Laser-Assisted Vascular End to End Anastomosis of Elastin Heterograft to Carotid Artery With an Albumin Stent: A Preliminary In Vivo Study

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Background and Objectives: Laser-assisted end to end vascular anastomosis of an elastin heterograft to native artery may prevent problems associated with currently available vascular synthetic grafts and conventional suture anastomosis.
Study Design/Materials and Methods: A total of 21 anastomoses in the carotid arteries of 7 domestic swine were performed with an 800 nm laser and an albumin stent plus solder. There were 5 artery to artery and 16 elastin heterograft to native artery anastomoses. Operative parameters, vascular patency, and histology of the anastomoses were evaluated.
Results: Out of 21 anastomoses, 16 were patent for 3 hours. One artery to artery anastomosis was thrombosed and four elastin heterograft to artery anastomoses were excluded from the study due to heterograft burst. The average amount of applied energy was 212 J for artery to artery anastomosis and 273 J for elastin heterograft to native artery. Histology shows coagulative necrosis of the adventitia, hypereosinophilic contraction band in the media of native arteries and no changes in elastin heterografts.
Conclusions: Laser-assisted vascular anastomosis (LAVA) of elastin heterograft to medium size vessel using an albumin stent is feasible. Chronic studies are warranted to determine long-term patency and histology of the LAVA. Lasers Surg. Med. 35:201–205, 2004.
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Key words: lasers; welding; albumin; elastin; anastomosis

INTRODUCTION

The first successful use of laser tissue welding was reported by Jain and Gorisch who sealed 0.8–1.0 mm openings in small blood vessels with a Nd:YAG laser in 1979 [1]. Since then laser-assisted vascular anastomosis (LAVA) have been performed mostly in the micro vascular system, but only few reports discussed vascular anastomosis in medium and large size vessels [2,3]. Various types of lasers with different parameter settings have been used for laser-assisted vascular anastomoses in medium and large size vessels. White et al. reported that while CO_2, Argon, and Nd:YAG lasers are each effective in creating vascular anastomoses, only Argon energy is successful for anastomoses in medium and large size arteries [4]. This is due to limited tissue penetration of the CO_2 laser, while the Nd:YAG laser may have resulted in excessive energy delivery, with destruction of elastin and collagen within the media and adventitia. Argon laser-assisted vascular anastomoses were technically more demanding and required almost twice the time to perform as sutured anastomoses. Although the results of LAVA were comparable, not superior to conventional manual suturing [5], the potential use of this technique in laparoscopic and robotic surgery, in terms of simplifying operative mode, saving surgical time, and less foreign body reaction, has been attractive to both the clinician and physicist. Poppas et al. reported that use of human albumin solders improved the strength of the anastomosis [6]. The solder absorbs laser energy to denature and coagulate protein components and bonds to the native tissue, thus acting as biological glue. Our previous work has demonstrated that use of an albumin stent resulted in stronger welding strength and higher burst pressure compared to using albumin solder alone in laser-assisted ureteral anastomosis [7].

In this study, we performed end to end anastomosis in native carotid artery and an elastin heterograft to carotid artery using an 800 nm wavelength diode laser with an albumin stent and solder. We investigated the feasibility of using this technique for medium size blood vessel anastomosis, in particular, for the elastin heterograft to carotid artery anastomosis.

MATERIALS AND METHODS

Preparation of Elastin Heterograft

Elastin heterografts were obtained through a NaOH extraction process of porcine carotid arteries as described by Crissman [8]. Briefly, fresh porcine carotid arteries from local abattoir were defatted and washed in 0.9% saline, then placed in 80% ethanol for 24 hours at 4°C with shaking. The carotids were rehydrated in 0.05 M HEPES buffer (Sigma, MO) for 1 hour and elastin matrix was extracted using 0.25 M NaOH with sonication for 70 minutes. The extracted elastin appears white and translucent, histologically displays multi-laminar fiber structures, and is free from other extracellular matrix proteins and has no detectable cellularity. The elastin was rehydrated in 0.05 M HEPES buffer then autoclaved at 225°F for 30 minutes. The size of the elastin heterograft conduit for surgery was approximately 4 cm in length and 5 mm in diameter.

Preparation of Albumin Stent and Solder

The preparation details of albumin stent have been published previously [7]. Briefly, the commercial 25% human serum albumin (Michigan Dept. of Public Health, MI) was filtered through an ultra-filtration membrane (YM 30, Amicon Bioseparations, Millipore, MA), and concentrated to 50% (w/v) using an ultra-filtration system (Model 8400, Amicom, MA) under 35−45 psi pressure at room temperature. The 50% albumin was mixed extensively with a sterile 10 mM Indocyanine Green (ICG, Sigma, I2633, MO) solution at 1:100 ratio (v:v) such that the final ICG concentration was 0.1 mM in the mixture. The albumin and ICG mixture was placed in a sealed chamber at 24°C until it became moldable as excess fluid evaporated. The albumin was then molded to produce a hollow cylinder-like stent with a 3.5 mm outer diameter, 2.0 mm inner diameter, and 1.5 cm in length. The stent was packaged in an air-tight opaque container and stored at 4°C. The liquid solder was made with the same process as the albumin stent, but the procedure was stopped before the evaporation step. The final product of the solder consisted of 50% albumin with 0.1 mM ICG mixture. The solder was packed in a 1 cc syringe and stored in the dark at 4°C. The procedures were performed in sterile fashion.

Laser System

The laser used in this study was a diode laser (Coherent; Model:FAP system, 1001A; Santa Clara, CA) with a wavelength of 800 nm coupled to a quartz silica non-contact optic fiber (600 μm diameter). The laser operation mode was setup at 8 W with 0.1 seconds pulse width and 0.2 seconds pulse duration. The distance from optic fiber to target tissue surface was approximately 5 mm with a irradiative spot size 2 mm in diameter approximately. These parameters were selected from our preliminary experiments on laser-assisted anastomoses [7]. Before and after welding, actual energy was measured using an energy meter (Vector H310, Scientech, CA). Laser operative parameters were recorded, calculated during surgery, and are listed in Table 1.

Surgical Procedures

Of seven mixed gender domestic swine, body weight 130−187 lbs (average 151 lbs), six underwent end to end LAVA in bilateral carotid arteries, one animal received unilateral LAVA and the contralateral artery was surgically exposed but not be performed surgical procedure which served as no treatment control, refer to Table 2. Of total 21 anastomoses, five were performed as native artery to artery, sixteen were elastin heterograft to native artery which include both proximal and distal ends. Our surgical protocol followed the *Guidelines of the Care and Use of the Laboratory Animals* and was approved by the *Animal Care and Use Committee of Oregon Health Sciences University*.

Animals were fasted overnight and sedated with intramuscular injection of Telazol (1.0 mg/kg) followed by general endotracheal anesthesia, using 1–2% Halothane inhalant. An intravenous line was placed in the ear for administration of drugs and fluids. In supine position, the animal neck was shaved, prepped, and draped in a sterile fashion. Both carotid arteries were exposed via midline incision in the neck. Prior to dissection, Verapamil was given intravenously (0.5 mg per hour). Arteries were exposed and dissected free from the periadventitial tissue. Papaverine solution (120 mg in 60 ml of saline) was then applied locally at the site of incision to dilate the vessel and overcome the vasospastic response. Intravenous Heparin (5,000 U) was given before clamping the artery and repeated every 90 minutes until the procedure was completed on both sides.

The LAVA technique in native carotid artery to artery consists of exposure and division of the artery in the middle portion. A 4-mm diameter albumin stent was inserted into both ends. Two edges were approximated over the stent supported by three 7-0 Prolene stay sutures at 120 degree intervals. Albumin solder was painted on externally at the anastomotic site. Laser welding was first done anteriorly. The artery was then rotated with the help of sutures and the welding was done posteriorly. After completing laser welding, the distal and proximal clamps were released respectively. A similar technique was utilized at both ends

TABLE 1. LAVA Operative Parameters (Mean ± SD)

	Irradiation time (second)	Applied energy (J)	Irradiance[a] (J/mm^2)
Artery to artery (N = 5)	139 ± 104	212 ± 175	6.8 ± 4.5
Heterograft to artery (N = 16)	240 ± 163	273 ± 226	8.7 ± 5.7

[a]Irradiance equals total energy per total irradiated area.

TABLE 2. LAVA Results In Vivo

Animals	Side of carotid	Type of anastomosis	No. of anastomosis	Results
1	Left	A to A	1	Patent
	Right	A to H	2	Burst[a]
2	Left	A to A	1	Patent
	Right	A to H	2	Patent
3	Left	A to H	2	Burst[a]
	Right	A to A	1	Patent
4	Left	A to H	2	Patent
	Right	A to A	1	Thrombus
5	Left	A to H	2	Patent
	Right	Non		Patent
6	Left	A to H	2	Patent
	Right	A to H	2	Patent
7	Left	A to A	1	Patent
	Right	A to H	2	Patent

A to A, artery to artery anastomosis; A to H, artery to heterograft anastomosis.

[a]It was excluded from ultrasound sonography.

of elastin heterograft to carotid anastomosis. After clamping artery, the elastin heterograft preinstalled with albumin stents at both ends was used as an interposition graft. Three 7-0 Prolene sutures at 120 degree interval were placed at both proximal and distal ends of the carotid artery to retract the vessel opening to assist stent insertion. Then the stent preinstalled heterograft was approximated first into the proximal carotid artery with the help of three sutures. After the anastomosis was accomplished with solder at the proximal end, the graft was retracted caudally, and the preinstalled stent was carefully inserted into the distal end of the carotid artery where LAVA was carried out. After completing the anastomoses sutures were removed, and the distal and proximal clamps were released, respectively. When an anastomotic leak occurred, the artery was reclamped, solder was re-applied, and the leaking spot was re-welded. Circulation was restored for 3 hours after completion of the anastomoses. Doppler sonography (ATL HDI 5000, Philips Co, Bothell, WA) was performed at 3 hours with the wound open to determine the patency of the anastomosis, vessel inner diameter, and blood flow pattern through the native artery and the graft. Upon completion of the experiment, the anastomotic segment of the artery was explanted just prior to euthanasia. The specimen was stored in 10% buffered Formalin and fixed for Movat's pentachrome, and Hematoxylin & Eosin staining.

Statistics

All data are expressed as mean $\pm$ standard deviation. Parametric data of two groups was compared using Student's t-test. P-values < 0.05 were considered statistically significant.

RESULTS

The LAVA was achieved in every case in accordance with the above mentioned laser mode, which includes 5 anastomoses in native carotid artery to artery and 16 in elastin heterograft to carotid artery. There was no statistical significant difference of irradiation time, applied energy, and irradiance in artery to artery and elastin heterograft to artery anastomosis (Table 1). The average clamp time was 31 ± 16 minutes for a single native artery to artery anastomosis and 39 ± 25 minutes for both anastomoses of elastin heterograft to carotid artery. Four anastomoses, one artery to artery anastomosis (1/5, 20%) and three heterograft to artery anastomoses (3/16, 19%), had to be re-clamped and re-welded as described above to control anastomotic leakage. Also, in one instance, a small hole in the elastin graft was sealed with external laser soldering. After releasing arterial clamps, circulation was immediately restored in all cases. Out of five native artery to artery anastomoses, one thrombosed 1 hour after LAVA. The remaining artery to artery anastomoses remained patent without anastomotic leakage through the 3-hour observation period. All heterograft to native artery anastomoses were patent at initial LAVA. Unfortunately, two elastin heterografts burst on the posterior surface just below the distal anastomosis approximately 1 hour 45 minutes post the LAVA procedure. These two vessels were ligatured so that four heterograft to artery anastomoses were excluded from the final Doppler sonography assessment. The contralateral carotid arteries were still patent after the incidents through the 3-hour study period (Table 2). The anastomotic site appeared as a crusty ring due to denaturation of the albumin solder. The control artery was patent for 3 hours.

Vascular Doppler sonography showed that the majority of heterograft to native artery anastomoses had turbulent flow through the anastomoses and graft due to size mismatch. The average vessel inner diameter of the porcine carotid artery and the elastin heterograft were 4.4 mm and 5.8 mm, respectively. No turbulent flow pattern was found in artery to artery anastomoses and the control artery.

Histology demonstrated coagulative necrosis of the adventitial collagen in the native carotid artery. There was

patchy necrosis of the media characterized by shrunken, hypereosinophilic contracted smooth muscle cells called contraction band necrosis (Fig. 1). Albumin presented on the adventitial and luminal aspect of the anastomotic site. The albumin on adventitia had a vacuolated appearance due to laser effect. The intraluminal albumin appeared as a homogenous thin layer and did not obstruct the lumen. In native artery to artery anastomosis, an albumin-filled gap of 100−300 microns between the anastomotic edges was noted (Fig. 2). The elastin heterograft is characterized by dark Movat staining (Fig. 3). Due to size mismatching of elastin heterograft to native carotid, the anastomotic edges were slightly overlapped. Small amounts of non-occlusive fibrin thrombus were noted at the anastomotic sites in most cases. Thermal changes were noted on the heterografts. In the single occluded artery to artery anastomosis, the anastomosis was occluded by fresh thrombus.

DISCUSSION

Although LAVA and laser-assisted microvascular anastomosis (LAMA) techniques have existed for more than 20 years, many problems still prevent its use in clinical practice. These include low welding strength, poor reproducibility, and extensive tissue thermal damage. In addition, the difficulty of maintaining the precise circumferential apposition of an end to end tubular anastomosis
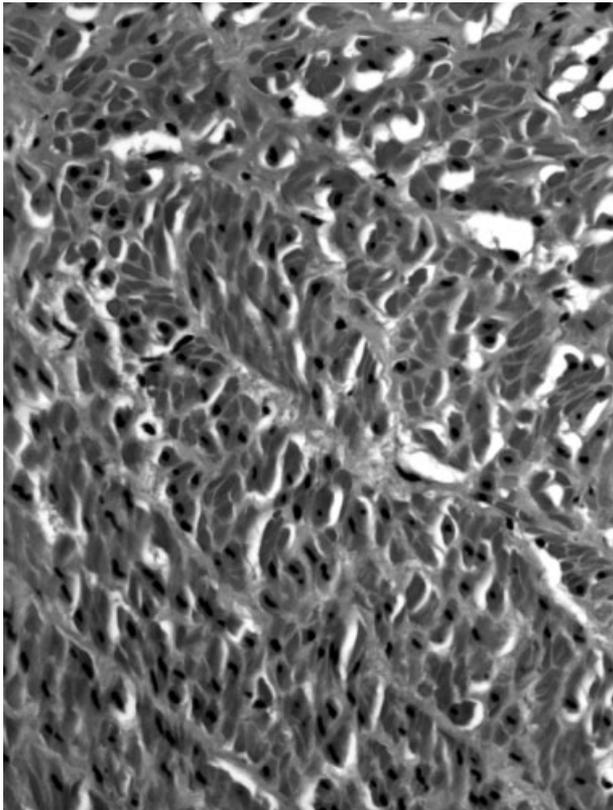

Fig. 1. Native artery media (H & E stain, 400×). It shows scattered, shrunken, hypereosinophilic contracted smooth muscle cells in the vessel media.

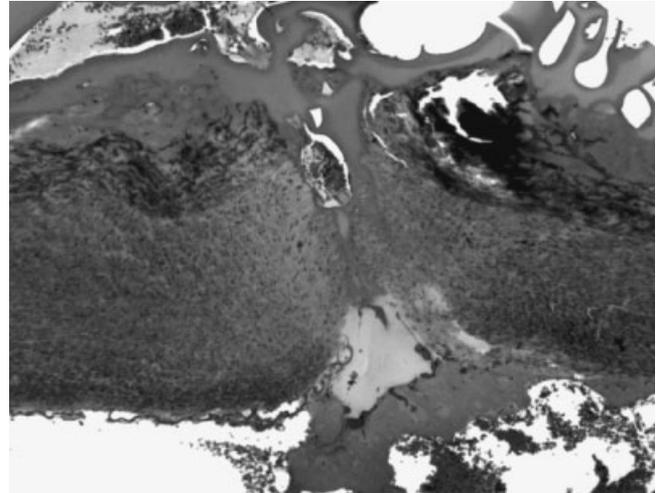Fig. 2. Native artery to artery anastomosis (Movat stain, 100×). The albumin coats the incision, most prominently at the adventitial surface (**top**). There is a small amount of albumin-fibrin-platelet mixed thrombus in the lumen (**bottom**).

during the laser tissue welding is a technical challenge [9]. Other challenges include limiting thrombogenicity, preventing heat-induced intimal hyperplasia and anastomotic aneurysm formation [10]. For laser welding to become practical, laser parameters for the specific physiological and geometric characteristics of the welded tissues must first be optimized. The underlying mechanism of laser tissue welding is generally accepted to be thermal remodeling of tissue proteins, interdigitation of collagen fibrils, and formation of biological glue from collagen cross-linking [11]. There are thus even more challenges to overcome if laser welds are to be effective on collagen-free grafts, such as the elastin heterograft or a Gortex graft. The use of protein solder may help the welding strength in such
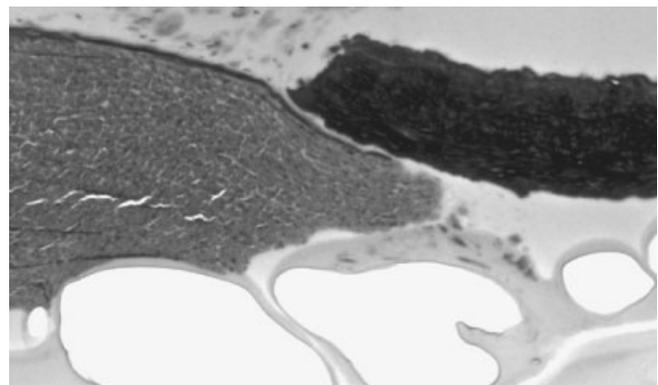

Fig. 3. Elastin heterograft to carotid artery anastomosis (Movat stain, 100×). The weld site shows a small amount of thrombus in lumen (**top**) with albumin vacuolization at the adventitial aspect (**bottom**). The media of the native artery (right) shows shrunken, hypereosinophilic cells consistent with coagulative necrosis. There are no changes in elastin heterograft (**left**).

applications. In our knowledge, this is the first report of using LAVA combined with albumin stent in vivo to anastomose a collagen-free elastin heterograft to a medium size native carotid artery.

In this study, we used an 800 nm wavelength diode laser in pulse mode associated with an intravascular albumin stent and external solder to improve the welding strength and limit the thermal damage. We expect that this technique will reduce suture use and provide a watertight anastomosis. One of the few technical difficulties we faced during this study of laser welding a heterograft to an artery in vivo was to overcome the severe vasospastic response in the porcine carotid artery. This caused mismatching of the end to end approximation edges between the native artery and heterograft. The heterografts were bigger in size than the native vessel because they were prepared from proximal end of carotid artery of larger animals (up to 400 lbs). Hence we have to manage the mismatch of the heterograft to the native artery by making a pleat at one point of the elastin graft. This pleat point became the most common site of hemorrhage after the anastomosis. This size discrepancy was also responsible for the longer time of surgical manipulation and laser irradiation, as well as for the turbulent pattern of blood flow across the anastomosis and through the heterograft. All these variables could be minimized if the proper sized graft is used. One thrombotic occlusion occurred at the artery to artery anastomosis. In this case, the artery was mechanically dilated to insert an albumin stent due to severe vasospasm. The thrombosis may result from the stent insertion process caused arterial intimal injury [12]. That two elastin heterografts burst during the study had demonstrated the weakness of the graft. The mechanical strength of the elastin heterograft has to be improved.

In our laboratory, the albumin stent has been used in ureteric anastomosis [7] but not in LAVA. Our experience demonstrated that using the albumin stent improved laser welding strength and technical ease in end to end laser-assisted anastomosis [13]. While anastomosing native carotid artery to artery we noted significant retraction of the two ends and stay sutures were necessary. The stents were very helpful in keeping the ends opposed while laser welding the vessels. These stents rapidly dissolve in blood and therefore did not cause any obstruction to blood flow. The histological images show that the denatured or coagulated albumin remnants from the stent co-mingled with the external solder through the anastomotic gap to form a sandwich structure at the edges that may improve the strength of the anastomosis. Long-term consequences of the intraluminal albumin in LAVA have not yet been studied.

In conclusion, using LAVA technique with the intravascular albumin stent is feasible for a medium sized arterial anastomosis, particularly in anastomosing a non-collagen containing graft, such as an elastin heterograft, to native artery. We recognize the limitations of this study including its acute nature and the relatively small number of subjects. Further chronic study is necessary to evaluate the long-term patency and chronic histological changes of the anastomosis using this technique.

ACKNOWLEDGMENTS

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