Optimization of in vitro sutureless laser-assisted vascular anastomosis

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End-to-end scaffold-enhanced laser-assisted vascular anastomosis: ex vivo proof-of-concept in porcine arteries

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**Abstract:**

**Objective:** To investigate the feasibility of ex vivo sutureless end-to-end laser-assisted vascular anastomosis (LAVA) in medium-sized arteries.

**Summary background data:** LAVA in combination with solder and polymeric scaffolds (ssLAVA) has been optimized in vitro. Proof-of-principle of the optimized modality needs to be established in an ex vivo model.

**Materials and methods:** Scaffolds composed of poly(ε-caprolactone) (PCL) or poly(lactic-co-glycolic acid) (PLGA) were impregnated with semi-solid solder and placed over coapted aortic segments. ssLAVA was performed with a diode laser. In the 1st substudy, the optimum number of laser spots was determined by bursting pressure (BP) analysis. The 2nd substudy investigated the resilience of the welds in a Langendorf-type pulsatile pressure setup, monitoring the number of failed vessels. The type of failure (cohesive vs. adhesive) was confirmed under SEM and thermal damage was assessed histologically. The 3rd substudy compared breaking strength (BS) of aortic repairs made with PLGA + semi-solid genipin solder to repairs made with BioGlue.

**Results:** ssLAVA with 11 lasing spots and PLGA scaffolds yielded the highest BP, (923±56mmHg vs. 703±96mmHg with PCL-ssLAVA) and exhibited the fewest failures (30% vs. 80% for PCL-ssLAVA). PLGA anastomoses broke adhesively, whereas PCL welds failed cohesively. Both modalities exhibited full thickness thermal damage. Repairs with PLGA scaffold yielded higher BS than BioGlue repairs (323±28N/cm² vs. 25±4N/cm², respectively).

**Conclusions:** PLGA-ssLAVA yields greater anastomotic strength and fewer anastomotic failures than PCL-ssLAVA. Aortic repairs with BioGlue were inferior to those produced with PLGA-ssLAVR. PLGA-ssLAVA should therefore be pursued as a clinically viable alternative to conventional suturing.
Introduction

Laser-assisted vascular anastomosis (LAVA) is an experimental, non-mechanical anastomosis technique that has the potential to replace suturing in minimally invasive and endoscopic surgery.\textsuperscript{1-3} Mechanistically, laser irradiation of coapted vessel segments causes absorption and conversion of incident light to heat by the chromophores (typically water or added pigments, depending on the chosen wavelength), heat diffusion, and thermal denaturation of mainly collagen in the vascular wall. The subsequent cross-linking of collagens in the adjoining vessel segments ultimately causes the tissue to fuse.\textsuperscript{1,2,4,5} Compared to mechanical closure such as by sutures, clips, or staples, LAVA is less traumatic, induces no foreign body reactions, and provides immediate liquid-tight sealing.\textsuperscript{1,2,5-10} However, LAVA is associated with relatively low welding strength, extensive thermal damage, poor reproducibility, and ambiguous end-points, which constitute major drawbacks that have hampered clinical application of the modality.\textsuperscript{1,2,5,11-14}

To improve welding strength and reduce thermal damage, much focus has recently been placed on scaffold- and solder-enhanced LAVA (ssLAVA). In ssLAVA, a biodegradable polymeric scaffold drenched in chromophore-containing proteinaceous solder is placed on the coaptation and irradiated. The photothermal processes underlying the welding effect in ssLAVA are similar to LAVA: the cross-linking between denatured tissue collagen and solder proteins (cohesive bonding) as well as between denatured proteins in the solder (adhesive bonding) account for the welding strength, the latter reinforced by the network of polymeric fibers that courses through the solder.\textsuperscript{15-22}

Our group has recently published several studies on ssLAV repairs (ssLAVR) using poly(\varepsilon\textsuperscript{-}caprolactone) (PCL) and poly(lactic-co-glycolic acid) (PLGA) scaffold material.\textsuperscript{18-20} The ssLAVR modality was optimized for both PCL and PLGA scaffolds in these studies, which revealed that (1) semi-solid bovine serum albumin (BSA) solder produces stronger welds than liquid BSA solder, (2) single spot pulsed lasing (SSPL) yields better results than single spot continuous lasing (SSCL), and (3) neither of the scaffolds is superior over the other with respect to post-ssLAVR breaking strength (BS) following 7-day hydration in physiological buffer, i.e., the most relevant parameter in regard to clinical translatability. The low melting point of the PCL material (62°C) accounted for a lower acute welding strength than achieved with the more thermoresistant PLGA (148°C) (248±54N/cm\textsuperscript{2} versus 409±79N/cm\textsuperscript{2}, respectively), but the hydrophobicity of PCL gave rise to more stable welds under quasi-physiological conditions, ultimately yielding BSs of 203±40N/cm\textsuperscript{2} versus 109±43N/cm\textsuperscript{2}, respectively (pg. 83-84). Apparently, PLGA scaffolds are more susceptible to hydrolytic degradation, as the material is less lipophilic than PCL.\textsuperscript{23} It was further demonstrated that addition of the protein cross-linking agent genipin to semi-solid solder enhanced welding strength following PLGA ssLAVR but not PCL ssLAVR. Aortas welded with PLGA + semi-solid-genipin solder exhibited post-hydration BS that was comparable to welds made with PCL + semi-solid solder, yielding 224±19N/cm\textsuperscript{2} versus 203±40N/cm\textsuperscript{2}, respectively (pg. 85-86).

The next step – and the aim of this study – was therefore to validate the optimized
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PCL and PLGA ssLAVA modalities in an ex vivo end-to-end anastomosis model using clinically representative, medium-sized porcine carotid arteries. The study was divided into three substudies. In the 1st substudy, the modalities were further optimized with respect to the number of lasing spots that were to be administered along the entire circumference of the coapted blood vessel segments. In the 2nd substudy, the ssLAVAed vessel segments were placed in a Langendorf-type setup and subjected to a 24-h pulsatile pressure test to determine the weld resilience and type of failure (adhesive versus cohesive) under quasi-physiological conditions. Finally, the 3rd substudy compared the most optimal ssLAVR modality from substudy 2 (PLGA ssLAVR) to BioGlue, a surgical adhesive used frequently in cardiovascular surgery for tissue adherence in aortic dissection and reconstruction,24,25 in terms of BS so as to ascertain the most suitable approach for further (pre)clinical testing.

Materials and methods

Tissue preparation

Fresh porcine carotid arteries (n=40) and aortas (n=3) were harvested at the slaughterhouse. After trimming the perivascular tissue, arteries were cut in 6-cm segments (n=80, external Ø=4.3-5.9mm) and aortas were cut along the longitudinal axis. Fourteen aortic strips (30×5mm) were punched out of aortic slabs using a parallel cutter. Vessels were stored in sterile PBS at 4°C and were used within 3d.

Preparation of PCL and PLGA scaffolds

All scaffolds were electrospun in a climate-controlled electrospinning cabinet (IME Technology, Eindhoven, The Netherlands) equipped with a 14-G capillary. Scaffolds were spun at a distance of 15cm between the capillary and the target drum (25×120mm). PCL (CAPA 6800, Perstorp UK, Cheshire, UK) was dissolved in chloroform to a 17% (w/w) concentration. The polymer-containing solution was electrospun for 25min at 23°C and 50% relative humidity and a flow rate of 60µL/min and 15kV to produce meshes consisting of fibers with a diameter range of 12-14µm.18-20 To produce PLGA meshes with a similar fiber diameter, PLGA (Purasorb PLG 82.18, Purac, Gorinchem, The Netherlands) was dissolved in chloroform at a 13% (w/w) concentration. The solution was spun for 60min at 23°C and 40% relative humidity at 16kV and a flow rate of 20µL/min.

Fiber diameter was determined by scanning electron microscopy (SEM, Quanta 600F ESEM-FEG, FEI Company, Hillsboro, OR) using analytical software (Xt Microscope Control, FEI Company). Scaffolds were punched out of the mesh in 18×5-mm arrays. Scaffold thickness was measured with a digital micrometer between two glass slides and scaffolds with 150-180-µm thickness were used for the experiments.

Solder preparation

Semi-solid albumin solder was prepared by dissolving 48% (w/v) BSA (Fraction V, Roche, Penzberg, Germany), 0.5% (w/v) methylene blue (MB, Sigma-Aldrich, St. Louis,
MO), and 3% hydroxypropylmethylcellulose (HPMC, Sigma-Aldrich) in MilliQ water.\textsuperscript{20,26} This solder was used for PCL ssLAVA as our previous study revealed that this solder yielded the strongest welds.\textsuperscript{20}

For PLGA ssLAVA a semi-solid genipin solder was employed in accordance with our previous study. Genipin (Sigma-Aldrich) was dissolved in MilliQ (1\% (w/v) final concentration) for 24h at 4°C under continuous stirring until a homogenous suspension was obtained. Next, the genipin solution was placed in a water bath at 38°C for 30 min to maximally dissolve the genipin\textsuperscript{27,28}. Semi-solid genipin solder was prepared by mixing 48\% (w/v) BSA, 0.5\% (w/v) MB, and 3\% (w/v) HPMC with the genipin solution (0.38\% (v/v) final genipin concentration).

All solders were stored at 4°C in the dark for up to 1wk.

**Experimental design**

**Substudy 1: Optimization of the number of lasing spots for PCL and PLGA ssLAVA**

The first substudy aimed to determine the number of spots required per coaptation to generate the strongest anastomoses with SSCL PCL and PLGA ssLAVA. The number of spots was varied from 5 (without spot overlapping), 7, 9, 11, to 13 (with maximum spot overlapping) spots (n=8-10/group). Intact arteries (n=8) were used as controls. The number of spots with which the highest bursting pressure (BP, ***Bursting pressure***) was achieved was used as the standard protocol for PCL and PLGA ssLAVA in the 2\textsuperscript{nd} substudy.
Substudy 2: 24-h pulsatile pressure test

In the 2nd substudy, arteries that had been ssLAVAed at the optimal parameters for PCL and PLGA (determined in substudy 1) were subjected to an ex vivo 24-h pulsatile pressure test (24-h closed-loop pulsatile pressure test) to mimic in vivo conditions. The pulsatile pressure test was performed at a physiological pressure of 120/80 mmHg, a flow rate of 100 mL/min, a pulse rate of 80 bpm, and a temperature of 38°C. Pressure waveforms were recorded for 24-h. A drop in pressure or a change in pressure waveform that was ensued by a burst in or leakage from the coaptation marked a failed anastomosis and constituted the endpoint of the experiment. Every 30 min, 6 cycles were extracted from the pressure waveform data and analyzed using MatLab (MatLab R2011b, MathWorks, Natick, MA). The extracted data were used to determine the exact time of leakage/burst. The vessels were video recorded to visually confirm anastomotic failure. At the end of the 24-h test, the arteries were carefully slid off the glass rod and prepared for SEM analysis (Scanning electron microscopy) to assess structural features of the solder-tissue interface. In a separate set of experiments, PCL and PLGA ssLAVAed arteries (n=5/group) were prepared for thermal damage analysis (Thermal damage analysis).

Substudy 3: PLGA ssLAVR versus BioGlue

The 3rd substudy compared the SSPL PLGA ssLAVRed modality (ssLAVA procedure) to repairs performed with BioGlue (n=10/group). Prior to ssLAVR, aortic strips (n=8/group) were pinned to a slab of silicone rubber (Sylgard 184, Dow Corning, Midland, MI) placed in a petri dish. The aortic strips were cut in half along the longitudinal axis and realigned, after which 50 µL of solder and scaffold was applied over the adventitial surface of the incised strip as described in the next section.

BioGlue (CryoLife International, Kennesaw, GA) was prepared in accordance with the manufacturer’s instructions. The applicator tip was connected to the syringe, de-aerated, and primed to ensure proper mixing of the glue. Aortic strips were prepared as described in the previous paragraph. The opposing ends were realigned and dried with gauze. Anastomoses were glued by applying approximately 1 mL of the BioGlue solution over the adventitial surface of the coaptation.

The strength of the repairs was determined by BS analysis (Breaking strength analysis).

ssLAVA procedure

To prepare solder-impregnated scaffolds, approximately 100 µL of solder was dispersed over a small area in a petri dish and PCL and PLGA scaffolds were soaked in semi-solid and semi-solid-genipin solder. The scaffolds were gently dabbed to enhance solder penetration.

The carotid artery segments were placed around a glass rod (Ø = 3.4 mm) to ensure complete contact between the opposing vessel edges after a circular incision was made, which cut the artery into half. The drenched scaffold was placed over the coaptation along its full circumference, extending laterally for approximately 2 mm at each flank. A continuous
wave 670-nm diode laser (model HPD7401, High Power Devices, North Brunswick, NJ) was used with a low-power red HeNe laser aiming beam for ssLAVA. The probe was fixed at a 3.0-cm distance from the vessel, perpendicular to the scaffold surface. The coaptations were lased in SSCL mode at an irradiance of 1.2W/cm² (spot diameter of 3.2mm) for 25s per spot, after which the glass rod was rotated (360° / # of spots) to set the adjacent spot. The color change of MB was used as a visual cue to proceed to the next spot.

In the 2nd substudy, SSCL ssLAVA was performed with the optimal number of spots as determined in the 1st substudy, on the basis of the bursting pressure. For both PCL ssLAVA and PLGA ssLAVA, 11 spots were used.

In the 3rd substudy, aortas were repaired with the combination of PLGA, semisolid-genipin solder and the previously established optimal irradiation regime (unpublished work), i.e., SSPL with three pulses at 25-10-15-10-10s, whereby the non-bracketed numbers designate the pulse length and the bracketed numbers indicate the length of the cooling interval.

**Bursting pressure**

The bursting pressure setup consisted of a syringe pump, an organ bath, and a pressure sensor. One end of the anastomosed or intact artery was submerged in an organ bath, cannulated with a polypropylene connector, and connected to a syringe pump, after which the syringe pump was set to a flow rate of 500mL/h. The submerged artery segment was de-aerated and the open end of the segment was sealed by a clamp. The intrarterial pressure was first brought to 100mmHg to measure the external diameter using a digital micrometer. Next, the bursting pressure measurement was started by gradually increasing the pressure to 950mmHg. A software module (LabView, National Instruments, Woerden, The Netherlands) was used to acquire the bursting pressure data. The pressure measurement was recorded until the anastomosis burst, started leaking, or the maximum pressure was reached.

**24-h closed-loop pulsatile pressure test**

A Langendorf-type setup (Fig. 2A) was used to test the resilience of the welded arteries under quasi-physiological conditions. Following ssLAVA (Substudy 2: 24-h pulsatile pressure test and ssLAVA procedure), polypropylene connectors were inserted into both ends of the welded vessel segment and fitted onto the stainless steel connectors of the organ chamber (Fig. 2B). The organ chamber was connected to the actuator module via silicone tubing in a closed loop configuration. The actuator module consisted of a pressure pump that was driven by a proportional pneumatic valve (Festo, The Netherlands) to set the desired pulsatile pressure. The pressure was measured by a pressure transducer (P10EZ, BD, USA) and the flow was controlled with an ultrasound clamp-on flow transducer. A reservoir module was connected to the circulatory system to provide the circuit with sterile PBS (PBS, Sigma-Aldrich). The entire setup was maintained at 38°C using a water jacket and a polyurethane insulation. Dedicated software was employed to regulate hemodynamic parameters and to acquire and store pressure and flow data.
The systolic and diastolic pressures, flow, and pulsatility were set to 120/80 mmHg (Fig. 2C), 100 mL/min (Fig. 2D), and 80 bpm. These settings represent normal coronary hemodynamic conditions. Once pressure and flow pulses were stable, the anastomosis was tested under these conditions for 24 h.

**Scanning electron microscopy**

SEM analysis was performed to ultrastructurally investigate the adhesive bonding at the solder-tissue interface and the state of the coagulated solder (cohesive bond). Immediately following the 24-h pulsatile test, the arteries were fixed in 1.5% glutaraldehyde, cut in half at the coaptation to expose the solder-tissue cross-section, and desiccated by CO₂ critical point drying. The dried sample was mounted on a metal stump and placed inside the SEM (Quanta 600F ESEM-FEG, FEI Company). The specimens were imaged in a high vacuum system at an accelerating voltage of 1kV.

**Thermal damage analysis**

To determine the extent of thermal damage, histological samples were prepared from PCL and PLGA ssLAVAed arteries obtained from the 2nd substudy. Immediately after ssLAVA, vessel segments were fixed in 4% buffered formalin, dehydrated in graded steps of ethanol (70%, 80%, 90%, and 100%), cleared in methyl benzoate and benzene, and impregnated with paraffin wax. Of each sample, 8-μm sections were stained with hematoxylin and eosin (H&E) and Masson’s trichrome (MT) for light microscopy, and picrosirius red (PR) for polarization microscopy. Images were acquired with an Olympus microscope (model BX51, Olympus, Osaka, Japan) equipped with a polarizer and analyzer and an Olympus DP70 camera. Olympus imaging software was used for image acquisition and processing.
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Breaking strength analysis

BS tests were performed in the 3rd substudy after every 5 consecutive ssLAVR or gluing procedures. The repaired aortas were kept moist in PBS-soaked gauzes between the repair procedures and BS testing. The strips were mounted in the metal clamps of a tensiometer (Zwick Z101, Ulm, Germany). The distance between the clamps was 10mm. BS analysis was performed at a test speed of 10mm/min. BS was defined as the force (N) required to completely tear two halves of the strip, divided by the cross-sectional area (in cm²) of the scaffold and solder.

Statistical analysis

Means, standard deviations (SDs), standard error of the means (SEM), Mann-Whitney U tests, Kruskal-Wallis tests, two-tailed homoscedastic student’s t tests, and ANOVA tests were performed in GraphPad Prism (GraphPad Software, La Jolla, CA). Parametric tests were only performed on normally distributed data sets, confirmed by a D’Agostino Pearson omnibus test in GraphPad. The number of symbols throughout the text designates the strength of the p-value, i.e., (*), (**), and (***) designates a p-value of ≤0.05, ≤0.01, and ≤0.001, respectively.

Results

Substudy 1: Optimization of the number of lasing spots for PCL and PLGA ssLAVA

Using a single external laser probe, end-to-end ssLAVA was performed in SSCL mode. On the basis of MB color change, each spot was irradiated for 25s. ssLAVA with PCL and PLGA was first optimized with respect to the number of spots required to produce welds of maximum strength in a clinically representative model. The arteries had an outer diameter of 4.3-5.9mm (mean±SD of 5.1±0.4mm), corresponding to a circumference of 13.5-18.2mm (mean±SD of 15.9±1.1mm), which is comparable to human coronary, internal carotid, renal, and brachial arteries. The glass rod around which the vessel segments were placed as well as the scaffold ensured full contact between the vessel edges without the use of stay sutures. In an in vivo and clinical setting, proper fixation of the coaptation can be established with for example a balloon catheter or a polyvinyl alcohol stent.

Fig. 3 depicts the mean±SD BP of PCL and PLGA ssLAVA as a function of the number of lasing spots. In both PCL and PLGA ssLAVA, the mean±SD BPs were highest when 11 spots were used, indicating that partial overlap of the lasing spots improves welding strength. In the 11 spots group, the combination of PLGA + semi-solid genipin solder yielded a BP of 922±56mmHg compared to 703±96mmHg in the PCL group (###). A further increase in the number of lasing spots considerably decreased the anastomotic strength of PCL ssLAVA but not PLGA ssLAVA. With the exception of the 9 spots group, all welds created with PLGA ssLAVA were stronger than those created with PCL ssLAVA (#, Fig. 3A vs. B). Seven out of 10 welded arteries in the PLGA ssLAVA group remained intact up to an intralumenal pressure of 950mmHg, which was similar to the intact arteries.
In contrast to PLGA ssLAVA, none of the PCL ssLAVAed arteries reached the maximum pressures. The 11 spots regime produced the strongest welds in both PCL and PLGA ssLAVA and was therefore used in the 2nd substudy.

**Substudy 2: 24-h pulsatile pressure test**

The welded arteries were tested in a Langendorf-type setup for their ability to withstand quasi-physiological conditions. Representative pressure traces of an intact and failed artery are provided in Fig. 4A, and the percentage of intact arteries in the PCL and PLGA groups is plotted as a function of time in Fig. 4B. The 24-h pulsatile pressure test revealed the superior strength of PLGA over PCL scaffolds following ssLAVA. Eighty percent of the anastomoses in the PLGA + semi-solid genipin solder group sustained their integrity during 24h, whereas only 30% of the anastomoses in the PCL + semi-solid solder group were intact after 24-h pulsatile perfusion (Fig. 4B). The two failed PLGA ssLAVAed arteries leaked at 19 and 22h, whereas the 7 PCL ssLAVAed arteries burst between 12-23h.

Fig. 4C-F shows representative macroscopic images of intact and failed PCL and PLGA ssLAVAed arteries. No significant shrinkage of the lased sites was observed in either PCL and PLGA ssLAVAed arteries (Fig. 4C and E, respectively). PCL and PLGA ssLAVAed vessels showed different types of failure. Anastomoses made with PCL + semi-solid genipin solder group exhibited bursting from mainly the solder/scaffold coagulum (i.e., cohesive failure, Fig. 4D), whereas arteries welded with PLGA + semi-solid genipin solder failed by leakage from the solder-tissue interface (i.e., adhesive failure, Fig. 4F).

Ultrastructural analysis of intact PCL- and PLGA-mediated welds revealed seamless adhesive bonding (Fig. 5A and E). PCL fibers appeared melted and coalesced with the coagulated solder (Fig. 5B, inset), whereas PLGA fibers were intact and interspersed throughout the coagulated solder (Fig. 5F, inset). SEM imaging confirmed the different types of failure observed during the pulsatile pressure tests. Deterioration of the PCL scaffold was found in all failed PCL ssLAVA arteries (Fig. 5C and D, arrows). In contrast,
detachment of the scaffold-solder coagulum from the adventitia was observed in the failed PLGA ssLAVA specimens (Fig. 5E and F, arrows).

To determine the extent of thermal damage and structural changes in the vascular wall, PCL and PLGA ssLAVAed arteries were stained with PR, MT, and H&E (Fig. 6). Native arteries were used as controls (Fig. 6A, D, G, and J). No qualitative histological differences were observed between welds made by PCL and PLGA ssLAVA with respect to thermally damaged tissue. The histological descriptions below therefore apply to both ssLAVA groups.

PR-stained sections revealed full thickness thermal damage and thinning of the vascular wall (Fig. 6B and C). The adventitia contained homogenized and compacted collagens (Fig. 6E and F, white arrows), whereas partial loss of yellow-stained collagen type I and green-stained collagen type III was observed in the thermally damaged media (Fig. 6E and F, white circles, insets).
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The MT-stained samples of the irradiated vessels revealed shrunken muscle cells with pycnotic nuclei (Fig. 6H and I, white circles and yellow arrows), i.e., clear markers of thermal damage. The nuclear-cytoplasm ratio was increased in comparison to the normal arteries (Fig. 6G vs. 6H and I). The H&E-stained samples demonstrated vacuolization of smooth muscle cells of irradiated arteries (Fig. 6K and L, orange arrows) that were absent in cells of control samples (Fig. 6J, blue arrows). Furthermore, elastin fibers in the ssLAVA-treated arteries were considerably more compressed (Fig. 6K and L, orange arrowheads) than the elastin fibers in control arteries (Fig. 6J, blue arrowheads).

Substudy 3: PLGA ssLAVR versus BioGlue

In correspondence to our previous work (pg. 85, 90), the 2nd substudy evinced that ssLAVA with PLGA produced the strongest and most stable welds. Consequently, the 3rd substudy compared SSPL PLGA ssLAVR of aortas with BioGlue repairs. BioGlue is an FDA approved surgical adhesive that is standardly used in cardiovascular surgery to facilitate hemostasis in large vessel suture lines, tissue adherence in aortic dissection and reconstruction, reinforcement of friable tissue, and to control bleeding through large puncture holes and suture lines.\textsuperscript{24,25} The glue contains 45% purified BSA and 10% glutaraldehyde, and creates a mechanical seal via glutaraldehyde-induced cross-linking of BSA and tissue proteins.\textsuperscript{24} The considerably higher BS obtained with PLGA ssLAVR compared to BioGlue, namely 323±29N/cm\textsuperscript{2} and 25±4N/cm\textsuperscript{2} (***) respectively (Fig. 7), demonstrates the viability of PLGA ssLAVR as an alternative for large vessel repair.
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Figure 6. Photomicrographs of native, PCL-, and PLGA ssLAVAed arteries stained with picrosirius red (PR, A-F), Masson’s trichrome (MT, G-I), and hematoxylin and eosin (H&E, J-L). Low magnification images of PR-stained samples exposed full thickness thermal damage in both PCL and PLGA ssLAVAed arteries (B and C) with considerable thinning/compression of the vessel wall. A higher magnification image of PR-stained control artery (D) showed intact, birefringent collagen in the adventitia (black arrows) and media (green circle and inset), as opposed to the ssLAVAed-treated arteries (E and F), which exhibited partial loss of collagen birefringence in the adventitia (white arrows) and partial loss of yellow- and green-stained collagen type I and III, respectively, in the media (white circles and insets). The normal MT-stained artery (G) was characterized by intact smooth muscle cells with normal nuclei (green circle and green arrowhead, respectively), whereas the thermally damaged arteries (H, I) were characterized by shrunken smooth muscle cells (white circles) and pycnotic nuclei (yellow arrows). The H&E-stained thermally damaged arteries (K, L) revealed vacuolization of smooth muscle cells (orange arrows) and compression of elastin fibers (orange arrowheads), which were absent in non-irradiated smooth muscle cells (blue arrows) and elastin fibers (blue arrowheads) in the control artery (J).
This study demonstrated the feasibility of sutureless ssLAVA of medium-sized vessels. The number of spots required to produce maximally stable welds in vessels of 4.3-5.9mm in external diameter was 11 in both the PCL and PLGA ssLAVA groups. However, anastomoses produced with PLGA + semi-solid genipin solder obtained higher acute BS and were more resilient under ex vivo pulsatile pressure conditions than anastomoses made with PCL and semi-solid solder. ssLAVA with PLGA produced an acute BP of 923±56mmHg versus 703±96mmHg in the PCL group, i.e., values that are well above malignant hypertension levels (250mmHg) and that compare favorably to the pressure that intact porcine carotid arteries can withstand (1420±99mmHg\textsuperscript{36}). During the pulsatile pressure test, 20% of PLGA ssLAVAed arteries failed adhesively, whereas 70% of PCL ssLAVAed arteries underwent cohesive failure. Unfortunately, welds in both scaffold groups exhibited notable thermal damage following ssLAVA. Apart from its application for vascular anastomosis, this study demonstrated the considerable greater welding strength obtained in aortic repairs made with PLGA ssLAVR than with BioGlue. Due to the significantly inferior results obtained with BioGlue, the discussion will focus only on ssLAVA/R.

The finding that the highest welding strength was achieved with 11 spots in both scaffold groups indicates that a common thermodynamic component dictates the most favorable conditions for protein denaturation and cross-linking, irrespective of the polymeric scaffold. This is most likely related to the generation of optimal temperatures for protein denaturation and cross-linking (pg. 88-89, 95).\textsuperscript{37,38} Collagen (i.e., adventitia) denatures between 59-64°C, whereas BSA denaturation starts at 60°C and peaks at 82°C, whereby complete denaturation occurs at above 75°C. In previous work we reported that the 50-s irradiation regime induced a temperature increase to 80-85°C, whereby a temperature of \~80°C was achieved during the first 25s of irradiation (pg. 88-89). The 25-s irradiation therefore created temperatures at which both collagen and BSA denaturation was complete, likely allowing maximum cross-linking between adventitial and solder proteins.

In addition to the favorable thermodynamic conditions of the 11-spot regime, the scaffolds also played an important role in weld reinforcement and weld sustenance during quasi-physiological conditions, albeit the contribution of PLGA scaffolds was superior to
that of PCL scaffolds. The end-to-end anastomoses were performed with a previously established optimal solder composition for every scaffold, namely genipin-containing semi-solid solder for PLGA and semi-solid solder for PCL (pg. 84-86). ssLAVA with PLGA yielded higher acute BPs than ssLAVA with PCL, which was in agreement with previous tensile strength results obtained with PLGA and PCL LAVR. However, when the ssLAVR-generated welds were subjected to hydration in PBS at 37°C for 7 days, the PLGA coaptations deteriorated markedly to ultimately yield a similar tensile strength as PCL welds. PLGA weld deterioration concurred with water-induced ultrastructural defects at the solder-tissue interface, which could be reduced to some extent by the addition of genipin to the semi-solid solder. In contrast to welds made with PLGA, such ultrastructural defects were generally absent in PCL welds, as PCL is more hydrophobic than PLGA and hence more water-repellant (pg. 86-87). It was therefore not expected that the PCL welds, would exhibit a considerably higher failures rate than PLGA welds during 24-h hydration.

The present study with ssLAVA confirmed our previous observations that PLGA ssLAVR produces stronger cohesive bonds whereas PCL ssLAVR produces stronger adhesive bonds. Due to the thermal stability of PLGA (melting point of 148°C), PLGA scaffolds remain intact during ssLAVA/R. As such, the intact PLGA fibers support cohesive bonding. Conversely, PCL has a melting point of 62°C (pg. 80, 82). Inasmuch as temperatures of 80-85°C were generated at the solder-tissue interface during welding conditions that mimicked a normal hemodynamic environment, the PCL fibers melt and coalesce with the denatured albumin in the solder and collagens in the adventitia (pg. 84, 87). As such, PCL fibers mainly promote adhesive bonding. These material properties clearly translated to the type of failure observed during the pulsatile pressure test, i.e., adhesive failure after PLGA ssLAVA and cohesive failure during PCL ssLAVA. In that respect, a dual-layer scaffold composed of an inner PCL layer and outer PLGA layer should be ideal for ssLAVA. The melting of PCL fibers secures the adhesive bonding while the intact PLGA fortifies the cohesive bonding.

Regardless of the high welding strengths achieved in this study, minimizing the extent of thermal damage is fundamental for the successful clinical application of sutureless ssLAVA. The 11-spot irradiation regime inflicted full thickness thermal damage to the vessel wall. Although several studies reported normal healing to occur after full thickness thermal damage, thermal damage that extends beyond the internal elastic lamina has been associated with intimal hyperplasia and aneurysm formation. A reduction in thermal damage can be achieved in several ways, including adjustment of lasing parameters (i.e., beam diameter, irradiance, pulse duration, and pulsing regime) (pg. 88-89), by employing a scaffold embedded with chromophore-containing nanoshells, or by means of photochemical vessel bonding. However, any change in welding protocol may concur with a reduction in welding strength and will need to be re-evaluated in a clinically representative model as for instance presented here.

The fact that the ssLAVA modalities described in this study resulted in BPs that are, by far, the highest reported in sutureless laser anastomoses of medium-sized vessels does not exempt the modality from implementation of further improvements. In light of the results and discussion above, further optimization of the modality should focus on (1) the
development of a dual-layer scaffold composed of PCL and PLGA to support both cohesive and adhesive bonding, respectively, and (2) the fine-tuning of the thermodynamics at the solder-tissue interface to control the extent of heat deposition and thermal damage. When adhesive and cohesive bonding is significantly improved and the thermal damage is considerably reduced, the potentially application of ssLAVA include distal arterial bypass in the lower extremities, coronary artery bypass grafting, and microvascular (replantation and freeflap) surgery.\textsuperscript{1}

**Conclusions**

This study has demonstrated the feasibility of employing ssLAVA as an alternative method to make end-to-end vascular anastomoses. The most ideal ssLAVA modality entailed the use of PLGA scaffolds with semi-solid genipin solder and 11 lasing spots for vessels with a circumference of 4.3-5.9mm. The very high welding strengths notwithstanding, the ssLAVA modalities should be fine-tuned so as to reduce the extent of thermal damage to the vascular wall. In addition to its future application as the primary means of anastomosis, ssLAVR demonstrated potential advantage to substitute the clinically used BioGlue as intra- and extravascular sealant.

**References**


