Optimal Dye Concentration and Irradiance for Laser-Assisted Vascular Anastomosis

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ABSTRACT

Objective: This investigation was done in order to find optimal indocyanine green (ICG) concentration and energy irradiance in laser vascular welding. Background Data: Many studies have shown that laser tissue welding with albumin solder/ICG may be an effective technique in surgical reconstruction. However, there are few reports regarding optimal laser settings and concentrations of ICG within the albumin solder in laser-assisted vascular anastomosis. Materials and Methods: Porcine carotid artery strips (n = 120) were welded in end-to-end by diode laser with 50% albumin solder of 0.01, 0.1, and 1.0 mM ICG at irradiance of 27.7, 56.7, and 76.9 W/cm², respectively. Temperature was measured by inserting thermocouples outside and inside the vessel. Tensile strength and histology were studied. Results: Temperature and strength of the anastomosis significantly decreased (all p < 0.05) with increasing ICG concentration at 56.7 W/cm². Histological study showed minimal thermal injury limited to adventitia and no appreciable difference between all groups. Conclusions: ICG concentration within solder is the most important factor affecting both vascular temperature and tensile strength. The optimal balance between strength and minimal thermal injury may be achieved primarily at 56.7 W/cm² and 0.01 mM ICG.

INTRODUCTION

SINCE JAIN AND GORISCH first reported successful laser-assisted vascular anastomosis (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 seal,2 reducing operative time,1,3 faster healing,4 ability to grow,4 and reducing intimal hyperplasia owing to the absence of foreign body reaction to suture material.5 However, the main disadvantages of the laser-assisted procedure are the low strength of the resulting anastomosis,2,3 especially in the acute healing phase up to 4 days postoperatively,4 and tissue thermal injury, including increased anastomotic pseudoaneurysm rate.5,6,8 These disadvantages and lack of satisfactory objective criteria for optimal laser exposure parameters have limited wide clinical application of laser anastomosis.

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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,9,10 urinary tract,11 and skin.12 The results of laser tissue welding may be affected by laser settings,13 concentration of albumin solder,14,15 ICG concentration in the solder,16,17 and temperature of the tissue surface.18,19 It is known that albumin solder with higher concentration results in significantly stronger tensile strength than albumin solder with lower concentration.16,17 However, there are few reports regarding optimal laser settings and concentrations of ICG in albumin solder in LAVA.

This study was carried out in vitro by measuring temperature profiles outside and inside the vessel, testing tensile strength and studying histology of the specimen while varying both ICG concentrations.
concentration within 50% albumin solder and diode laser irradiance in order to find optimal ICG concentration and laser parameters in LAVA.

MATERIALS AND METHODS

Tissue and albumin solder preparation

Fresh porcine carotid arteries were harvested from animals being sacrificed from 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 artery wall thickness ranged from 1.1 to 1.3 mm (1.2 ± 0.01 mm, there was no significant difference among the groups).

Human serum albumin at 25% (Baxter Health Corp., Glendale, CA) was filtered through an ultrafiltration membrane (YM 30, Millipore Corp., Bedford, MA) and concentrated to 50% (w/v) by using an ultrafiltration system (model 8400, Amicon, MA) under 60 psi on a stirrer/hotplate (model PC-320, Corning Corp., Corning, NY) at 37°C. The albumin was then mixed well for 10 min with indocyanine green (Sigma Chemical Co., 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 50% albumin solder with different ICG concentration was stored in 1-mL syringes at 4°C in the dark until use (less than 14 days).

Diode laser system

Laser treatments were performed with a 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 utilized consists of a phased array of gallium-aluminum-arsenide semiconductor diodes, and produces an invisible laser beam at 810-nm wavelength. A red aiming beam allowed the operator to visualize the spot size of the laser during activation.

The laser spot diameter was 2 mm at a distance of 15 mm between the tip and the tissue. The laser was set as a continuous wave (CW) at output powers of 870, 1800, and 2300 mW resulting in laser irradiance of 27.7, 56.7, and 72.9 W/cm², respectively. The output power of the laser settings was measured by a Laser Energy/Power Meter (EPM 1000, Moleclectron Detector Inc., Portland, OR) before and after welding in each experiment. Laser exposure time (indicated by the solder changing to a tan color) was recorded during the operation.

Experiment design and grouping

The vascular strips (n = 120) were randomly divided into two groups: Group A involved vascular strips that were welded by diode laser in end-to-end at 56.7 W/cm² with 50% albumin solder at varying ICG concentrations of 0.01, 0.1, and 1.0 mM, respectively. Group B involved vascular strips that were welded by diode laser in end-to-end at 0.1 mM ICG concentration within the 50% albumin solder and at laser irradiances of 27.7, 56.7, and 76.9 W/cm², respectively. Direct temperature measurements were obtained by placing thermocouples on the vascular strip surface within the albumin solder and beneath the strip during the laser welding. The endpoint of the laser welding was indicated by the solder changing to a tan color. Tensile strength was tested immediately and histological study was performed after the welding.

Temperature measurement

The 36-gauge Type T thermocouples (Omega Engineering Inc., Stamford, CT) were placed on the vascular surface (the albumin solder was directly placed above it) and inside the vascular lumen. They were then connected to a data acquisition system controlled by LabVIEW software (National Instruments, Austin, TX). 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. The program converted the input voltage to temperature (10 mV/°C), recorded temperature versus time for each thermocouple input, and then saved the data to an ASCII text file for further analysis. The thermocouples were cleaned with alcohol between each measurement.

Tensile strength test

The welded vascular strips were loaded on the universal tester (Chatillon Materials Testing, Vitrodyne 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 metal 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 failed. The strength was then divided by the area of the solder to yield tensile strength.
Optimal Diode Laser Vascular Soldering

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 μm were stained with hematoxylin-eosin (H&E) and Movat pentachrome. Specimens were examined under a light microscope and photographed.

Statistical analysis

All statistical analysis was performed with SPSS software (SPSS Inc., Chicago, IL). Data was expressed as mean ± standard error 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 irradiance and ICG concentration among the different groups. A value of $p < 0.05$ was considered significant.

RESULTS

Temperature profile

There were significant ($p < 0.05$) differences of peak temperature between outside and inside the vascular strips in all groups (Figs. 2A and 3A). In group A (56.7 W/cm²), the peak temperatures (both outside and inside vascular strips) significantly decreased (111 ± 3°C vs. 91 ± 6°C, $p < 0.01$; 83 ± 4°C vs. 60 ± 2°C, $p < 0.01$) with the increasing of ICG concentration (0.01, 0.1, and 1.0 mM) within the solder (Fig. 2A). However, in group B (0.1 mM of ICG concentration), there was no significant difference ($p > 0.05$) of peak temperature among the different irradiance groups (Fig. 3A).

![Graph A](image1)

**FIG. 2.** At irradiance of 56.7 W/cm² and variable ICG concentrations in albumin solder, peak temperatures during laser tissue welding (A) and tensile strength (B). *$p < 0.01$, 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, Scheffe’s F-test. mM, millimole; N, Newton; M², meters².

![Graph B](image2)

**FIG. 3.** At 0.1 mM ICG concentration in albumin solder and variable laser irradiance, peak temperatures during laser tissue welding (A) and tensile strength (B). *$p < 0.01$, compared with that within solder; *$p < 0.05$, compared with that at 56.7 W/cm²; analysis of variance, Scheffe’s F-test. W, Watt; cm², centimeters²; N, Newton; M, meters.
Immediate tensile strength

At irradiance of 56.7 W/cm², the tensile strength significantly decreased (2700 ± 350 N/m² vs. 1600 ± 160 N/m², p < 0.05; 2700 ± 350 N/m² vs. 1340 ± 260 N/m², p < 0.01) with increasing ICG concentration (Fig. 2B). At ICG 0.1 mM, the tensile strength was significantly higher at 56.7 W/cm² than that at 72.9 W/cm² (2330 ± 310 N/m² vs. 1270 ± 220 N/m², p < 0.05) (Fig. 3B).

Histology

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 groups (Fig. 4).

DISCUSSION

The application of indocyanine green dye (ICG)-enhanced protein solder began a new era in laser tissue welding. Laser soldering techniques rely on the laser energy, through the mechanism of covalent or noncovalent bonding of protein substrates 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. The solder may be able to bridge small gaps in coaptation that would otherwise produce a tear-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. Furthermore, diode laser energy at 810-nm wavelength is specifically absorbed by ICG dye in the solder. It thus focuses the energy in the target solder at the site of welding and limits thermal injury to the tissues. However, the relationships among ICG concentration, laser power, temperature, and strength have been little understood.

This study has showed that, based on solder color change as the laser endpoint, at laser irradiance of 56.7 W/cm² with increasing ICG concentration, the peak temperature and tensile strength of the vascular anastomosis both significantly decreased; and at ICG 0.1 mM with increased irradiance, both peak temperature and strength did not increase, but decreased. Furthermore, we noticed that laser exposure time gradually decreased with increasing ICG concentration or irradiance (Fig. 5). The

FIG. 4. Histological studies. (A) Photomacrophotograph of the sample carotid showing the repaired zone with fused indocyanine green layer on the anterior surface of the vessel. (B) Posterior view of A, showing fusion site with good apposition of the carotids tissue on the luminal surface. (C) Movat stain of sample with minimal thermal injury limited to adventitia. (D) 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.
results suggest that temperature of the tissue and strength of the anastomosis in laser welding were mainly related to the ICG concentration within the solder and furthermore, to laser exposure time. We found that there were negative correlations between ICG concentration and laser exposure time needed, tissue temperature and strength. The higher ICG concentration in the solder, the shorter laser exposure time needed resulting in the lower temperature in the tissue and the lower tensile strength. We also found that increasing laser irradiance did not proportionally improve the tensile strength of vascular anastomosis. These results correspond with other reports. 37

Identifying the proper laser endpoint is very important during laser welding. Some investigators have 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 the inability to accommodate variations in tissues. Difference in weld strength or thermal injury between afferent control and an experienced operator, even in a reproducible laboratory model where afferent control would be expected to perform ideally, has not been demonstrated. Others have applied a thermal-based feedback control system to get an optimal endpoint. But accuracy, response time, and sampling errors (both spatial and temporal) are factors affecting the practicality of such a system. Preferred tissue temperature remains controversial, as it may vary with apposition pressure, tissue type, and chemical composition.

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. Solder color change has been the major determinant for the completion of laser tissue soldering when ICG is a solder component. Previous studies have shown that green solder color represented no appreciable changes, tan color change 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 predicts failure. In this study, we used tan color change as a laser endpoint considering that it is easy to reproduce, and simple to perform.

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 115°C within solder, or 83°C within vessel), there was no elastic lamina injury, it has been considered one of the main causes of anastomotic pseudoaneurysm formation. Since elastin is not denatured until 140°C is reached, we prevented destruction of the elastic lamellae by controlling the laser welding and obtained maximum strength (2700 ± 350 N/m²) without appreciable thermal injury at irradiance 56.7 W/cm² and 0.01 mM ICG within the solder. However, the long-term strength and the effects on vascular healing still need to be investigated in future studies.

CONCLUSION

The present study indicates that, ICG concentration within albumin solder is the most important factor affecting both tissue temperature and tensile strength during laser vascular welding; there is a negative relationship between ICG concentration and tissue temperature and tensile strength based on solder color change as laser endpoints; the optimal balance between stronger strength and minimal thermal injury of the vessel may be achieved primarily by using irradiance of 56.7 W/cm² at 0.01 mM ICG within the solder during LAVA.

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