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***In vivo* argon laser vascular welding using thermal feedback: open and closed loop patency and collagen crosslinking**

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ABSTRACT

An *in vivo* study of vascular welding with a fiber-delivered argon laser was conducted using a canine model. Longitudinal arteriotomies and venotomies were treated on femoral vein and artery. Laser energy was delivered to the vessel wall via a 400 μ m optical fiber. The surface temperature at the center of the laser spot was monitored in real time using a hollow glass optical fiber-based two-color infrared thermometer. The surface temperature was limited by either a room-temperature saline drip or direct feedback control of the laser using a mechanical shutter to alternately pass and block the laser. Acute patency was evaluated either visually (leak/no leak) or by *in vivo* burst pressure measurements. Biochemical assays were performed to investigate the possible laser-induced formation or destruction of enzymatically mediated covalent crosslinks between collagen molecules. Viable welds were created both with and without the use of feedback control. Tissues maintained at 50°C using feedback control had an elevated crosslink count compared to controls, while those irradiated without feedback control experienced a decrease. Differences between the volumetric heating associated with open and closed loop protocols may account for the different effects on collagen crosslinks. Covalent mechanisms may play a role in argon laser vascular fusion.

Keywords: tissue welding, tissue fusion, infrared thermometry, feedback control

1. INTRODUCTION

Various laser wavelengths with and without the use of extrinsic media and feedback control have been employed in the developing practice of laser tissue welding.¹ The argon laser accompanied by a cooling saline drip has yielded successful results in both animal and human *in vivo* models without feedback control.²⁻⁴ Recent studies have demonstrated that temperature-controlled laser tissue welding yields increased consistency and strength compared to open loop procedures.⁵ However, the mechanisms responsible for the creation of a viable weld remain unknown. This preliminary single animal study compared the effects of temperature feedback control on argon laser vascular tissue welding to procedures done without feedback control, and investigated the influence of argon laser irradiation on the collagen crosslink content. A hollow glass optical fiber-based two-color infrared thermometer⁶ was used to monitor the surface temperature, and a feedback control loop was employed to maintain a preset temperature by intermittently blocking the laser beam with a mechanical shutter. Biochemical assays were performed to quantify the concentrations of three specific covalent crosslinks between collagen molecules.

2. MATERIALS AND METHODS

The animal used in this study received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

2.1. Welding procedures

A 30 kg mongrel dog was intubated and placed under general anesthesia. Both femoral veins and the right femoral artery were isolated for welding. Once proximal and distal control of the vessels was attained, longitudinal incisions 1 cm or 0.5

cm in length were made with a scalpel. Either stay sutures or clips were placed at the proximal and distal ends of the incisions, and a stay suture was placed at the center of the 1 cm incisions. In the case of the 1 cm incisions, the proximal and distal segments, each 5 mm in length, were welded separately.

Argon laser energy was delivered via a 400 μm optical fiber held 5 mm above the tissue, yielding a spot size of about 3 mm (FWHM). The surgeon moved back and forth along the incision at a rate of approximately 1 mm/s. The open loop procedures were done at 0.7 W using 5 s pulses with 0.5 s intervals, with delivered energy ranging from 35 J to 75 J (laser exposures from 50 s to 105 s) for each 5 mm segment and average fluence of 22 J/cm². A continuous drip of room-temperature saline was applied to the tissue surface at a rate of approximately 1 drop/s for cooling during the irradiation. The closed loop procedures were done in continuous-wave (CW) mode using laser powers of 0.36 W for 30 s or 0.7 W for 60 s for each segment. Though the laser was operated in CW mode, the mechanical shutter used to control the laser effectively pulsed the delivery at a rate of about 1 Hz, yielding laser exposure times of about 15 s at 0.36 W and 30 s at 0.7 W (total energy delivered was 5.4 J and 21 J, respectively). Average fluences were 10 J/cm² at 0.36 W and 22 J/cm² at 0.7 W. No saline was applied in the closed loop trials.

2.2. Temperature feedback

The surface temperature within a 1 mm diameter spot at the center of the laser spot was monitored in real time during the laser procedures using a hollow glass optical fiber-based two-color infrared thermometer.⁶ A 700 μm bore hollow glass optical fiber^{7,8} collected the radiation from the tissue surface, and a reflective chopper modulated and split the incident radiation into two paths, each of which contained a thermoelectrically cooled infrared photoconductor. Lock-in amplifiers were used to recover the signals from the two photoconductors, which had spectral bandpasses of 2–6 μm and 2–12 μm , respectively, and a computer calculated the surface temperature and emissivity based on a previous blackbody calibration. A handpiece containing both the laser and sensing fibers and a guide wire allowed the operator to maintain a preset fiber-to-tissue distance of 5 mm. The two fibers subtended an angle of 25°, and the guide wire extended to the point where the two fibers would intersect. A mechanical shutter was used in the feedback loop to control the laser delivery in a binary fashion (on/off) and maintain the surface temperature near 50°C for closed loop procedures. No audio or video prompts were provided to the operator to indicate arrival at the temperature setpoint. The shutter was kept in the open position during open loop procedures. A diagram of the tissue welding feedback system is shown in Figure 1.

Fig. 1. Diagram of the surface temperature feedback system used to control the vascular tissue welding procedures.

2.3. Weld integrity assessment

Patency of each weld was determined immediately following completion of both proximal and distal segments either by qualitative observation or *in vivo* burst pressure measurement. Visual evaluation consisted of allowing the blood to resume flowing through the vessel and identifying the presence of any leak. Quantitative evaluation was done by infusing anti-

coagulated blood via a syringe pump at 30 cc/min while the pressure was monitored by a physiologic transducer connected to a bioamplifier and chart recorder. In either case, the segment (i.e., proximal or distal) in which any leak occurred was noted for the 1 cm incisions.

2.4. Collagen crosslink measurement

The irradiated femoral vein tissues from the 1 cm venotomy repairs were harvested for biochemical analysis to quantify the concentrations of three specific enzymatic covalent crosslinks between tropocollagen molecules: the intermediate difunctional crosslink dehydrohydroxylysine (HLNL); the intermediate difunctional crosslink dehydrodihydroxylysine (DHLNL); and the mature trifunctional product of DHLNL, hydroxypyridinium (OHP). Enzymatic crosslinks confer strength and stability to the collagen matrix, and HLNL, DHLNL, and OHP, in particular, are found in newly formed collagen in wound healing,⁹ suggesting a possible correlation with tissue fusion. Each sample was divided into several smaller samples prior to analysis. High-performance liquid chromatography (HPLC) was used to analyze the samples. HLNL and DHLNL were analyzed by HPLC using an isocratic solvent,^{10,11} and OHP was analyzed using a modification of the method of Eyre et al.^{12,13}

3. RESULTS

3.1. Surface temperature

A sample of the surface temperature data for both open and closed loop 1 cm incision procedures is shown in Figure 2. The open loop temperatures never exceeded approximately 60°C, while the closed loop temperatures were clamped around 50°C for the duration of every weld.

Fig. 2. Open and closed loop surface temperature histories during argon laser femoral vein repair. The laser was turned off following completion of the distal segment, and then turned on again several seconds later to begin welding the proximal segment.

3.2. Weld patency

Table 1 shows the open and closed loop patency results for the 1 cm femoral vein welding procedures. For the relatively small number of trials, the success rate for the closed loop welds was slightly higher compared to the open loop welds. Table 2 shows the closed loop patency and burst pressure results for femoral artery welding procedures. A previous study using the same open loop protocol used in this study reported burst pressures for 0.5 cm open loop arterial welds to be 114 ± 66 mmHg.³

TABLE 1. Acute Patency and Success Rates for Open and Closed Loop Argon Laser Femoral Vein Welding of 1 cm Incisions

	Acute segment patency (proximal / distal)			Segment success (%)
	Trial 1	Trial 2	Trial 3	
Open loop	no leak / leak	no leak / leak	no leak / no leak	67
Closed loop (50°C)	no leak / no leak	no leak / no leak	—	100

TABLE 2. Acute Patency, Burst Pressure, and Success Rate for Closed Loop Argon Laser Femoral Artery Welding at 50°C

Acute segment patency (proximal / distal)				Segment success (%)
Burst pressure (mmHg)				
1 cm incision			0.5 cm incision	
Trial 1	Trial 2	Trial 3	Trial 4	
leak / leak	no leak / no leak	no leak / no leak	no leak	71
—	140	140	>110*	

*The suture holes leaked at this pressure; the weld remained intact.

3.3. Collagen crosslink assays

Biochemical assays of HLNL, DHLNL, and OHP collagen crosslink concentrations were done for open and closed loop femoral vein procedures. The open loop 5 mm segments were repaired using eleven 5 s laser pulses at 0.7 W (total irradiation time = 55 s) with a saline drip. The closed loop procedures maintained the surface near 50°C using a laser power setting of 0.7 W for 60 s (total irradiation time = 30 s). Tissues maintained at 50°C using feedback control contained significantly greater concentrations of DHLNL and OHP compared to controls; the HLNL concentration did not change with respect to controls. Tissues treated in the open loop format contained significantly lower concentrations of all three analyzed crosslinks compared to controls.

4. SUMMARY AND CONCLUSIONS

Viable vascular welds were created *in vivo* in a canine model using the argon laser both with and without shutter-based temperature feedback control. A hollow glass optical fiber-based two-color infrared thermometer monitored the surface temperature at the center of the laser spot in real time. Though no direct feedback control was used in the open loop procedures, the surface temperature was limited by the saline drip and pulsed energy delivery. The closed loop femoral vein procedures resulted in an increased patency rate and an elevated DHLNL and OHP collagen crosslink concentration, while the open loop procedures experienced a decrease in HLNL, DHLNL, and OHP crosslinks. These results may be correlated with the different sub-surface temperatures achieved with open and closed loop procedures. Modeling results indicate that, for equivalent surface temperatures in the open and closed loop formats, the subsurface temperatures achieved using the open loop format with a saline drip are up to 20°C greater than those in the closed loop. The closed loop femoral artery procedures resulted in acutely stronger welds compared to previous results. The tissue fusion process may involve collagen crosslinking, as well as other covalent bonding mechanisms. Further work needs to be done to identify the roles of the temperature history and laser delivery parameters on weld patency and crosslink formation and destruction.

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