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Patch Welding with a Pulsed Diode Laser and Indocyanine Green

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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. An elastin-based biomaterial patch was welded to the intimal surface of porcine aorta in the present study. 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 the 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 to 20 N cm^{-2} for 5 ms pulse durations and 83 mJ mm^{-2} radiant exposure. Bleaching of the indocyanine green by as much as 85% was observed after exposure laser irradiation. Finally, successful welds required 5 N cm^{-2} of pressure between the elastin biomaterial and aorta.

INTRODUCTION

Laser tissue welding is the process of uniting or fusing two pieces of tissue by heating with a laser. Upon cooling, a bond is established between tissue edges. Tissues that have been successfully welded include gallbladder, intestine and artery (1). The advantages over traditional suture and staple methods are no foreign body reaction, less scar formation, no leakage and shorter operating times (2). 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. This paper integrated several welding improvements into a single study; using an exogenous dye, using pulsed laser heating, using patches and using an elastin-based biomaterial.

Laser tissue welding is primarily a thermal process. The authors would like to heat only the area to be welded to minimize collateral tissue damage. By applying an exogenous light absorbing chromophore, it is possible to localize the deposition of the laser energy (3, 4). Since tissue absorption in the infra-red is relatively low, indocyanine green (ICG) was chosen as the chromophore. By adding ICG at the interface between the tissues and irradiating with 808 nm light, the difference in absorption between tissue and ICG ensures that the majority of the laser energy will be deposited at the site of welding.

To minimize the collateral thermal damage, it is also necessary to deposit the laser energy faster than it can thermally diffuse (5). Consequently, the pulse duration required must be less than $d^2\ \kappa^{-1}$, where d is the thickness of the heated layer and κ is the thermal diffusivity. Since $d \sim 200\,\mu m$, this means that the pulse duration must be less than 300 ms. Laser pulse durations of 1–5 ms were used for all the experiments. One final advantage of using pulsed laser delivery is that a welding feedback system becomes simpler to engineer; there is a relatively long period between pulses during which the decision to deliver another pulse can be made.

The combination of a small area to be welded and an awkward geometry make joining the ends of two vessels together with a laser a challenging problem. The problem of creating viable vessel or nerve anastomoses

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has been the focus of the majority of welding studies (1). The present authors chose to weld patches to flat surfaces to simplify the welding process.

Another advantage of the patch geometry is that the pressure between the two surfaces during the welding process can be easily monitored. From preliminary work, it had been observed that firm contact between the two surfaces was necessary to create strong welds.

The elastin-based biomaterial used in these welding studies has had preliminary success in cardiovascular, urological and gastrointestinal repair (6, 7). 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.

MATERIALS AND METHODS

Porcine aorta

All aortic tissues were obtained at Carlton Packing Co., Carlton, OR, USA. They were freshly cut and placed on ice. Frozen samples were kept at -10 °C until thawed for use.

Elastin-based biomaterial

The elastin-based biomaterial is made using 280 mg of filtered insoluble elastin that has been swollen in an excess of phosphate buffer. Only 40 μm or smaller particles were used. The mixture was vortexed and centrifuged, and the excess buffer was discarded. Next, 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 0.2 ml of 14 mg ml^{-1} thiourea solution. Thirty-three 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° C water bath for 30 min. The biomaterial was stored in 33% ethanol solution at 4 °C. One hour before use it was transferred to a 0.9% sodium chloride solution at room temperature, and 15 min before use, the biomaterial was transferred to fresh saline solution.

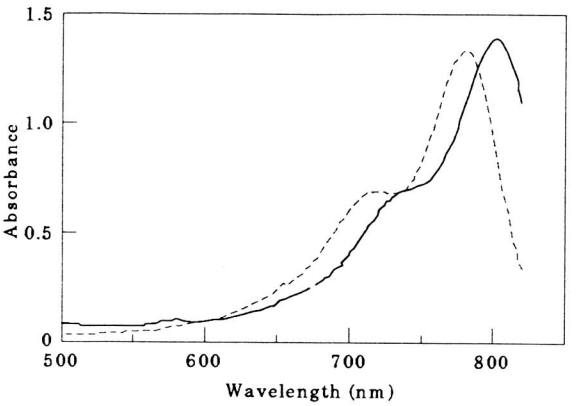

Fig. 1. The absorbance of 0.01 mM indocyanine green (ICG) in water and when bound to albumin. The laser emission from 790 to 810 nm coincides closely with the peak absorption of ICG bound to albumin. ——, ICG in albumin; – – –, ICG in water.

ICG deposition

Indocyanine green was previously widely used as a colorimetric indicator for circulatory studies (8). It has a peak absorption of light at 805 nm when bound to albumin, and a peak at 775 nm when dissolved in water (Fig. 1). To assess the absorption coefficient, it is necessary to know how deeply ICG penetrates into aorta. The penetration depth of a saturated solution of ICG in water, albumin and sodium hyaluronate solder was measured in fresh and previously frozen porcine aorta. A saturated solution of 5 mg ml^{-1} of ICG was used in water and in 25% human serum albumin. The sodium hyaluronate solder was made from 0.85 ml of 5 mg ml^{-1} ICG in 25% albumin and 0.85 ml of 10 mg ml^{-1} sodium hyaluronate (9). 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 min before being blotted away using cheesecloth. The samples were stored in the freezer for easier observation under the microscope to measure the penetration depth.

ICG degradation

It was 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^{-1} ICG solution in water. The intima of thawed aorta was stained with

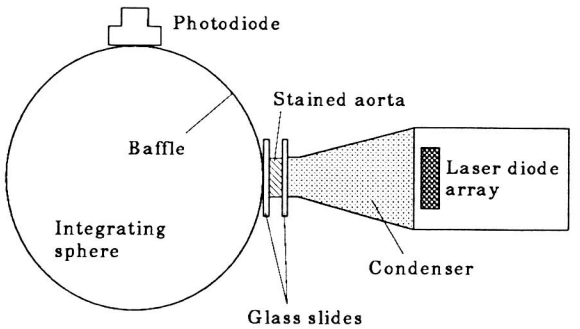

Fig. 2. Illustration of the diode laser, its condenser and the integrating sphere used to measure the transmission.

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.

The present laser source was a pulsed diode laser at wavelength between 790 and 810 nm from Star Medical Technologies, Pleasanton, CA, USA. A condenser was fitted to the handpiece of the laser. The non-imaging optical condenser was made of polished copper that was shaped to collect the light from the diode array and redistribute it over a small spot. Two condensers were used that uniformly illuminated either a 3 × 3 mm or a 6 × 6 mm square. The tip of 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.

An integrating sphere 6″ in diameter coated with 98% reflecting Spectralon from Labsphere (New Hampshire) was used to make transmission measurements of the stained aorta (Fig. 2). The sphere had four ports, each at 90° around the circumference of the sphere. A sample of stained aorta was mounted 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 at 2 Hz. 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. As the typical transmission through an unstained sample was more than 80%, with about 10% of the apparent losses due to surface reflections (top and bottom), it was assumed that the thin ICG layer could be treated as an absorbing-only surface layer. In this case, the transmission of the ICG layer is the ratio of the transmission of the stained layer to the transmission to the unstained layer.

Aorta-biomaterial welding

To determine the pressure needed to make a strong weld of biomaterial to aorta, a glass slide, biomaterial, aorta, slide sandwich was used. A scale that measured up to 2.5 kg, and a brass ring to exert the pressure were used. The condenser fit within the ring. The sample size of the aorta/biomaterial sample varied from 1 to 1.5 cm^{-2}. The laser was fired at 2 Hz until bleaching was observed.

Weld strengths were scored on a qualitative scale. A weak weld was defined as stickiness just at the corners of the sample. A medium weld was stickiness over the entire sample. A strong weld was reached when the corners of the biomaterial remained behind as the weld was pulled apart. An extremely strong weld occurred when the biomaterial ripped at all places on the sample as the weld was pulled apart.

RESULTS

ICG deposition

Indocyanine green in both albumin and sodium hyaluronate solder did not penetrate well or evenly into the aorta. The stained aorta was more lightly stained and appeared grainy with approximately 100 μm diameter spots. In water, ICG penetrated the intimal layer to depths varying between 100 and 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

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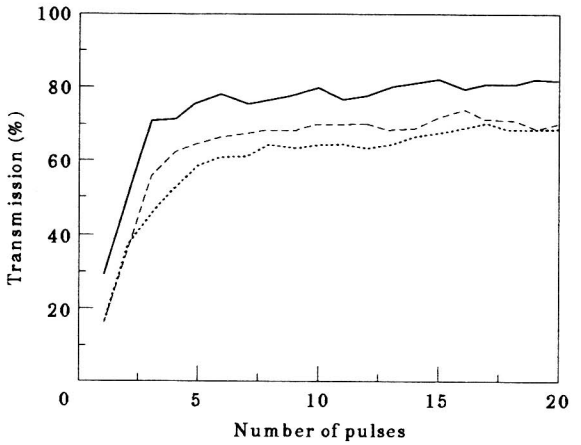

Fig. 3. Transmission at 800 nm after each 3 ms laser pulse using a 3×3 mm spot and three different radiant exposures. The radiant exposures shown are for a single pulse. The change is most dramatic for the first 4 or 5 pulses. ——, 94 mJ mm^{-2}; – – –, 56 mJ mm^{-2}; · · ·, 38 mJ mm^{-2}.

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.

ICG degradation

The bleaching effect observed consisted of a faint pale spot that deepened with exposure to more pulses into an orange square spot the size of the condenser (Fig. 3). 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. When bleaching occurred, a corresponding increase in transmission was noted. Bleaching was noted only for the highest radiant exposure for each condenser.

ICG temperatures

From transmission measurements of the ICG layer, the absorption coefficient μ_a can be calculated using Beer's law using a layer

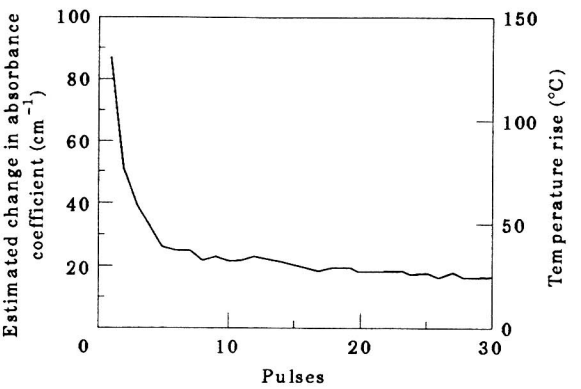

Fig. 4. The change in the absorption coefficient of the indocyanine green layer with successive 3 ms pulses and a 3×3 mm spotsize at 56 mJ mm^{-2}. The temperature rise is proportional to the absorption coefficient and is shown on the right-hand axis.

thickness of 200 μm. As the pulse duration is sufficiently short that the problem is thermally confined, the peak temperature increase ΔT caused by a radiant exposure E_0 is

$$\Delta T = \frac{\mu_a E_0}{\rho c} \quad (1)$$

where oc is the heat capacity of tissue (assumed equal to that of water). As the light transmission increases with each pulse, the absorption coefficient decreases, and consequently the peak temperature associated with each pulse will decrease (Fig. 4).

Aorta-biomaterial welding

Welds were successfully achieved with sufficient pressure. Under the conditions used, pressures greater than 5 N cm^{-2} were needed to achieve a minimum weld. Results are given in Table 1.

DISCUSSION

ICG deposition

Three methods of ICG delivery to tissue were tried: a saturated solution in water, albumin and sodium hyaluronate solder. The albumin and solder samples yielded a grainy stain on the tissue, while the ICG in water sample was more uniform. This arises probably because the ICG is bound to the proteins in the albumin and solder solutions. The majority of the ICG is consequently unable to bind to proteins in the tissue.

Table 1. Pressures and corresponding weld qualities

Pressure (N cm^{-2})	Area (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

No welds were achieved at any energy without indocyanine green.

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

ICG degradation

The transmission measurements were intended to monitor how ICG layer on aorta 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, the absorption coefficient and expected rise in temperature of tissue could be calculated.

At low pulse 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. This increase was permanent. At the highest pulse energies, bleaching occurred within 1 or 2 pulses, and transmission remained at the maximum level of 85%.

The transmission through unstained aorta decreases as the aorta is heated/cooked (10). This means that the estimated transmission will overestimate the actual transmission of the ICG. Decreases in the ICG transmission, therefore, cannot be attributed to underlying changes in the optical properties of the aorta.

Dimitrov et al have shown that 3 mM solutions of albumin-stabilized ICG were not thermally degraded by 2 h in a 100 °C water bath (11). Earlier studies by Gathje et al have shown that ICG bound to albumin is optically stabilized (12). Similarly, if the ICG degradation process were entirely photochemical, then 3 38 mJ mm^{-2} pulses should degrade more ICG than a single 94 mJ mm^{-2} pulse. This was not observed (Fig. 3).

Why does the ICG-stained aorta degrade? It seems likely that the ICG on the aorta is bound to proteins since the ICG cannot be washed or wiped off the aorta. Some stabilization (similar to that from albumin) is likely since the ICG on aorta does not bleach at low radiant exposures. This stabilization by albumin has been attributed to the anti-aggregation influence of albumin on ICG (13). Alternately, a solely thermal degradation process is unlikely because the temperature increases are for fractions of a second.

The authors postulate a synergistic effect between the thermal and optical processes. Indeed, basic chemical kinetics indicates that raising the temperature reduces the activation energy required for a photochemical degradation reaction. Low radiant exposures make a sufficiently small change in the temperature that very few absorbed photons have sufficient energy to exceed the activation energy for the degradation process. High radiant exposures lead to large temperature increases that allow a much larger fraction of the absorbed photons to cause photodegradation.

Aorta-biomaterial welding

A radiant exposure high enough for maximum bleaching over 4–5 pulses using a 3 × 3 mm condenser was used to weld aorta to biomaterial. No welds were achieved at any energy without ICG.

Bleaching of either the aorta or the biomaterial was often observed around the edges of the 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.

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In later experiments, it was 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 ICG layer on aorta was distinct when viewed under a microscope after welding. In fact, in some cases, this layer could be peeled away beforeand 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.

Dye-enhanced laser welding of aorta and elastin biomaterial proved to be a complicated problem. Bleaching of ICG was an unexpected result and is a result of the high fluences and temperatures associated with pulsed laser welding. However, once the behavior and concentration of ICG is known, and induced heating is predictable and reproducible, pulsed diode laser welding shows promise as a viable method for wound closure and healing.

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