Optimal dye concentration and power density for laser assisted vascular anastomosis (LAVA)

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ABSTRACT

Laser tissue welding with albumin solder/indocyanine green (ICG) dye is an effective technique in surgical reconstruction. This study was carried out in vitro to find optimal ICG concentration and power density (PD) in laser assisted vascular anastomosis (LAVA). Fresh porcine carotid arteries incised into vascular strips (n=120) were welded by diode laser in end-to-end with 50% albumin solder of 0.01, 0.1, and 1.0 mM ICG and at power density of 27.7, 56.7, and 76.9 W/cm^2. Direct temperature was measured by inserting thermocouples outside and inside vessel. Tensile strength was tested immediately and histological study was performed. Temperature (both outside and inside vessel) significantly gradually decreased ($p<0.01$) with the increasing of ICG concentration at PD 56.7 W/cm^2. Tensile strength significantly gradually decreased ($p<0.01$) with increasing of ICG concentration at PD 56.7 W/cm^2. Histological study showed minimal thermal injury limited to adventitia of vessels and no appreciable difference in all groups. We find that ICG concentration within solder is most important factor affecting both tissue temperature and tensile strength during laser vessel welding. The optimal balance between stronger strength and minimal thermal injury of vessel may be achieved primarily by using PD 56.7 W/cm^2 at 0.01 mM ICG within solder during LAVA.

Key words: laser welding, albumin solder, vascular anastomosis, indocyanine green, power density

1. INTRODUCTION

Since Jain and Gorisch first reported successful laser assisted vascular anaostomosis (LAVA),[1] many studies have been published on vascular anastomosis by laser welding. In comparison with conventional suture technique, LAVA has remarkable advantages such as providing an immediate watertight sealant,[2] reducing operative time,[3-5] faster healing,[6] ability to grow,[4] and reducing intimal hyperplasia owing to no foreign body reaction to suture material.[7] However, the main disadvantages of the laser-assisted procedure are the low strength of the resulting anastomosis,[2,8] especially in the acute healing phase up to 4 days postoperatively,[8] and tissue thermal injury including increased anastomotic pseudoaneurysm rate.[3,5,6,9] These disadvantages and lack of satisfactory objective criteria for optimal laser exposure parameters have limited wide clinical application of laser anastomosis.

From a surgical viewpoint, a satisfactory requirement of laser tissue welding is to obtain maximum weld strength with minimal tissue thermal injury. It has been shown that laser tissue welding using diode laser and albumin solder with indocyanine green (ICG) is an effective technique in surgical reconstruction such as in blood vessels,[10,11,12] urinary tract, [13] and skin[14,15] etc.. The results of laser tissue welding may be affected by several factors such as laser settings,[2,15] concentration of albumin solder,[16,17] ICG concentration in the solder,[10-14] and temperature of the tissue surface[18,19] etc.. Among these effective factors, it is known that the albumin solder with higher concentration resulted in significantly stronger tensile strength than the albumin solder with lower concentration.[16,17] However, there are little reports regarding optimal laser settings and concentrations of ICG in the albumin solder in LAVA.

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This study was carried out in vitro by describing temperature profiles of outside and inside vessel, tensile strength test and histology study while varying ICG concentration and diode laser power density (PD) in order to find optimal ICG concentration and laser parameters in LAVA.

2. MATERIALS AND METHODS

2.1 Tissue and albumin solder preparation

Fresh porcine carotid arteries were harvested from the animals sacrificed from our other research projects. The arteries were immediately placed in sterile 0.9% saline solution and transported to the laboratory at 4°C. Fat and excess adventitial tissues were removed. The arteries were incised longitudinally into strips. The arteries wall thickness ranged 1.1-1.3 mm (1.2 ± 0.01 mm, there was no significant difference among the all groups).

25% human serum albumin (Baxter Health Corporation, Glendale, CA) was filtered through an ultrafiltration membrane (YM 30, Millipore Corporation, Bedford, MA) to concentrate to 50% (w/v) by using an ultrafiltration system (Model 8400, Amicom, MA) under 60 psi pressure on a Stirrer/Hotplate (Model PC-320, Corning, USA) at 37°C. The albumin was then mixed well for 10 min with indocyanine green (Sigma Chemical Company, St. Louis, MO) solution on a Stirrer/Hotplate at 37°C. The final ICG concentration in the 50% albumin solder was 0.01 mM, 0.1 mM, and 1.0 mM, respectively. The solder (50%) was stored in 1 ml syringes at 4°C in the dark until use.

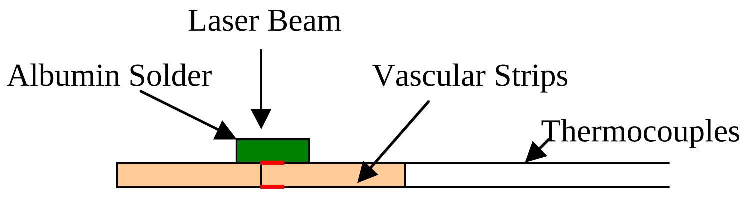
2.2 Diode laser system

Laser treatments were performed with diode laser module (Coherent, Model: FAP system, 1001A, Santa Clara, CA) coupled to a rounded quartz silica optic fiber (600 μm diameter). The laser system consists of phased array of gallium-aluminum-arsenide semiconductor diodes, and produces an invisible laser beam at 810 nm wavelength. A red aiming/pilot beam allows operator to visualize the spot size of the laser during activation.

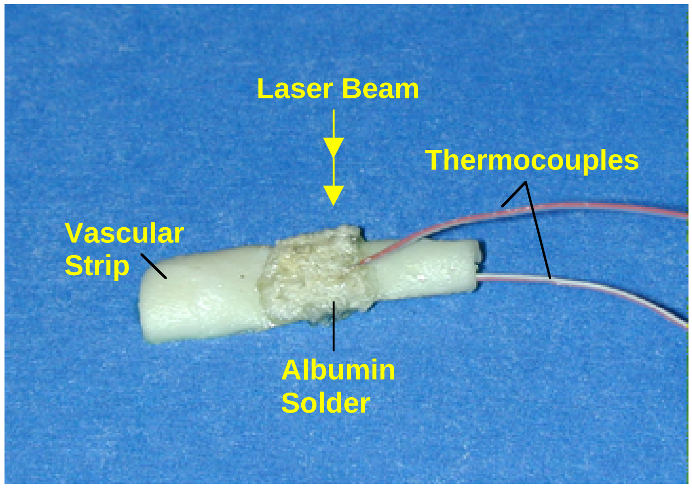
The spot diameter was 2 mm at a distance of 15 mm. The laser was set as a continuous wave (CW) at output powers of 870, 1800, and 2300 mW resulted in power densities (PD) of 27.7, 56.7, and 72.9 W/cm^2, respectively. The output power of the laser settings was measured by a Laser Energy/Power Meter (EPM 1000, Molectron Detector, Inc., Portland, OR) before and after welding in each experiment. Laser exposure time (the solder color change from green to tan) was recorded during the operation.

2.3 Experiment design and grouping

The vascular strips (n=120) were randomly divided into two groups. Group A: vascular strips were welded with diode laser in end-to-end at PD 56.7 W/cm^2 with 50% albumin solder at varying ICG concentration (0.01, 0.1, and 1.0 mM); Group B: vascular strips were welded with diode laser in end-to-end at 0.1 mM ICG concentration in the 50% albumin solder and at varying PD (27.7, 56.7, and 76.9 W/cm^2). Direct temperature measurements were obtained by placing thermocouples on the vascular strip surface within the solder and beneath the strip during the laser welding (Fig.1). The endpoint of laser welding was tan color change of the solder.[13,15] Tensile strength (TS) was tested immediately and histological study was performed after the welding.



(A)



(B)

Fig. 1. (A) and (B) show placement of the thermocouples relative to the solder joint.

2.4 Temperature measurement

36-gauge Type T thermocouples (Omega Engineering Inc., Stamford, CT) placed on the vascular surface (the albumin solder was directly placed above it) and inside the vascular lumen were connected to a data acquisition system controlled by LabVIEW. Signals from the thermocouple were processed by an AD595CQ monolithic chip amplifier (Analog Devices, Norwood, MA). This chip combines an ice point reference (0°C) with a precalibrated amplifier to give a 10 mV/°C output signal. The chip was powered by a +5 volt power supply. The output voltage was wired through a BNC adapter (BNC 2081), to a 12 bit, 100 kS/s data acquisition board, PCI-1200 (National Instruments, Austin, TX). A LabVIEW program sampled the data at a rate of 10 Hz. It then converted the input voltage to temperature (10mV/°C), recorded temperature vs. time for each thermocouple input, and then saved the data to an ASCII text file for further analysis. The thermocouples were cleaned up with alcohol between each measurement.

2.5 Tensile strength test

The welded vascular strips were loaded on the universal tester (Chatillon Materials Testing, Vitrodyna V1000, Liveco Inc, Burlington, VT). This tester consisted of a computer-controlled motorized actuator in a vertical position. The standard load cell was 500 g. The welded vascular strip was placed in the tester grips that consisted of two sliding mental plates backed with screws to secure two ends of the strip. A tensile strength test was performed by pulling on the sample at a constant speed of 200 μm/sec, and measuring the tension exerted by the sample. The yield maximum strength was recorded with LabVIEW when the weld was broke.

2.6 Histological studies

The vessel samples were immediately fixed in 10% formalin. The specimens were sliced longitudinally, dehydrated, and embedded in paraffin wax. Tissue sections cut at 5 microns were stained with hematoxlyin-eosin and Movat pentachrome. Specimens were examined under a light microscope and photographed.

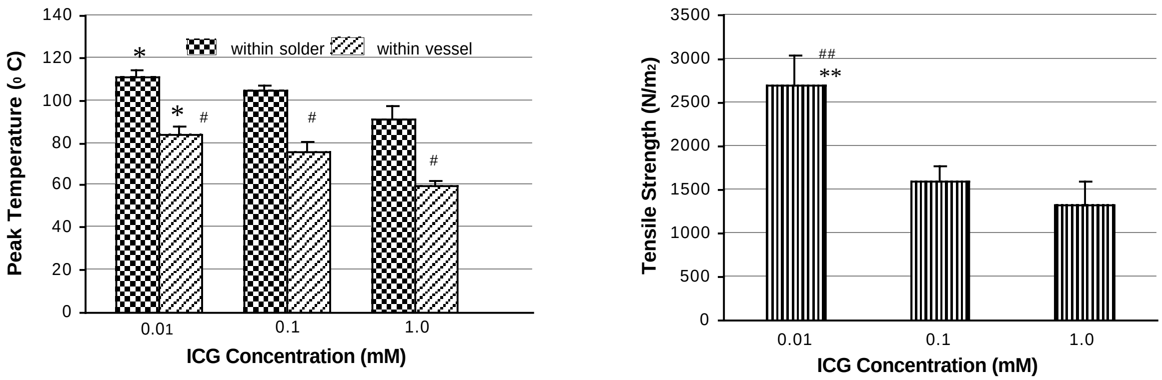
2.7 Statistical analysis

All statistical analysis was performed with SPSS software (SPSS Inc, Chicago, Ill). Data were expressed as mean ± SE of the mean. One-way analysis of variance (ANOVA) was used to test statistical significance among groups. Scheffe's *F*-test was used as a *post-hoc* test between the varying laser power densities and ICG concentrations among the different groups. $P < 0.05$ was considered significant.

3. RESULTS

3.1 Temperature profile

There were significant ($p<0.05$) differences of peak temperature between outside and inside the vascular strips in all groups (Fig. 2 A and Fig. 3 A). In group A (56.7 W/ cm^2 of PD), the peak temperatures (both outside and inside vascular strips) significantly gradually decreased (111.2 ± 3.2°C vs. 91.2 ± 5.8°C, $p<0.01$; 83.4 ± 4.2°C vs. 59.9 ± 1.9°C, $p<0.01$) with the increasing of ICG concentration (0.01, 0.1, and 1.0 mM) within the solder (Fig. 2 A). However, in group B (0.1 mM of ICG concentration), there was no significant difference ($p>0.05$) of peak temperature among the different power density groups (Fig. 3 A).

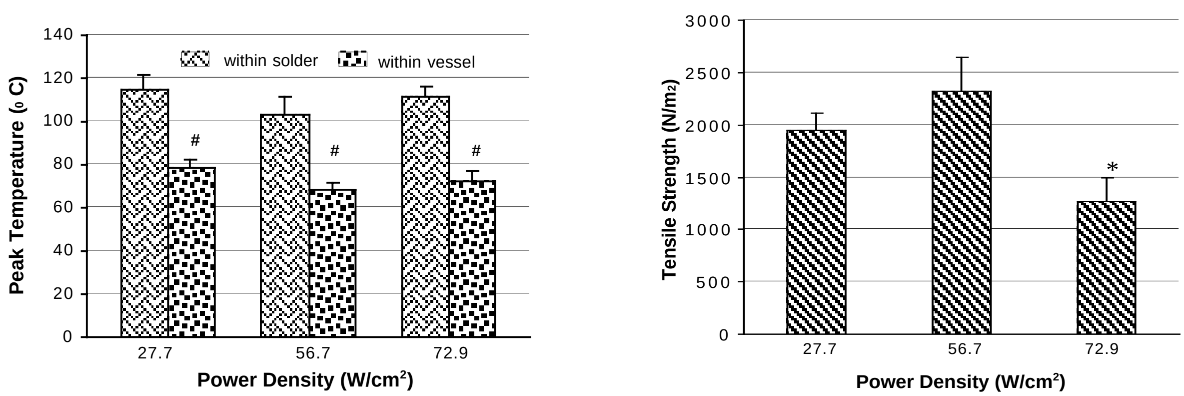


(A) (B)

Fig. 2. Peak temperatures during laser tissue welding (A) and tensile strength (B) at irradiance of 56.7 W/cm^2 and variable ICG concentrations in albumin solder. #$p<0.01$, compared with that within solder; *$p<0.01$, compared with that at ICG 1.0 mM; ##$p<0.05$, compared with that at ICG 0.1 mM; **$p<0.01$, compared with that at ICG 1.0 mM; analysis of variance, Scheff's *F-test*. mM, millimole. N, Newton. M^2, miters^2.

3.2 Immediate tensile strength

At PD 56.7 W/cm^2 **t**he tensile strength significantly gradually decreased (2695 ± 352.8 N/m^2 vs. 1603.3 ± 156.8 N/m^2, $p<0.05$; 2695 ± 352.8 N/m^2 vs. 1335.7 ± 254.8 N/m^2, $p<0.01$) with the increasing of ICG concentration (Fig. 2 B). At ICG 0.1 mM the tensile strength was significantly higher at PD 56.7 W/cm^2 than that at PD 72.9 W/cm^2 (2326.5 ± 313.6 N/m^2 vs. 1272 ± 215.6 N/m^2, $p<0.05$) (Fig. 3 B).



(A) (B)

Fig. 3. Peak temperatures during laser tissue welding (A) and tensile strength (B) at 0.1 mM ICG concentration in albumin solder and variable power density. #p<0.01, compared with that within solder; *p<0.05, compared with that at 56.7 W/cm^2; analysis of variance, Scheff's *F-test*. W, Watts. cm^2, centirmiters^2. N, Newton. M^2, miters^2.

3.3 Histological results

Thermal injury was minimal and limited to the tunica adventitia without having appreciable effect on the underlying externa and interna elastic laminae. There was no significant difference of thermal injury among the all groups (Fig. 4).

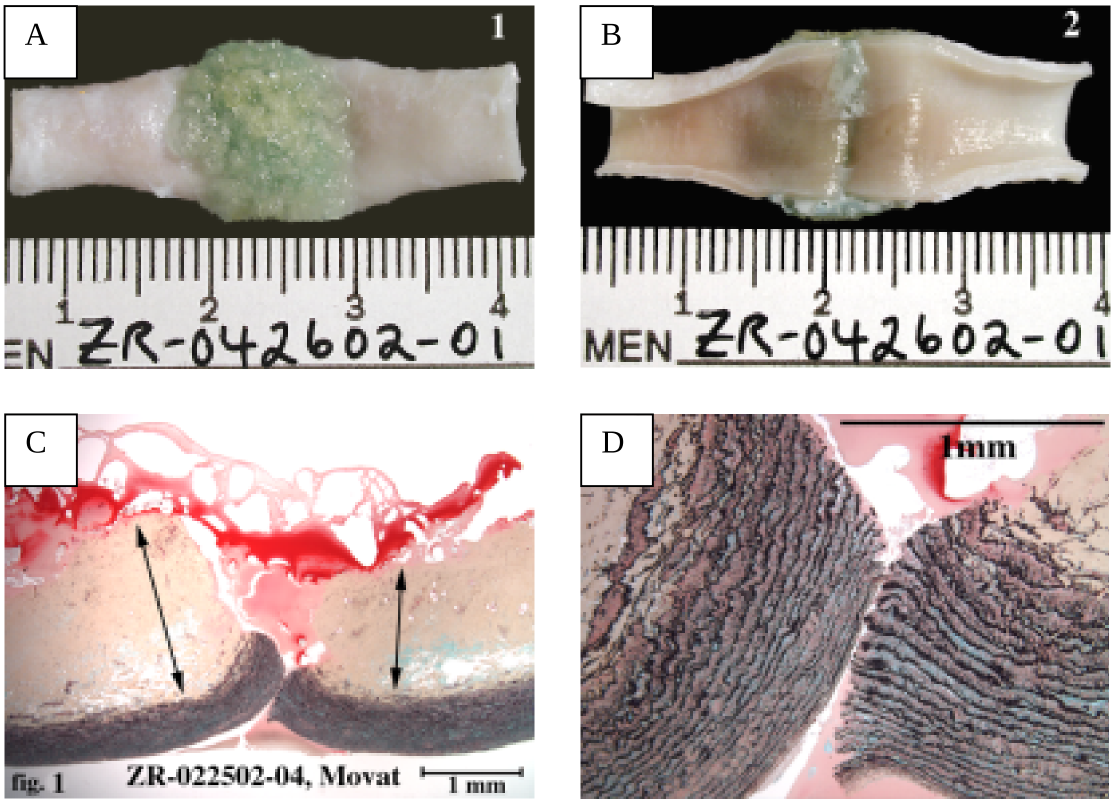
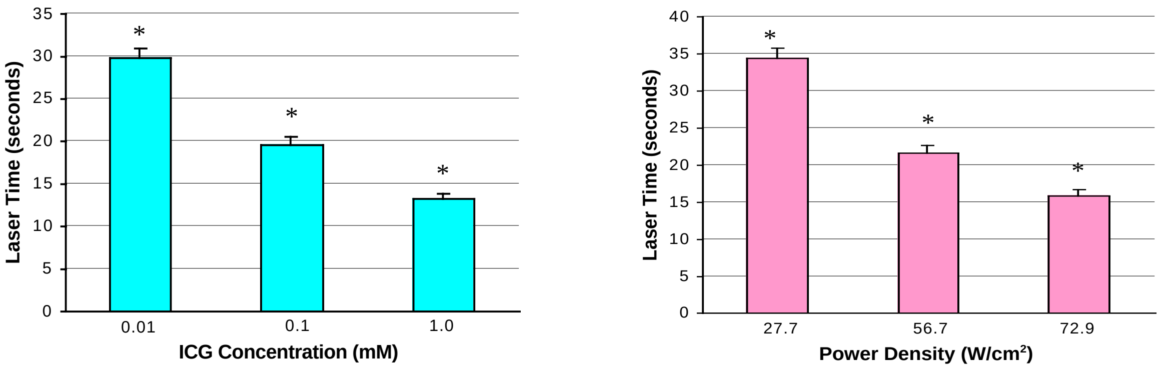


Fig 4. Histological studies. **A** is a photomacrograph of the sample carotid showing the repaired zone with fused indocyanine green layer on the anterior surface of the vessel. **B** is a posterior view of A, showing fusion site with well apposition of the carotids tissue on the luminal surface. **C** shows a Movat stain of sample with minimal thermal injury limited to adventitia. **D** is a higher magnification of the boxed area in **C**, showing the carotid fusion site without appreciable thermal injury on the underlying externa and interna elastic laminae.

4. DISCUSSIONS

The application of indocyanine green dye (ICG)-enhanced protein solder began a new era in laser tissue welding.[10] Laser soldering techniques rely on the laser energy, through the mechanism of covalent or noncovalent bonding of protein substrates,[2] to produce activation or fixation of the solder to the vessel edges and also to the adventitial surface of the vessel adjacent to the actual anastomosis. In this way, a sleeve type of joint is formed by the solder, which is mechanically much stronger than a simple edge to edge joint.[2] And the solder may be able to bridge small gaps in coaptation that would otherwise produce a lead-point for separation of the weld. Solder also may be beneficial in that it can protect the underlying vessel wall from the thermal damage seen with nonsolder techniques.[20] Furthermore, diode laser with 810 nm wavelength are specifically absorbed by ICG dye[10] in the solder and thus focus the energy in the target solder at the site of welding and limits the thermal injury. However, the relationships among ICG concentration, laser power, temperature, and strength have not been well understood.

From this study we found that, based on solder color change as laser endpoint, at PD 56.7 W/cm^2 with increasing of ICG concentration the peak temperature and tensile strength of the vascular anastomosis both significantly gradually decreased, while at ICG 0.1 mM with increasing of PD the both peak temperature and strength did not increase but decreased. Furthermore, we noticed that laser exposure time gradually decreased with increasing of ICG concentration or PD (Fig. 5). The results suggested that temperature of the tissue and the strength of the anastomosis in laser welding were mainly related to the ICG concentration in the solder and furthermore, to laser exposure time. We found that there were negative correlations between them --- the higher ICG concentration in the solder, the shorter laser exposure time needed, consequently the lower temperature in the tissue and the lower tensile strength.



(A) (B)

Fig. 5. Laser exposure time at variable ICG concentrations (A) and power densities (B) based on the solder color change from green to tan. * $p<0.01$ among all groups. mM, millimole. W, Watts. cm^2, centirmiters^2.

Proper laser endpoint is very important during laser welding. Some investigators[2] tried to use automated dosimetry (afferent control) that relates to input of tissue parameters as in the Dew system (tissue type, thickness), which allows the computer prospectively to control pulse width and power density. However, the disadvantages of this approach are inability to accommodate variations in tissue and they have been unable to demonstrate a difference in weld strength or thermal injury between afferent control and an experienced operator even in a very reproducible laboratory model where afferent control would be expected to perform ideally.[2] Others[21,22] applied thermal-based feedback control system to get optimal endpoint. But accuracy, response time, and sampling errors (both spatial and temporal) are factors affecting the workability of such system. Also, exactly what the preferred tissue temperature is remains controversial, varying with apposition pressure, tissue type, and chemical composition and so on.

Clinically, in a typical laser assisted anastomosis procedure, the surgeon looks for a subtle visible change in the tissue, either blanching or some discoloration of the tissue surface, as an endpoint for completion of the weld. Thus solder color change has far been the determinant for the completion of laser tissue soldering when ICG is a solder component.[15] Previous studies showed that green represented no appreciable changes, tan represented smooth drying of the solder, brown represented a gritty drying of the solder, and black represented carbonization. A tan color change indicates a successful tissue weld while carbonization indicates failure.[13,15] In this study we used tan color change as a laser endpoint and considered that it is easy to be operated, practicable, and cost-effective.

The histological study showed that thermal injury was minimal and limited to the tunica adventitia without having appreciable effect on the underlying externa and interna elastic laminae. Even though at the highest peak temperature (average of 114.7°C within solder, or 83.4°C within vessel), there was no elastic lamina injury that has been considered one of main causes of anastomotic pseudoaneurysm formation.[3,5,6,9] Since elastin is not denatured until 140°C is reached,[23] we prevented destruction of the elastic lamellae by controlling the laser welding and obtained maximum strength (2695 ± 352.8 N/m^2) without appreciable thermal injury at PD 56.7 W/cm^2 and 0.01 mM ICG within the solder. However, the long-term strength and the effects on vascular healing will be investigated in future.

5. CONCLUSIONS

We found from the present study that, ICG concentration within the solder is the most important factor affecting both tissue temperature and tensile strength during laser vessel welding; there is negative relationship between ICG concentration, tissue temperature, and tensile strength based on solder color change as laser endpoints; the optimal balance between stronger strength and minimal thermal injury of vessel may be achieved primarily by using the power density of 56.7 W/cm^2 at 0.01 mM ICG within the solder during LAVA.

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