Optimization of in vitro sutureless laser-assisted vascular anastomosis

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Biodegradable polymer scaffold, semi-solid solder and single spot lasing to increase solder-tissue bonding in suture-free laser-assisted vascular repair
Abstract:

Objective: We have recently demonstrated the fortifying effect of poly(ε-caprolactone) (PCL) scaffold in liquid solder-mediated laser-assisted vascular repair (ssLAVR) of porcine carotid arteries, yielding a mean±SD leaking point pressure of 488±111mmHg. Despite the supraphysiological pressures, the frequency of adhesive failures was indicative of weak bonding at the solder-tissue interface. Therefore, the study aimed to improve adhesive bonding by using a semi-solid solder and single spot vs. scanning irradiation.

Materials and Methods: In the 1st substudy, in vitro ssLAVR (n=30) was performed on porcine abdominal aorta strips using a PCL scaffold, liquid or semi-solid solder, and a 670-nm diode laser for dual-pass scanning. In the 2nd substudy, the scanning method was compared to single spot lasing. The 3rd substudy investigated the stability of the welds following hydration under quasi-physiological conditions. The welding strength was defined by acute breaking strength (BS). Solder-tissue bonding was examined by scanning electron microscopy and histological analysis was performed for thermal damage analysis.

Results: Altering solder viscosity from liquid to semi-solid solder increased the BS from 78±22N/cm² to 131±38N/cm². Compared to scanning ssLAVR, single spot lasing improved adhesive bonding to a BS of 257±62N/cm² and encompassed less structural defects at the solder-tissue interface but more pronounced thermal damage. The improvement in adhesive bonding was associated with constantly stronger welds during 2wk of hydration.

Conclusions: Semi-solid solder and single spot lasing increased welding strength by reducing solder leakage and improving adhesive bonding, respectively. The improvement in adhesive bonding was associated with enhanced weld stability during hydration.
Introduction

The advent of polymeric scaffolds as reinforcement material in solder-mediated laser-assisted vascular anastomosis/repair (sLAVA/R) has brought laser-assisted anastomosis of medium-sized vessels closer to clinical practice.\textsuperscript{1-10} Scaffold-enhanced sLAVR (ssLAVR) has proven a viable alternative for sLAVR in that supraphysiological bursting pressures can be achieved without notable structural damage beyond the lower tunica media.\textsuperscript{1,3,5,6} Apart from these clinically favorable results, our previous studies on electrospun poly(\textit{ε}-caprolactone) (PCL) ssLAVR\textsuperscript{5,6} evinced that the modality could be further improved with respect to solder state and adhesive bonding strength.

Leakage of liquid solder from an adjoined vessel surface leads to difficulties in defining the surface and thickness of the solder layer and complicates the calculation of laser energy per unit mass of protein.\textsuperscript{11-14} This renders the controlled implementation of the modality in the clinical setting virtually impossible. Semi-solidification of the liquid solder by the addition of viscosity-enhancing solutes such as hydroxypropylmethylcellulose (HPMC) has been associated with reduced solder leakage and an increased in vitro welding strength in coapted intestine and skin,\textsuperscript{15} and thus constitutes a plausible solution to the leakage dilemma.

Welding strength is derived from the strength of the cohesive (solder-confined protein-protein or protein-scaffold) and adhesive (solder-tissue) bonds. The reinforcement effect of biodegradable polymer scaffolds is attributed to the fortification of intermolecular albumin bonds (cohesive bonding).\textsuperscript{5,6,8} Correspondingly, the weak point of the weld is shifted to the solder-tissue interface, i.e., the area where the fortifying properties of PCL have no influence.\textsuperscript{5,6} Regardless of the strengthening of the weld through improvements in cohesive bonding, a weak adhesive bond is detrimental to the stability of the coaptation.\textsuperscript{9} Research efforts should therefore be specifically directed to strengthening adhesive bonds in ssLAVR.

Consequently, this study was performed to further improve the ssLAVR modality using electrospun PCL as biodegradable reinforcement material. The first part of the study focused on minimizing solder leakage by increasing solder viscosity and determining its implications on welding strength. The second part of the study investigated the effect of scanning versus single spot irradiation on the strength of adhesive bonds. In the last part of the study, the stability of the welds was determined as a function of hydration time in phosphate buffered saline (PBS) solution to assess possible deterioration of welding strength under quasi-physiological conditions.
Materials and methods

The concentrations listed throughout the manuscript refer to final concentrations.

Tissue preparation

Fresh porcine thoracic and abdominal aortas (n=50) were harvested at the slaughterhouse. Aorta strips were stored in histidine-tryptophan-ketoglutarate preservative solution (Custodiol, Tramedico, Weesp, The Netherlands) at 4°C and were used within 3d. Perivascular tissue was trimmed and the aortas were cut along the longitudinal axis. Subsequently, 30×5mm strips (n=165) were punched from the unfolded slabs of vascular tissue. The thickness of the aorta strip was measured between two glass slides using a digital caliper.

Solder preparation

Liquid protein solder was prepared from 48% (w/v) bovine serum albumin (BSA, Fraction V, Roche, Penzberg, Germany) and 0.5% (w/v) methylene blue (MB, Sigma-Aldrich, St. Louis, MO) in MilliQ water. Semi-solid solder was prepared by adding 48% (w/v) BSA and 0.5% (w/v) MB to MilliQ containing increasing concentrations of hydroxypropylmethylcellulose (HPMC, Sigma-Aldrich) (1, 3, 5, or 7% (w/v)) as determined in substudy 1 (Substudy 1: Liquid vs. semi-solid solder).

Solders were stored at 4°C in the dark until further use for up to 1wk.

Preparation of poly(ε-caprolactone) scaffolds

PCL (Sigma-Aldrich) with an average molecular weight of 80kDa was dissolved in chloroform under gentle stirring to obtain a 17% (w/w) solution. The polymer-containing solution was delivered at a constant flow rate (60µL/min) to a metal capillary connected to a high-voltage power supply. The distance between the capillary and the target drum (25×120mm) was set to 15cm. As the jet fluid accelerated towards a grounded collector, the solvent evaporated and a charged polymer fiber was deposited on the rotating target in the form of a non-woven mesh. Electrospinning was performed at 15kV for 25min to produce a mesh consisting of fibers with a mean diameter of 14-µm. Fiber diameter was confirmed 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 a 5×5-mm array. Scaffold thickness was measured with a digital micrometer between two glass slides; the mean±SD thickness was 200±10µm.

Experimental design

Substudy 1: Liquid vs. semi-solid solder

Semi-solid solders were prepared by dissolving 48% (w/v) BSA and 0.5% (w/v) MB in MilliQ containing increasing concentrations of HPMC (1, 3, 5, or 7% (w/v)). Only the 1% and 3% HPMC concentrations yielded homogeneous semi-solid solders. The 5% and 7% HPMC concentrations were therefore not used in substudy 1.

To determine which solder composition produces the greatest welding strength,
aorta strips (n=10/solder composition) were pinned to a slab of silicone rubber (Sylgard 184, Dow Corning, Midland, MI) placed in a petri dish. The aorta strips were cut into half along the longitudinal axis and realigned, after which 50µL of solder and PCL scaffold were applied over the intimal surface\(^2\) of the incised strip.

For welding, a 670-nm diode laser (model HPD7401, High Power Devices, North Brunswick, NJ) was used in continuous wave mode with a HeNe red aiming beam. SsLAVR was performed by manually scanning a fiber optic handpiece perpendicularly to the scaffold surface in a zigzag movement, starting at the upper left corner of the scaffold.\(^6\)\(^,\)\(^18\) The laser power and spot diameter were set to 0.73W and 0.4cm, respectively, accounting for an irradiance of 5.8W/cm\(^2\).\(^5\)\(^,\)\(^6\) The scan speed was dictated by MB transiting into its leuco-form, i.e., irradiation of the subsequent scaffold volume was performed only after the prior scaffold area had turned white. This lasing regime was standardly applied twice per scaffold and is, from this point onward, described as scanning ssLAVR. The cumulative irradiation time was recorded for each procedure.

The optimum solder composition was defined by the highest breaking strength (BS) (Breaking strength testing and weak point analysis). SEM analysis of the solder-tissue interface was performed to investigate the quality of adhesive bonding (n=2/group).

**Substudy 2: Scanning vs. single spot ssLAVR**

Substudy 2 investigated the effect of lasing technique on welding strength and the type of failure (cohesive vs. adhesive). Aorta strips were prepared as described in Substudy 1: Liquid vs. semi-solid solder. Scanning ssLAVR was performed at the laser parameters and solder composition as established in substudy 1 (i.e., 3% (w/v) HPMC, 48% (w/v) BSA, and 0.5% (w/v) MB in MilliQ).

Single spot ssLAVR was performed by positioning the laser probe at a fixed distance perpendicular to the scaffold surface. Prior to the experiments, the optimal irradiance (W/cm\(^2\)) and radiant exposure (J/cm\(^2\)) were determined for single spot ssLAVR. The laser probe was fixed perpendicularly to the vessel surface at a distance of 1.8, 2.5, or 3.0cm to generate spot diameters of 0.8, 1.0, or 1.3cm, respectively. First, the samples were irradiated at 3 different laser powers per spot diameter to determine the optimal irradiance. The irradiation time was equal to the time required for MB to transit to its leuco-form in the entire scaffold-covered area. Subsequently, the optimal radiant exposure was determined by irradiating the coaptations for 30, 40, 50, 60, or 70s (n=5/group) at the previously established optimal irradiance. To prevent thermal damage to neighboring tissue, a black metal panel containing an 8×8–mm rectangular window was placed on the aorta strip before ssLAVR. The optimal laser parameters were defined by BS analysis as those yielding the highest BS (Breaking strength testing and weak point analysis).

For both techniques, the type of dissociation during BS analysis and the structural features of the welded coaptation at the solder-tissue interface (SEM analysis, Scanning electron microscopy) were used to assess the quality of the adhesive bond. The extent of thermal damage was determined by histological analysis (Thermal damage analysis) (n=5/group).
Substudy 3: Hydration study

In the 3rd substudy the stability of the welds was investigated as a function of hydration time in PBS. Aorta strips were irradiated on the adventitial surface using scanning or single spot ssLAVR (n=40/lasing technique). ssLAVR was performed at the optimal ssLAVR parameters as described in 5,6 and Substudy 1: Liquid vs. semi-solid solder for the scanning mode and in Substudy 2: Scanning vs. single spot ssLAVR for the single spot mode. These included a solder composition of 3% HPMC, 48% BSA, and 0.5% MB, and laser irradiation at a spot diameter of 13mm, an irradiance of 1.2W/cm², and a pulse duration of 50s. To prevent thermal damage to neighboring tissue, a black metal panel containing an 8×8-mm rectangular window was placed on the aorta strip before scanning ssLAVR. Twenty ssLAVRed specimens underwent BS analysis directly after the ssLAVR procedure as the 0-d control groups (n=10/lasing technique). Sixty ssLAVRed aorta specimens were submersed in sterile PBS and incubated at 37°C for 1, 7, or 14d (n=10/hydration period, per lasing technique). BS analysis (Breaking strength testing and weak point analysis) and SEM analysis (Scanning electron microscopy, n=2/hydration period, per lasing technique) were performed at the end of the hydration period.

Breaking strength testing and weak point analysis

BS analysis for the acute experiments (i.e., substudy 1, 2, and the 0-d hydration groups in substudy 3) were performed after 5 consecutive ssLAVR procedures. The ssLAVRed aorta strips were kept moist in PBS-soaked gauzes between the ssLAVR procedure and BS testing. The hydrated samples in substudy 3 were subjected to BS analysis at the end of the hydration period.

The ssLAVRed aorta strips were mounted between 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. The type of dissociation during the BS measurement, assessed visually, served as an indication of the weak point of the weld (i.e., cohesive vs. adhesive failure).

Scanning electron microscopy

SEM analysis was performed to investigate the structure of adhesive bonding at the solder-tissue interface in fixed specimen cross-sections. SsLAVR was performed on the adventitial layer of an intact aortic strip (n=2/group for each substudy). Immediately following ssLAVR or at the end of the hydration period the samples were fixed in 1.5% glutaraldehyde, cut in half along the longitudinal axis 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. Adhesive bonding was analyzed in a high vacuum system at an accelerating voltage of 1kV.
Thermal damage analysis

Histological samples were prepared from ssLAVRed aortas irradiated in scanning and single spot mode (n=5/group). Immediately after ssLAVR, vessel segments were fixed in 4% buffered formalin, dehydrated in ethanol, cleared in methyl benzoate and benzene, and impregnated with paraffin wax. Of each sample, 6-µm sections were stained with Masson’s trichrome (MT) for light microscopy and with picrosirius red (PR) polarization microscopy. Images were acquired with an Olympus microscope (Olympus 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.

Statistical analysis

Means, standard deviations, 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). A single asterisk (*) designates a p-value of ≤0.05 throughout the text, (**) designates a p-value of ≤0.01, (***) designates a p-value of ≤0.001, and (****) designates a p-value of ≤0.0001. Values are reported as mean±SD.

Results

Substudy 1: Liquid vs. semi-solid solder

To increase the viscosity of the solder, 48% BSA and 0.5% MB were added to MilliQ containing increasing concentrations HPMC. Only the 1% and 3% HPMC concentrations produced homogenous semi-solid solders. The solders containing 5% and 7% HPMC were therefore not used.

Fig. 1 presents the BS of ssLAVRed aorta strips welded using liquid and semi-solid solder. Addition of 1% HPMC to the solder did not increase welding strength compared to control (liquid solder), producing a BS of 78±22N/cm² vs. 71±20N/cm² (p=0.60), respectively. A significant improvement in welding strength was found when the HPMC concentration was increased to 3%, yielding a BS of 131±38N/cm² (*** vs. liquid solder ssLAVR and ** vs. semi-solid solder containing 1% HPMC) (Fig. 1). The semi-solid solder

Figure 1. Breaking strength of ssLAVRed aorta strips as a function of solder composition. Liquid solder-mediated ssLAVR was compared to semi-solid solder-enhanced ssLAVR. Liquid solder was composed of 48% (w/v) BSA and 0.5% MB in MilliQ water, while the semi-solid solders contained 48% BSA, 0.5% MB, and 1% or 3% of HPMC in MilliQ water. All coaptations were welded in combination with electrospun PCL scaffold and irradiated using the scanning technique. Data are presented as mean±SD.
did not leak following solder application. However, minimum leakage did occur during the application of scaffold. The results evinced that semi-solid solder containing 3% HPMC, 48% BSA, and 0.5% MB comprised the optimal solder composition. This solder composition was therefore used in the subsequent substudies.

Fig. 2 presents SEM images of the cross-sectional areas of aortas welded using (A) liquid solder, (B) 1% HPMC containing semi-solid solder, and (C) 3% HPMC containing semi-solid solder. Following ssLAVR, the irradiated scaffold was found to completely coalesce with the coagulated albumin solder. Multiple focal areas of separation were observed at the solder-tissue interface of all ssLAVRed aorta strips (Fig. 2A, B, and C, arrow).

**Substudy 2: Scanning vs. single spot ssLAVR**

Fig. 3 presents the BS as a function of irradiance (Fig. 3A) and exposure time (Fig. 3B). The highest BS (257±62N/cm²) was produced at a laser power of 1.6W, a 1.3-cm spot diameter, and an exposure time of 50s, accounting for an irradiance of 1.2W/cm² and a radiant exposure of 60J/cm².

Fig. 4 presents BS results of aorta strips welded by single spot ssLAVR (n=10) and scanning ssLAVR (results obtained in substudy 1, n=10). The time required to complete the dual pass irradiation in scanning ssLAVR was 82±31s, with ~3s of irradiation time per spot. Scanning ssLAVR produced a BS of 131±38N/cm². Single spot ssLAVR increased the welding strength by 2-fold, yielding a BS of 257±62N/cm² (**** vs. scanning ssLAVR). One out of 10 samples in the scanning ssLAVR group broke cohesively, whereas all samples in the single spot ssLAVR group broke adhesively, suggesting an enhancement in cohesive bonding by single spot ssLAVR.

Fig. 5 presents SEM images of the cross-sectional areas of scanning and single spot ssLAVRed aorta specimens. The irradiated PCL scaffold could not be distinguished from the albumin solder following ssLAVR, as the PCL scaffold had completely coalesced with coagulated albumin solder (Fig. 5A and B, S). The coalescence between scaffold and solder in the single spot ssLAVR group was more compact and homogenous than in the scanning ssLAVR group, where individual fibers were intermittently observed (Fig. 5A). In

![Figure 2. SEM images of cross-sectional areas of aortas welded using (A) liquid solder, (B) 1% HPMC-containing semi-solid solder, and (C) 3% HPMC-containing semi-solid solder. PCL scaffold was found to coalesce with the coagulated albumin solder (S). No ultrastructural evidence for the improvement in adhesive strength was found; multiple focal areas of separation were observed at the solder-tissue interface in all ssLAVRed aorta strips (panels A, B, and C, arrows).](image)

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the single spot ssLAVR, the coagulated solder at the solder-tissue interface exhibited homogenous coalescence with adventitial collagen (Fig. 5B, arrowheads). Contrastingly, multiple focal areas of separation were observed at the solder-tissue interface of the scanned ssLAVR aorta strips (Fig. 5A, arrows). In sum, structural analysis of the ssLAVRed aortas strongly suggests an improvement in the adhesive bonding quality when the single spot lasing technique was applied as opposed to the scanning ssLAVR technique.

Representative histology images of native, scanned, and single spot ssLAVRed aorta strips are presented in Fig. 6. The extent of thermal damage (white dashed line) in the PR-stained aorta specimens was marked by a loss of collagen birefringence in thermally afflicted tissue (Fig. 6B, C, E, and F). Thermal damage in the scanning ssLAVRed aortas was confined to the upper 1/3 portion of the vascular wall, whereas single spot ssLAVR induced thermal damage to the upper 2/3 of the vascular wall (Fig. 6B and C).

The loss of collagen type I was assessed by polarization microscopy of PR-stained sections. In the thermally afflicted adventitia, damaged collagen bundles appeared bright orange (Fig. 6E and F, white arrow) as opposed to the bright yellow polarizing collagen at undamaged (control) sites (Fig. 6D). Similarly, thermal damage of the media was visualized as a dark orange hue of the tissue, and loss off yellow-stained collagens type I and green...
stained collagens type III at the same sites (Fig. 6E and F, white encircled vs. control in Fig. 6D). As such, we found that PR-stained sections revealed more extensive thermal damage in the single spot ssLAVR group than in the scanning ssLAVR group (Fig. 6E vs. Fig. 6F).

The thermal damage profiles as observed in the PR-stained sections were structurally confirmed in the MT-stained samples, where thermally afflicted regions contained compacted and homogenized collagens (Fig. 6H and I, white arrow) in the adventitia and shrunken muscle cells in the medial layer (Fig. 6H and I, white encircled). Corroboratively, single spot ssLAVRed aorta strips showed more extensive thermal damage than those irradiated with the scanning technique. At 100x magnification all muscle cells appeared thermally afflicted in the single spot ssLAVRed aorta (Fig. 6I, white encircled), whereas in the scanning ssLAVRed aorta strips the muscle cells were either shrunken (Fig. 6H, white encircled) or unaffected (Fig. 6H, green encircled).

Substudy 3: Hydration study

To assess the stability of the welds under quasi-physiological conditions, ssLAVRed aorta strips were submersed in sterile PBS and incubated at 37°C for 1, 7, and 14d. BS measurements were performed at the end of the hydration period and compared to the acute BS (0 d hydration, i.e., control).

Fig. 7 presents the BS as a function of hydration period for scanning (A) and single spot (B) ssLAVR. Hydration of scanning ssLAVR aortas did not significantly reduce welding strength (p=0.11); BS were 116±36N/cm², 90±12N/cm², 91±37N/cm², and 81±26N/cm² for control, 1d, 7d, and 14d of hydration, respectively (Fig. 7A). Similarly, welding strength in the single spot ssLAVR groups remained stable for up to 2 weeks of hydration (Fig. 7B) as reflected by a BS of 250±61N/cm², 206±24N/cm², 204±31N/cm², and 191±56N/cm² for control, 1d, 7d, and 14d of hydration, respectively. The BS in the single spot
ssLAVR groups was significantly higher than the BS in the scanning ssLAVR groups for all hydration periods. Furthermore, the BS following 14d of hydration in the single spot ssLAVR group was higher than the BS of control samples in the scanning ssLAVR group, namely 191±56N/cm² vs. 116±36N/cm² (**), respectively.

Fig. 8 presents SEM images of the scanning and single spot ssLAVR aortas at 0, 1, 7, and 14d of hydration. SEM images of the cross-sectional area of the scanned ssLAVR aortas showed an inhomogeneous solder-tissue bond with detachment of the coagulated solder from the tissue surface in several areas at the solder-tissue interface (Fig. 8A). Despite the stable BS at longer hydration periods, the adhesive bond created by scanning ssLAVR weakened structurally as evidenced by the increased number and widening of clefts between coagulated solder and the tissue surface (Fig. 8E and G). In contrast, single
Figure 7. Breaking strengths of ssLAVRed aorta strips plotted as a function of hydration period. Aorta strips were welded using electrospun PCL scaffold and a semi-solid solder (3% HPMC, 48% BSA, and 0.5% MB) using either (A) scanning or (B) single spot ssLAVR. Following ssLAVR, aorta strips were submersed in sterile PBS and incubated at 37°C. No significant reduction in BS was observed as a result of hydration in both groups.

Figure 8. SEM images of cross-sectional areas of scanned and single spot ssLAVRed aorta strips, with emphasis on the structural quality of the solder-tissue bond following hydration. PCL scaffold was found to coalesce with albumin solder (S). White arrowheads are pointing to the solder-tissue interface. White arrows indicate the areas where coagulated solder was loosely attached to the tissue surface (T).
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spot ssLAVRed aorta groups showed well-adhered and homogenous solder-tissue bonds up to 7d of hydration (Fig. 8B, D, and F). A minimal degree of detachment of coagulated solder from the tissue surface was found only after 14d of hydration (Fig. 8H), although this did not translate into weakening of the coaptation.

Discussion

In previous work\(^5\,^6\) we have demonstrated the potential utility of electrospun PCL scaffolds in ssLAVR of medium-sized arteries. In this follow-up study we have shown that welding strengths can be further enhanced by 1) increasing the solder viscosity (~1.7-fold increase in welding strength) and 2) by using single spot irradiation of the coaptation rather than scanning irradiation (~2.0-fold increase in welding strength). SEM analysis confirmed that the increase in welding strength in single spot ssLAVR is attributable to enhanced adhesive bonding. Although quasi-physiological conditions did not have negative effects on welding strength in both scanning and single spot ssLARed aortas, the enhancement of adhesive bonding in single spot ssLAVR gave rise to stronger welds.

Increases in welding strength by changing the state of the solder have been reported before,\(^8\,^19\,^20\) albeit not for modalities that combined laser-mediated tissue welding with biodegradable polymeric scaffolds as reinforcement material. Aside from solving liquid solder-associated issues such as solder leakage,\(^11\,^21\) alterations in the physical properties of the solder material have been shown to increase welding strength by 1.2-fold\(^11\) for semi-solid solder composed of 3% HPMC- 48% BSA and by approximately 2.7-fold\(^19\) for solid solder composed of 60% BSA. Although solid albumin-based solders provided greater control of solder thickness and resulted in stronger welds, the solders were considered too brittle and rigid for LAVA/R.\(^2\,^8\) A constricted and rigidified vascular wall following LAVA/R may give rise to compliance mismatch between the welded and native vascular segments, which increases the risk of aneurysm formation.\(^22\,^23\)

Contrastingly, a semi-solid solder is less rigid and effectively reduces solder leakage while maintaining acceptable welding strength inasmuch as semi-solid solders provide greater protein density for cohesive bonding than liquid solders.\(^11\) In this study, SEM analysis evinced that the improvement of welding strength was not attributable to HPMC-mediated increases in adhesive bonding quality. Additionally, mechanical tests confirmed welding strength enhancement with increasing HPMC concentration, but without a reduction in adhesive breakage, yielding credence to the relationship between protein density and welding strength.\(^11\) Furthermore, a semi-solid composition has better adhesive properties than its solid counterpart\(^11\) and semi-solid solders are more compact than liquid solders, particularly when used in combination with a porous scaffold. Consequently, a semi-solid solder constitutes the most suitable type of solder for clinical ssLAVA/R, especially in light of the ‘circumferential’ surgical approach to end-to-side and end-to-end anastomoses.

An important finding related to further optimization of welding strength was that scanning over the coaptation produces substantially inferior breaking strengths compared to single spot irradiation. The scanning technique was previously selected due to its applicability
in clinical practice\textsuperscript{5,6,18} and, in the case of MB, due to the favorable thermodynamics of the chromophore. The photobleaching of MB during laser irradiation is not only used as a visual cue to advance lasing to an adjacent spot, but also as a preventive measure against excessive heat build-up in the solder. After transit to the leuco-form the chromophore loses its absorptive properties in the near-infrared spectrum,\textsuperscript{24} i.e., the wavelength range in which MB is irradiated, and thus becomes incapable of generating additional heat. Since the chemical modifications of MB are heat-driven, the temperatures generated at the nucleation centers (where the light is absorbed) are constant and hence provide a certain degree of control over the welding protocol. The overall temperature distribution in the solder is therefore primarily dependent on the combination of irradiation time per spot and the radiant exposure. These parameters are more stringently controlled in the single spot regime, whereas in the scanning mode the temperature profiles depend strongly on the collective heat diffusion and cooling of tissue, that in turn are chiefly dictated by the manner in which the lasing is performed.

Additionally, it has been reported that the optimal welding strength is reliant on the temperature at which protein cross-linking takes place,\textsuperscript{19,25,26} corresponding to the denaturation temperature of vascular collagen\textsuperscript{25,27} and albumin,\textsuperscript{15,28,29} i.e., 62–65°C. It is therefore arguable that temperatures outside this range were generated in the scanned vessel segments, accounting for the poorer breaking strengths through predominantly adhesive failures, and that the optimized single spot laser parameters yielded a more optimal ‘thermal milieu’ and thus substantially greater breaking strengths.

Evidence for the contention that suboptimal thermal profiles are responsible for the low breaking strengths achieved in the scanning ssLAVR group was provided by the structural analysis of the solder-tissue interface following ssLAVR. In the single spot ssLAVR group the heat-induced coalescence between denatured collagen and albumin was practically seamless, whereas the solder-tissue interface in the scanning ssLAVR group contained numerous zones of separation. These structural defects were most likely the result of thermal effects and could not have arisen from the manner in which the solder was applied (as solder application was performed uniformly in both groups) or from sectioning artifacts created during preparation of the samples for SEM. Compared to scanning ssLAVR, single spot ssLAVR delivered less irradiance (5.8W/cm\textsuperscript{2} vs. 1.2W/cm\textsuperscript{2}, respectively) with almost twice as much radiant exposure (34J/cm\textsuperscript{2} vs. 60 J/cm\textsuperscript{2}). The volumetric heat production, which is the product of the absorption coefficient (\(\mu_a\)) and the fluence rate (\(\phi\), J/cm\textsuperscript{2} in an infinitesimal tissue area below the irradiated tissue surface)\textsuperscript{30} was therefore different at the solder-tissue interface in the scanning ssLAVR group vs. the single spot ssLAVR group. The latter corroborates the previous argument regarding optimal welding temperatures and corollary welding strength.

The positive ramifications of homogenous adhesive bonding on the clinical applicability of ssLAVAR are underscored by the final substudy. Although hydration did not significantly alter welding strength of scanning ssLAVR groups, SEM analysis demonstrated deterioration/separation of albumin solder from the tissue in the scanning ssLAVRed aortas throughout the hydration period. Contrastingly, SEM analysis of the single spot ssLAVRed aortas
revealed firmly adherent solder-tissue bonds up to 7d of hydration. Slight detachments were found only after 14d of hydration, but these were not associated with a decrease in breaking strength. The quality of adhesive bonds in the scanning ssLAVR groups resulted in consistently lower breaking strength compared to single spot lasing. Previous studies reported significant decrease in welding strength after the first day of hydration.\textsuperscript{1,9,20,31} Therefore, with respect to welding strength, both ssLAVR modalities presented in this study produced the most stable welds reported to date.

The most significant albeit not uncircumventable drawback to the single spot ssLAVR modality was the extensive thermal damage to the vascular wall. Major thermal damage to mural tissue has been linked to intimal hyperplasia and aneurysm formation at 120d of clinical follow up.\textsuperscript{32} Although the high breaking strengths yielded by the single spot ssLAVR modality may withstand blood pressures above malignant hypertension levels (>250mmHg), the modality cannot be introduced into the clinical setting if extensive thermal damage cannot be restricted. Applying single spot ssLAVR in multiple pulses rather than in a single pulse may prevent excessive thermal damage while still generating sufficient heat at the solder-tissue interface. Moreover, spreading the laser energy over multiple pulses allows the tissue to cool down before subsequent reheating. Alternatively, a higher power but shorter single pulse may also be beneficial if the pulse duration is set such that heat build-up at the solder-tissue interface (by diffusion) is confined to the optimal temperature range for collagen and albumin denaturation and never exceeds the upper limit.

Another technical improvement that might enhance adhesive bonding and concomitantly reduce thermal damage is the addition of protein linker to the semi-solid solder.\textsuperscript{33} A protein slow linker, i.e., genipin, has two aldehyde chains that, upon thermal activation,\textsuperscript{34,35} can cross-link albumins to tissue collagens. The addition of protein cross linker may enable us to reduce the irradiance and exposure time and hence reduce thermal damage.

**Conclusions**

The approaches employed in this study improved the outcome of ssLAVR. Solder leakage was minimized by using a semi-solid solder while adhesive bonding was improved by employing single spot lasing rather than scanning ssLAVR. The improvement in adhesive bonding was beneficial for the long term stability of the weld under quasi-physiological conditions. However, before applying the current modality in vivo, additional work should focus on reducing the extent of thermal damage.
References


