

Thermal Damage Control of Dye-Assisted Laser Tissue Welding: Effects of Dye Concentration

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ABSTRACT

Successful laser-assisted tissue welding was implemented to provide proper weld strength with minimized tissue thermal injury. We investigated and compared the weld strengths and morphologic changes in porcine small intestinal submucosa (SIS) and porcine ureteral tissues with various concentration of indocyanine green (ICG) and with a solid albumin sheet. The study showed that the tissues were welded at lower ICG concentration (0.05 mM) with minimized tissue thermal damage using an 800-nm wavelength diode laser.

Keywords: Laser welding, Laser soldering, in vitro, albumin

1. INTRODUCTION

Laser tissue welding or soldering is very attractive novel techniques that are highly interested in various surgical fields. The baseline to satisfy the surgical requirements is to get stronger welding strength and to minimize tissue thermal injury. To reach this requirement, researchers are developing new techniques which decrease energy output or/and reduce energy absorption or localize thermal transmission, such as, 1. Using short pulse laser and target thermal feedback system to limit energy output. 2. Selecting laser wavelengths to limit tissue energy absorption. 3. Applying solders and chromophores to locate energy absorption. Although those techniques have been quite successful in reducing for the tissue thermal injury, the problems still retained which limit those techniques practicing in clinic. The major technical difficulties are the welding strength unstable and surgical end point uncontrollable. The balance of welding strength and tissue thermal injury is always considerable in clinical practice. In this study, we used various parameters of indocyanine green (ICG) concentration to reduce thermal injury in our applications.

2. MATERIALS AND METHODS

2.1. Preparation of Solid Solder

25% Human serum albumin (Michigan Dept. of Public Health, MI) was filtered through an ultrafilter membrane (YM 30, Amino) using an ultrafiltration system (Model 8400, Amicom, MA) to concentrate it to 52~55% (w/v). 1, 5, 10 and 50 mM indocyanine green (ICG) (Sigma, I2633, MO) solutions were filtered for sterilization (Gameo 25ES, Fisher) and added to the concentrated albumin at 1:100 (v/v) and mixed well for 3 min. The albumin and ICG mixture were exposed in a dark chamber at room temperature until the solvent evaporated and became moldable. The moldable albumin was pressed to sheets with 100-150 μm thickness. The albumin sheets were stored at 4 °C in the dark until use.

2.2. Laser System

Laser treatments were performed with an 800 nm diode laser (Coherent, US) coupled to a quartz silica non-contact fiber optic (600 μm diameter) with an optic collimated micro-lens (Coherent, US). The optic micro-lens allows laser beam maintaining uniform and collimated. The light spot size was 4 mm diameter and 3 cm at distance. The laser was setting at 14 joules output and 100 ms pulse length, the energy density was measured at 4.35 W/mm².

2.3. In Vitro Experiments

Fresh porcine ureters harvested from our other experimental sacrificed animals. The ureters were stored to 4 °C until use.

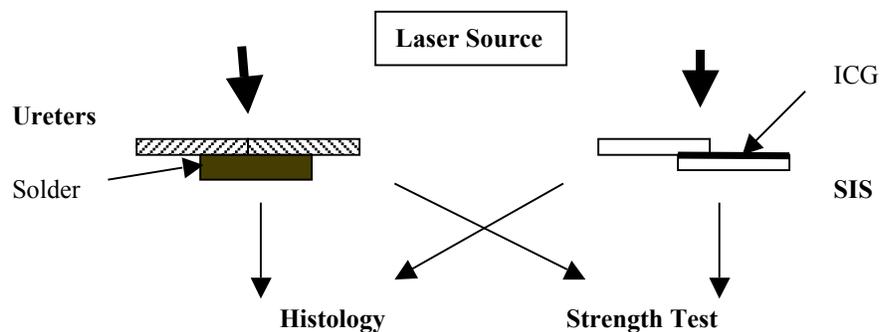
Harvesting of small intestine submucosa (SIS) has been previously described¹. The SIS segments were decellularized in a solution of 2 mM SDS and 0.1 N NaOH and then washed in 0.01M PBS (pH 7.0). The SIS was stored at 4 °C in 1% Neomycin / Polymyxin B solution until use. Indocyanine Green (ICG; I2633, Sigma, MO) was solved and diluted in double deionized water at 0.01, 0.05, 0.1 and 0.5 mM, and painted onto SIS intraluminal side for 15 minutes staining. The ICG stained SIS were kept in dark before use.

The study was divided into two groups (Figure 1); In group 1, two pieces of ureter welded at edge to edge with the solid albumin sheet. The solid albumin sheets were laid on ureteral intraluminal side. In group 2, two pieces of SIS were overlapped and welded together. The laser light passes through unstained SIS piece. In all the groups, the samples were divided for tensile strength testing and histological examinations.

The welded samples were loaded on Strength tester (Vitrodyna V1000, Liveco, VT). The standard load cell was 500 g.

The rest of samples were immediately fixed in 10% formalin solution. The specimens were dehydrated and embedded with paraffin wax and then sliced longitudinally for H & E and Trichrome staining. The slides were observed with a Leica microscope (Leica DMRB, Germany) under normal light and polarization reflected light. The area of thermal damage was distinguished by a color change and loss of birefringence under the light microscopy.² The thermally damaged area was measured under 50 X magnification.

Figure 1: In Vitro Experimental Design:



2.4. Statistical Analysis:

Statistical comparisons of all groups were examined using Student T-test on SPSS program. All data are expressed as average \pm standard deviation. P values <0.05 were considered statistically significant.

3. RESULTS

3.1. Tensile Strength of Laser Welding:

In SIS groups (Figure 2), the welding strength are significantly higher in ICG stained groups. In comparison with ICG stained groups, there were no significant differences among the groups although that was slightly great strength at 0.01 and 0.05mM concentrations. In ureteral welding groups, the greatest tensile strength outcome at 0.1mM solid solder group, however, we did not found the significant differences among the groups (Figure 3).

3.2. Solid Solder Geometric Changes:

The solid solder shows that the "bubbling" phenomena occurred when the ICG concentration goes up (Picture 1).

3.3. Tissue Thermal Injuries:

Ureteral tissue welding with solid solder shows the trends which increasing ICG concentration in solid albumin sheet was producing larger thermal injury area in welded tissues (Figure 4). However, there are no statistical differences among the study groups.

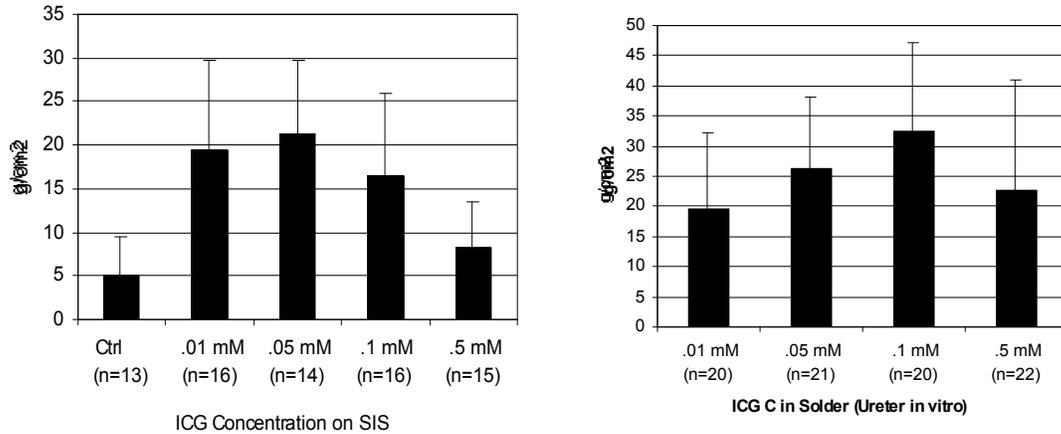


Figure 2 (Right) & 3 (Left): Welding strength and ICG concentration on SIS and ureteral Laser welding

4. DISCUSSIONS

To reduce tissue injury are still major issues for laser tissue welding applications, particularly in some fine tissue repair surgeries, as microsurgery, small diameter's vessels anastomosis using the laser welding / soldering techniques. The unexpected thermal injury may cause welded vessel stricture or stenosis after surgery due to increase scar tissue formation in the wound healing process. Currently, several existed techniques are used to produce greater tensile strength in tissue welding. One of the techniques is using solid solder to increase the welding strength³. In our previous study, a solid albumin stent was developed to assist small vessel anastomosis⁴. For the stent development, we are testing the stent with different ICG concentrations for producing a suitable tensile strength and limiting the tissue thermal injury in ureteral anastomosis.

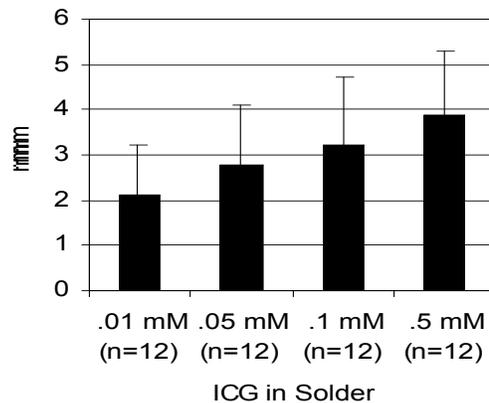
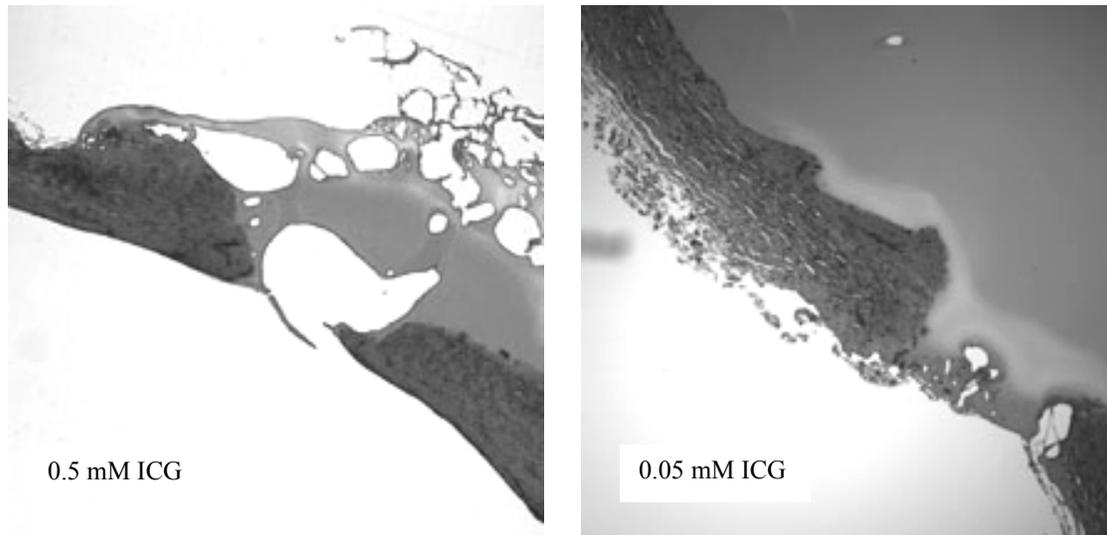


Figure 4: Thermal injury on ureteral tissues using laser welding with solid solder.

In our study, we noticed that the welding strength was significantly higher in ICG stained SIS groups. In comparison with ICG stained groups, there were no significant differences among the groups although that was slightly great strength at 0.01 and 0.05mM concentrations. In ureteral welding groups, the greatest tensile strength outcome at 0.1mM solid solder group, however, the results also showed that 0.5

Picture 1. Porcine ureteral welding with solid albumin solders: Solder with 0.5 mM ICG produced "bubbling" phenomena during laser welding (right); with 0.05 mM ICG solder appeared a uniform bonding surface during the lasering (left).



mM ICG concentration stained SIS and solid solder with 0.5 mM ICG did not provide stronger strength. It may cause by tissues inter-bonds destroyed due to energy absorption with high ICG concentration in tissues. The geometry of the solid solder appeared "bubbling" phenomena in high ICG concentration. In the inter-surface of the bonding tissue, the air bubbles could reduce the bonding area between the welding tissue or/and solder that compromised the tissue welding. Our study demonstrated that using 0.05-0.1 mM ICG in the solid albumin stent is the best combination to build our stent for ureteral anastomosis. Furthermore, in vivo experiments are carrying on to test the ICG concentration in the solid solder applications.

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