Green

ICG in both albumin and healon solder did not penetrate well or evenly into the aorta. The stained aorta appeared grainy with approximately 100 μm diameter spots. ICG in water penetrated the intimal layer to depths varying between 100–400 μm. The depth was difficult to measure because the ICG appeared to soak through in layers—the inner layers were lighter or less concentrated than outer layers. Nevertheless, ICG in water penetrated about 200 μm independent of time soaked.

Direct staining of aorta varied with technique. A dark stain is achieved when ICG is dropped onto the aorta and allowed to soak. A lighter less uniform stain is achieved when ICG is dropped onto and then massaged into the aorta.

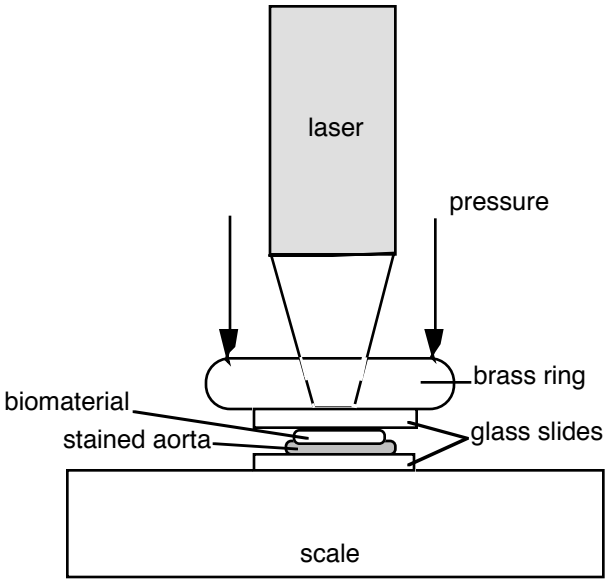

Figure 3. Welding with pressure monitored by a scale.

3.2. ICG Transmission Measurements

The bleaching effect observed consisted of a faint pale spot that with more pulses deepened into an orange square spot the size of the condenser. The first appearance of pale orange was defined as faint bleaching. At sufficient radiant exposure, the orange bleaching appeared more reflective with each successive pulse. This visual change in appearance was defined as maximum bleaching. Further laser pulses darkened the orange spot, and the spot spread approximately 1 mm beyond condenser dimensions. This effect was noted only for the highest radiant exposure for each condenser.

Transmission measurements were calculated from voltage data taken from the oscilloscope. When bleaching occurred, a corresponding increase in transmission was noted.

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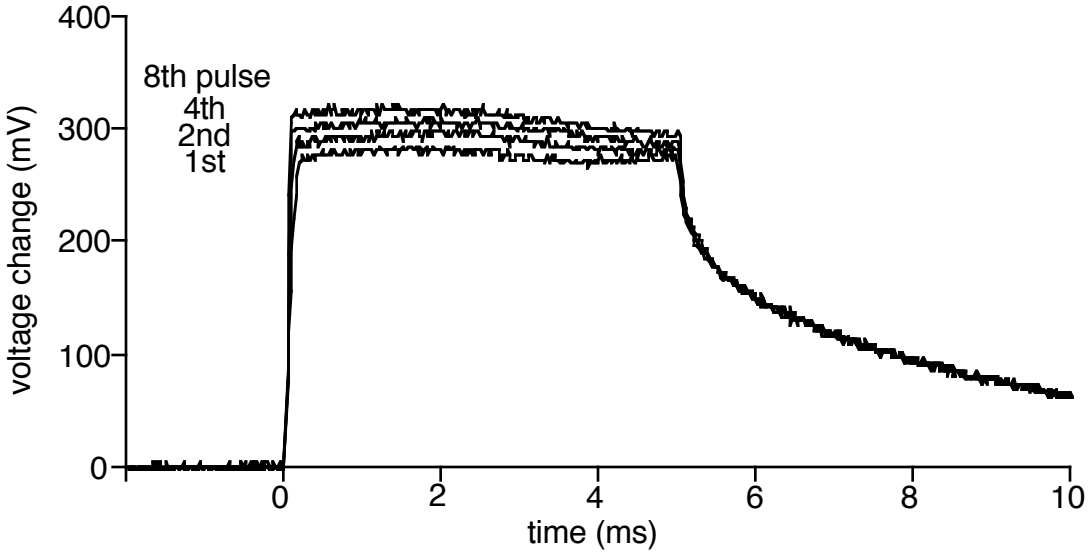

Figure 4. Increase in transmission over several pulses using the 3×3 mm condenser at 1.1 J per pulse

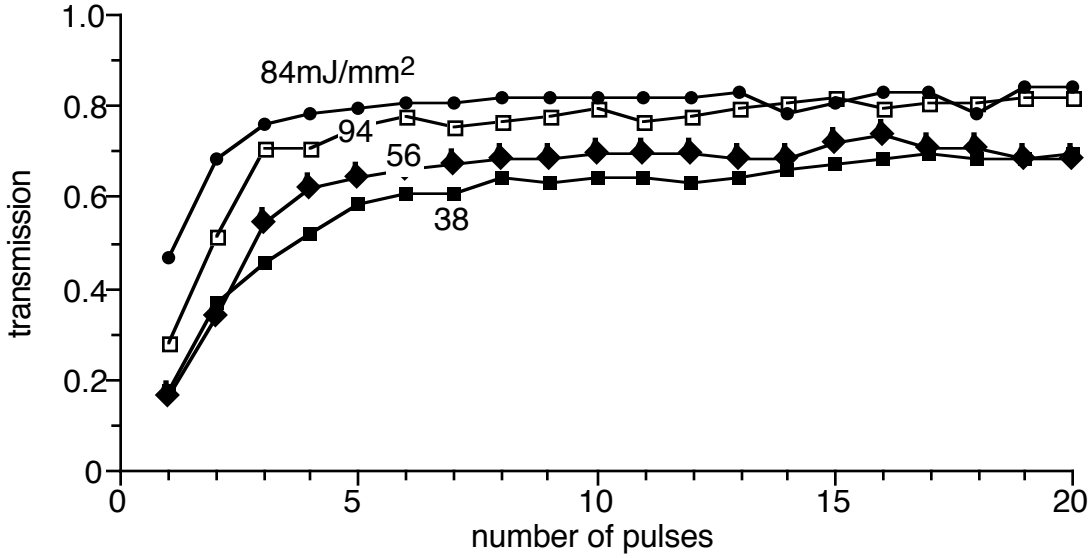

Figure 5. Transmission for 3 ms pulse durations, 3×3 mm condenser. Notice increase in transmission over first several pulses.

3.3. Welding of biomaterial and aorta with pressure

Welds were successfully achieved with sufficient pressure. Under the conditions we used, pressures greater than $5\,\mathrm{N/cm^2}$ were needed to achieve a minimum weld. Results are shown below.

Pressure ($\mathrm{N/cm^2}$)	Area ($\mathrm{cm^2}$)	Weight (kg)	Weld Quality
4.8	3.06	1.5	*no weld*
5.0	1.95	1.0	*very weak*
6.3	1.87	1.2	*medium*
8.9	1.32	1.2	*medium*
11.6	1.69	2.0	*extremely strong*
11.7	1.68	2.0	*medium*
12.6	1.56	2.0	*strong*
14.8	1.32	2.0	*strong*
19.6	1.00	2.0	*strong*

Table 1. Pressures and corresponding weld qualities.

To determine how water content affected the pressure needed for welding, the biomaterial was blotted on a paper towel, and then welded without pressure, yielding a medium strength weld. Squeezing the biomaterial first, and then welding yielded a weak weld on the interior of the sample, but a strong weld along the edges.

4. DISCUSSION

4.1. Penetration Depth of Indocyanine Green

We tried three methods of ICG delivery to tissue: a saturated solution in water, albumin (blood protein) and healon solder. The albumin and solder samples yielded a grainy stain on the tissue, while the ICG in water sample was more uniform. This may arise because the ICG already bonded to the proteins in the first two solutions, and was unable to bond to proteins in the tissue. Because water yielded a more uniform stain, we decided to use a saturated solution of ICG in water in this series of experiments. The thickness of the stained layer was measured to be approximately $200\,\mu$m.

The light-absorptive properties of the dye localize and help heating. We need to know how much dye is in the tissue, and how deep the dye penetrates to predict temperatures at the weld site and in the surrounding tissue. By measuring the transmission of 808 nm light through the tissue, we can use these results to calculate a concentration of ICG in the stained layer. One measurement we need for these calculations is the thickness of the stained layer.

4.2. ICG Transmission Measurements

The transmission measurements were intended to monitor how ICG-tissue layer behaves when subjected to laser light. The transmission measurements of this layer were made using only aorta and no biomaterial. By comparing the penetration depth and transmission measurements we could calculate the absorption coefficient and an expected rise in temperature of tissue.

At low energies no bleaching or change in transmission was noted, and transmission through the layer was around 50%. At higher energies the transmission increased with successive pulses until a maximum was reached. At highest energies bleaching occurred within one or two pulses and transmission remained at the maximum level of 85%. We postulate that ICG is slowly being destroyed pulse by pulse. At the low energies insufficient energy is delivered to induce this effect. At mid-range energies each pulse has enough energy to wipe out the top layer of ICG, and so, since there is less ICG to absorb the light, the transmission through the sample increases. It takes several pulses to completely bleach through the layer. At highest energies, there is enough light to disintegrate the layer within one or two pulses. That is why transmission remains high over several pulses and no increase is seen.

From transmission, we can calculate corresponding absorption coefficient for each pulse. And then we can calculate expected increase in temperature.

$$\mu_a = -\frac{\ln T}{d}$$

$$\Delta\text{Temp} = \frac{\mu_a E_0}{\rho c}$$

T is transmission, d is the penetration depth of ICG, E_0 is radiant exposure, and ρc is the heat capacity of water. Since transmission is increasing with each pulse, we expect absorption coefficient and the corresponding increase in temperature to decrease with successive pulses.

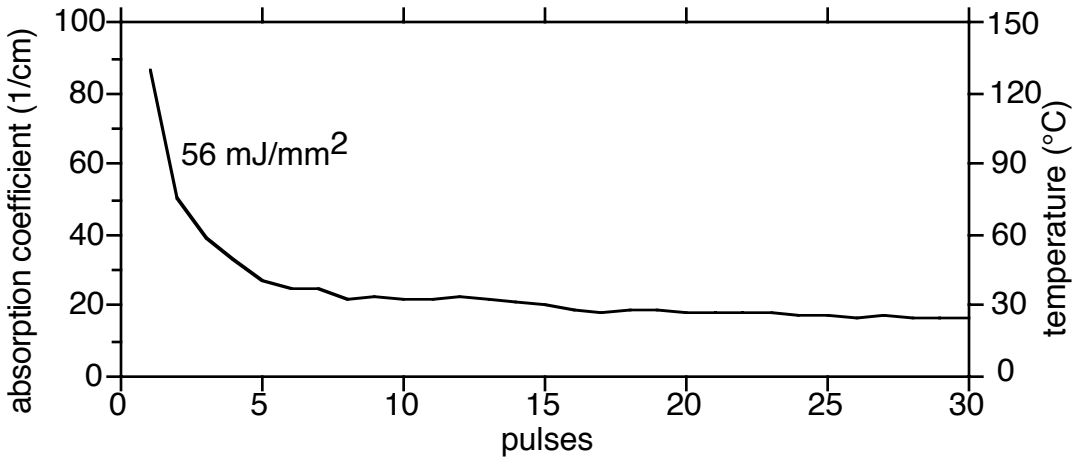

Figure 6. Absorption coefficient and expected increase in temperature for 3 ms pulse duration, 3×3 mm condenser.

4.3. Preliminary Welding

We decided to attempt to weld aorta to biomaterial using the highest energy setting for the 3×3 mm condenser. Welds without pressure were unsuccessful. It is important to note that no welds were achieved at any energy without ICG.

Bleaching of either the aorta or the biomaterial was often observed around the edges of our welding sample. Welds were strongest in these areas. Insufficient pressure prevented welding regardless of bleaching. Bleaching may indicate when enough light has reached that particular area, and when the operator may move to the next area to be welded.

In later experiments we noticed that the more dehydrated the biomaterial, the less pressure was needed to weld. One possible explanation is that squeezing out the water allows for more contact between biomaterial and aorta. This reduces the heat capacity and therefore the sample reaches a higher temperature. The layer of ICG stained aorta was distinct when viewed under the microscope after welding. In fact, in some cases this layer could be peeled away before and after welding. It is not clear if this was due to dehydration of the aorta, or an effect of the ICG on the intimal layer.

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