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ORIGINAL ARTICLE

Endoluminal laser-assisted vascular anastomosis—an in vivo study in a pig model

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Abstract Microvascular surgery is time consuming and requires high expertise. Laser-assisted vascular anastomosis (LAVA) is a promising sutureless technique that has the potential to facilitate this procedure. In this study, we evaluate the handling of our soldering material and the 1-week patency rate in a porcine model. Six pigs were subjected to LAVA. For each pig, the saphenous artery on one side was transected while the contralateral side was used as control. A porous polycaprolactone scaffold soaked in 40% (*w/w*) bovine serum albumin solution in combination with 0.1% (*w/w*) indocyanine green was wrapped at the anastomosis site and at the control site. Both sides were then soldered with a diode laser coupled into a light diffuser fiber emitting radiation with a wavelength of 808 nm and a power of 2–2.2 W. Vessels were successfully soldered with a 100% immediate patency rate. The 1-week patency rate was 83% for the anastomoses versus 67% for the control side. Vessels irradiated for 80 to 90 s tended to maintain the highest patency rate. Macroscopically, there was no difference between the two sides. The patch was easy to handle provided that the environment could be kept dry. This study shows the potential and the limitations of endoluminal LAVA as a one-step procedure without the use of stay sutures. Further studies are needed to improve the soldering material, the long-term patency rate, and standardized irradiation parameters. The long-term effects of laser soldering on the vessel wall remain to be determined.

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Introduction

The difficulties in vascular surgery were already clearly cited in the classic paper by Alexis Carrel in 1902 regarding the technique for vascular end-to-end and end-to-side anastomosis [1]. These difficulties are technical, including the coaptation of the vessel walls, the meticulous handling of the vessel, and precise suturing. In addition, the vessel has to be entirely tight while being fully patent [2]. Vascular anastomoses require high experience from surgeons since they have to be accomplished in decent time to maintain organ vitality. Finally, due to the intrinsic nature of the vessel wall, anastomoses are prone to thrombosis and hence failure. Carrel's original paper described the use of three-stay sutures to help with the proper coaptation of the two vessel ends, which is still done today, including for laser-assisted vascular anastomosis (LAVA) [3]. Other non-suture methods have also been described such as the use of staples, clips, and glue but none of them have been able to show superiority over standard surgical vascular anastomosis [3].

In the 1970s, LAVA has been introduced as a promising tool that could overcome the surgical challenges during vascular anastomoses. Laser applied at the site of anastomosis produces a photothermic reaction which reinforces the tissue through heat absorption. This process of melting tissues together is named laser welding. Studies have shown that the far-red to near infrared region (NIR) of the spectrum with wavelength between 650 and 900 nm allows safe application due to minimal tissue absorption [4]. The addition of a chromophore such as indocyanine green (ICG) allows furthermore selective energy absorption and hence reduces damages to the surrounding tissue. Nevertheless, with long exposition time, tissue alteration and injury do occur [5]. Hence, another problem faced with LAVA is the low bonding strength following laser welding. To overcome this difficulty, solders were introduced to strengthen the site of anastomosis in a process termed laser soldering. It also acts by denaturing proteins of the tissue and the solder by thermal effect. They thus coagulate into a seal strong enough to withstand the blood pressure. As true for each technique, clear and unanimous guidelines have yet to be put in place to allow easy reproducible results in the hands of surgeons and to ensure a 100% reliable anastomosis.

We have previously developed a new soldering material, consisting of a polycaprolactone (PCL) based scaffold in combination with bovine serum albumin (BSA) and ICG [6]. This patch enhances the bonding strength due to the addition of the gluing protein albumin. Furthermore, our team has introduced a technique using an intraluminal laser source in combination with an external solder [7]. In this previous article, we were able to show ex vivo that this technique allows a homogeneous irradiation of the whole vessel circumference and did indeed cause minimal tissue damage. We subsequently applied this technique on a pig model [8] and observed excellent patency rates over a 3-h period.

The goal of this new study would be to show that the 1-week patency rate of transected vessels treated with endoluminal LAVA is equivalent to uninjured control vessels on which the same procedure is applied. Furthermore, we aim to assess the performance of our new soldering composition and set the foundations for further studies.

Materials and methods

Scaffold and solder preparation

The scaffold production protocol and the solder preparation have been extensively described in a previous article by Bregy et al. [6]. Briefly, porous PCL scaffolds (Mn 80,000 (GPC), Sigma–Aldrich Chemie GmBH, Steinheim, Germany) were produced using the solvent casting and particulate leaching technique. 501.45 mg of PCL were dissolved in 15 ml of chloroform (purity 99.0–99.4%, MERCK, Darmstadt, Germany) using a magnetic stirrer (MR2002 Heidolph Instruments GmbH & Co.KG, Schwabach, Germany). A porous membrane was then obtained by adding 5.546 g of sodium chloride (reinst, Ph Eur III, Dr. Grogg Chemie, Stettlen, Switzerland), casting the mixture into a petri dish (Sterilin ™ Thermo Fisher Scientific, Waltham, MA, USA), and leaving it to dry under the fume hood for 24 h. For the solder preparation, 40% (*w/w*) BSA (Sigma–Aldrich, Saint Louis, MI, USA) were dissolved in ultrapure water together with 0.1% (*w/w*) ICG (Acros Organics-Janssen Pharmaceuticals, Geel, Belgium) using the same magnetic stirrer and a water bath at 37 °C. The PCL scaffold was washed in pure water to remove the salt. The scaffold was then immersed in 20 ml of the solder for 4 h and cut to 5 mm × 5 mm to obtain the desired soldering patch of thickness 200 to 400 μm.

Surgical protocol

All animal experimentations were performed after approval from the animal care committee of the Canton of Bern, Switzerland (Nr. BE72/07) and in agreement with the guidelines for the care and use of experimental animals of the National Institutes of Health. Six white adult pigs with an average weight of 40 kg underwent standardized surgery on both of their hind limbs forming two equal groups of the six hind limbs. One side was used as the experimental side while the other was used as a control. They received a prophylactic antibiotic therapy along with 100 mg of acetylsalicylic acid (aspirin®, Bayer, Münchenbuchsee, Switzerland). General anesthesia was induced with 10-mg/kg body weight ketamine (Pfizer, Zurich, Switzerland) given intramuscularly. Ten minutes later, 5-mg/kg metomidate (Hypnodil®, Jaansen-Cilag, Zug, Switzerland), 0.05-mg/kg atropine (Labatec Pharma, Meyrin, Switzerland), and 2-mg/kg azaperon (Stresnil®, Biokema, Crissier, Switzerland) were administered intravenously for tracheal intubation. Anesthesia was maintained with halothane and 79% nitrous oxide in oxygen. Inhaled and exhaled concentrations of halothane and nitrous oxide were continuously monitored with a multi gas analyzer (Hellige SMU 611; Hellige, Freiburg, Germany). The animals also received volume-controlled ventilation with a positive end expiratory pressure of 5-cm H_2O (Tiberius 19; Drägerwerk, Lübeck, Germany), and the body temperature was kept constant through the whole experiment. Ringer's lactate was administered intravenously at a rate of 10–15 ml/kg/h. They were then placed in supine position and prepped and draped in a sterile manner. All interventions were performed by the same surgeon. The saphenous artery was accessed by an incision at about the medial part of the thigh, extending distally. It was then dissected free for a length of 6 cm to the bifurcation. Side branches were clipped or ligated under microscopic view, and 10,000 units of heparin were administered IV. A bulldog clamp was then placed proximally interrupting the blood flow, and an arteriotomy was performed with microscissors on a distal side branch to allow the introduction of a balloon catheter (Fox Plus, PTA catheter, max ø = 3 mm, AP12003, Abbott, ALVE Ltd. Beringen, Switzerland). The saphenous artery was transected and the balloon catheter was retrogradely advanced in a way to lie just at the level of the section. The two arterial stumps were carefully adjusted over the balloon catheter and held in place with an Acland double microvascular clamp (Synovis Micro Companies Alliance, Inc. Birmingham, USA). The custom-made laser fiber with diameter of 0.8 mm with a light diffuser at its distal end (cylindrical light diffuser, model RD, Medlight

SA, Ecublens, Switzerland) was then introduced through the working channel of the PTA catheter and advanced to the repair site. Proper alignment was verified with the guiding beam. The balloon was thereafter inflated with saline water. Finally, the semi-solid albumin solder patch of length adapted according to the vessel diameter was uniformly placed at the soldering site in a way to cover the entire diameter of the vessel. On the contralateral hind limb used as control, the identical surgical procedure was performed; however, the saphenous artery was not transected and the solder was applied over the intact saphenous artery. This was done in order to reduce the variables between the two groups and only have the vessel transection as the main difference.

Soldering process and follow-up

A GaA1As (gallium aluminum arsenide) diode laser system (DL50, FISBA Optik, St Gallen, Switzerland) emitting continuous wave irradiation at wavelength $\lambda = 808$ nm and with a power of 2–2.2 W. Laser irradiation was performed until the solder was melted, and a color change was noticed. The temperature during soldering was assessed with an infrared camera placed above the anastomosis area at a minimal distance of 70 cm (Fig. 1) (Radiance HS, Raytheon, Waltham, MA). It was calibrated for a temperature range between 8 and 145 °C, allowing an accuracy of 1%. The camera controlling and data recording were performed using Image Desk II software (Harris Geospatial Solution, Gilching, Germany). Analysis and data processing were performed using IDL 6.2. The patency of the vessel was assessed clinically; flow was recorded with a flowmeter (TS420, Transonic System Inc., Ithaca, NY); and the anastomosis was evaluated for any eventual leakage. The same soldering procedure was applied on the contralateral side. After soldering, the clamp, the fiber, the balloon catheter, and the bulldog clamp were removed. For both sides, hemostasis was followed by closure of the wounds on two layers and the subsequent application of Opsite spray® (Smith & Nephew, Baar, Switzerland). Animals were then extubated and discharged to the animal unit for the follow-up period. They were then re-operated 1 week later using the same incisions to assess the 1-week patency rate.

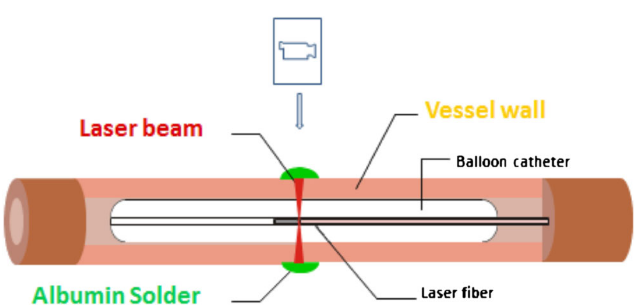

Fig. 1 Schematic representation of the experimental set up

Statistical analysis

Statistical analysis was performed using SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, version 21.0. Armonk, NY: IBM Corp). Wilcoxon test analysis and Kruskal-Wallis post hoc test were carried out. Values were given as mean ± standard deviation, and statistical significance was given to values with $p < 0.05$. Post hoc power analysis was also carried out showing a power of 0.24.

Results

Soldering was stopped according to visual observation. We had a 100% patency rate after surgery for both the experimental and the control group, which was confirmed clinically by the presence of blood flow. Figure 2 illustrates the appearance of the soldered vessel. One week after surgery, one soldered vessel on the experimental arm of the study had become occluded and two in the control arm of the study (Table 1). This was confirmed clinically and macroscopically by the presence of a thrombus visualized distal to the anastomosis. The overall 1-week patency rate was hence 75%, with an 83% patency rate for the anastomosis arm (five out of six) and 67% for the sham arm (four out of six). We observed that vessels that remained in majority patent were the ones that had been irradiated for 80 to 90 s (Table 1). Flow measurements were recorded before surgery and directly after LAVA was completed. It showed a decrease in comparison with the mean preoperative value from 27.64 to 10.75 ml/mn ± 5.3 ($p > .05$, $n = 4$) postoperatively on the transected side and 8.9 ml/mn ± 5.0 on the sham side ($p > .05$, $n = 3$) (Fig. 3). Systolic blood pressure measurements preoperatively and postoperatively showed a decreasing trend after the surgery with values ranging from 138 to 60 mmHg preoperatively and from 111 to 51 mmHg postoperatively. Macroscopically, there was no difference between the experimental and the sham-operated side (Figs. 4 and 5).

Recording of the temperature revealed different curves throughout the experiment for all treated vessels. Figure 6

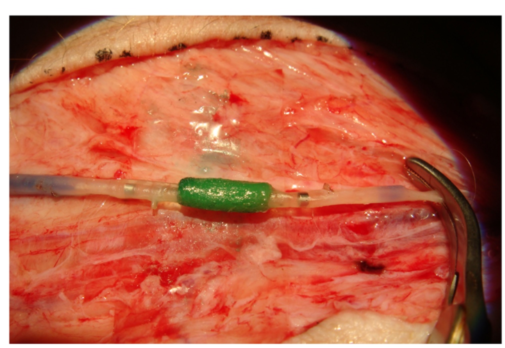
Fig. 2 Soldering in vivo

Table 1 Summary of the individual surgical outcome

	Pig 1	Pig 2	Pig 3	Pig 4	Pig 5	Pig 6
Observation time	7	7	7	7	7	7
Irradiation time anastomose (s)	81	90	90	76	90	61
Irradiation time sham (s)	81	65	90	76	77	58
Patency anastomose	Yes	Yes	Yes	No	Yes	Yes
Patency sham	No	No	Yes	Yes	Yes	Yes

shows an example where the temperature curve of the control side exhibits one peak while the curve on the anastomosis side shows several intervals of increase and decrease. Nevertheless, the average temperature was 68 °C for both groups.

Discussion

For the past 40 years, the use of laser as an adjunct to vascular surgery has been a hot topic, but the technical aspect and the absence of consensus prevent its routine use in operating rooms. Vascular surgery is very tedious and not without complications even in the hands of a trained specialist. Following open arterial repair of a major arterial vessel after trauma, the failure rates are as high as 5.2% [9], and close to 1% for microsurgical anastomoses for free flap transfer [10]. The main complications after arterial anastomoses are early occlusion, bleeding, ischemia, infection, and failure of repair with a need for re-intervention, the formation of fistula or pseudoaneurysm [9]. A swine model was chosen for the experiment because of their close anatomical resemblance to humans [11].

To accomplish a sutureless intraluminal laser soldering, an arteriotomy has to be performed. There are several advantages with using this approach. First of all, the severed vessel can be accessed distally or proximally through another location such as the femoral artery (as is the case during cardiac catheterization), or through a side branch as was the case during our procedure. The balloon catheter can then be endoluminally advanced to reach the site of anastomosis. Once completed, the entry site can simply be closed, or as in our experiment, the collateral vessel can be clipped with standard microsurgical clips. Furthermore, this technique allows an even distribution of the laser beam at the site of the anastomosis while keeping the two vessel stumps in contact without the need for stay stitches. This reduces the amount of trauma to the actual anastomosis site.

Before soldering, the scaffold was placed at the chosen site, and the irradiation was performed according to the protocol. Due to its nature, the patch is extremely flexible and can be easily applied on vessels. One difficulty faced is that the solder becomes very sticky and harder to handle when in contact with liquids. We will address this issue in future studies by further modifying the composition of our solder material. For the current study, this problem was addressed by the use of a surgical peanut to isolate the vessel from contiguous structures. Another variable is the different water content of the vessels during surgery, which leads to different temperatures during soldering. The temperature measurements showed an average soldering temperature identical for both sides. The value of 68 °C is under the ideal soldering temperature of 80 °C which has been shown to be slightly above the temperature required to denature the ICG doped albumin scaffold and allow stronger tissue bonds [12]. This was sufficient as we also had periods with higher irradiation temperature. This average underestimates the actual effective soldering temperature for two reasons: first of all, the temperature recording set up used allows us to only measure the temperature of the

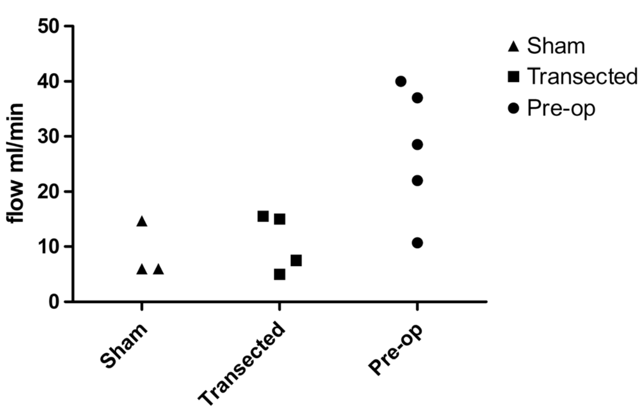

Fig. 3 Pre and postoperative blood flow recorded before surgery and directly after the LAVA procedure. We observed a decrease in blood flow following laser soldering ($p > 0.05$)

Fig. 4 Macroscopic appearance of the soldered vessels, patent anastomosis

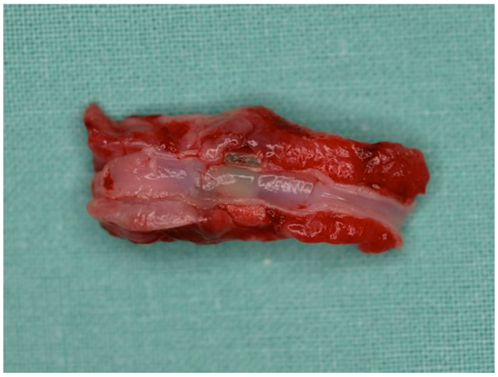

Fig. 5 Macroscopic appearance of the soldered vessels, patent vessel sham

external surface of the patch and not the interface solder/vessel. Nevertheless, previous simulations from Bogni et al. showed that repetitive irradiation under 30-s duration allowed to maintain the intima temperature under 65 °C while the external surface of the patch could be heated above 80 °C. Second of all, it takes into account the time when the scaffold is heating up until the time the temperature decreases below 75 °C [12]. The analysis of the temperature curves on both the control and the anastomosis side shows two patterns: one pattern is an ascending phase to reach the soldering temperature of 80 °C, after that, the effective soldering phase where denaturation of the solder takes place, and finally the cooling period until the solder temperature regains its starting temperature. The other pattern observed is that previously described; there is a crescendo decrescendo aspect due to repetitive exposure without an actual effective soldering phase. In both cases, the soldering was nevertheless successful.

The irradiation time on both the control side and the experimental side showed an important fluctuation ranging from 58 to 90 s on the control side and from 61 to 90 s on the anastomosis side. The difficulty in standardizing the irradiation time lies in the fact that the temperature of the anastomosis is influenced by the humidity of the solder and how the solder is wrapped around the vessel. These factors are extremely hard to keep constant. Temperature might therefore increase faster

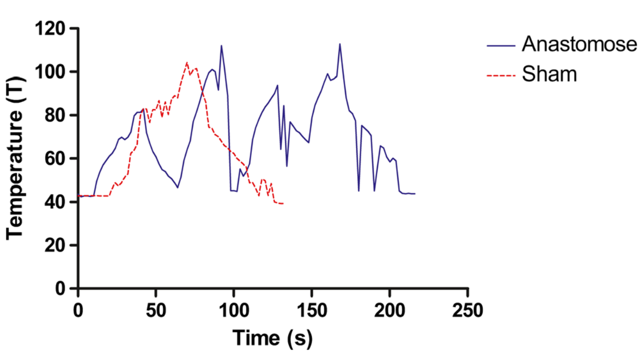

Fig. 6 Example of the temperature profile during soldering. The analysis of the temperature curves on both the control and the anastomosis side exhibited two patterns

or slower. In the case of the former, a brief interruption to allow cooling and prevent vessel damage is therefore necessary [7, 13].

We achieved a 1-week patency rate of 83% on the anastomosis side and of 67% on the sham side despite an initial 100% patency rate. The decrease in patency in our follow-up period can have various etiologies. One reason could be thrombus formation due to the laser-induced inflammation of the vessel wall. Nightingale et al. showed that the re-endothelialization period takes up to 2 weeks, and formation of thrombus takes place at the site of vessel handling. This was the case during the coaptation process with forceps over the fiber which may have led to injury [14]. The use of a new custom-made catheter with a diameter more suited to the vessel width in conjunction with the use of a prophylactic antithrombotic treatment postoperatively might therefore be a key element in further studies. The fact that the patency rate was even lower on the control side than on the transected vessels indicates, however, that the coaptation process was not the main reason for the observed obstructions. This is supported by the observation that the flow rate and systolic pressure were comparable between the two groups. Stenosis at the anastomosis site could be another reason for a lower patency rate. The goal of the intraluminal balloon catheter is to prevent the vessel's lumen from being obstructed during the soldering process but this might have occurred after its removal due to the weakened vessel wall. This unexpected result also outlines the difficulty with laser soldering and could simply signify that despite our experimental set up we were unable to reduce the number of variables involved.

We used a flowmeter to evaluate the patency of the vessel in order to obtain blood flow velocities and have a better understanding of the effect of the procedure on the vessel. Furthermore, we also looked at the systolic pressure distal to the site of soldering. A study performed by Marchese et al. showed that an increased flow of more than 10% distally to the anastomosis and a decreased systolic blood pressure could indicate the presence of a stenosis [15]. The reduction of both the flow rate and the systolic pressure that we observed postoperatively can be explained by the fact that the postoperative blood pressures were obtained directly after surgery and are therefore prone to values lower than normal. The reduction of flow observed postoperatively on both the sham side and the anastomosis side was not significant compared to the preoperative values. Several publications have also mentioned the long-term risk for aneurysm formation associated with lower bursting pressure possibly due to weakening of the arterial wall with damage to the elastic lamina and an alteration of the media from thermal damages [16–18]. In one case (pig number 6), a lesion was accidently made to the vessel wall in the experimental side while introducing the fiber. It was successfully repaired by soldering but increased the irradiation time needed and might have weakened the vessel wall. Interestingly, this vessel remained patent throughout the observation period, and we did not observe any vessel dilatation at 1 week.

The use of the contralateral side as the control side without transecting the vessel allowed us to point out difficulties with this technique that will have to be addressed in further studies while reducing the number of animals needed for the experiment. We cannot rule out that the non-significance between the results obtained is not caused by the limited size of our study which directly reduces its power. A post hoc power analysis using the effect size obtained in the current study ($d = 0.37$) showed that a sample size of 25 pigs would have been needed to achieve a power of 0.80. We chose a small sample size for ethical reasons and to reduce the number of animals used for this study as it investigated new data with no reference values. This will allow the planning of further studies. Nevertheless, this study provides a good insight regarding the feasibility of this new LAVA procedure in vivo, possible limitations, and parameters to optimize. Further studies will also have to focus on a more accurate automatic temperature control and on the precise effect of endoluminal laser soldering on the vessel.

Conclusion

This study demonstrated the potential and limitations of LAVA by an endoluminal approach and the limitations of our new scaffold. With the exception of its stickiness, it was easy to handle. Additional studies on this technique are needed with a larger set of animals to standardize the irradiation parameters and improve the patency rate. The long-term effects of LAVA on the vessel wall also need to be assessed while comparing this technique against standard vessel suturing.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Informed consent This article does not contain any studies with human participants performed by any of the authors.

Ethical approval All animal experimentations were performed after approval from the animal care committee of the Canton of Bern, Switzerland (Nr. BE72/07) and in agreement with international guidelines and the guidelines for the care and use of experimental animals of the National Institutes of Health.

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