lower magnification images revealed the extent of thermal damage (Figure 8B.1, C.1, D.1, E.1, F.1, and G.1, white dashed line), whereas higher magnification images (Figure 8B.2, C.2, D.2, E.2, F.2, and G.2) showed homogenized and compacted thermally damaged adventitial collagen (white arrows) and loss of yellow-stained type I collagen in the thermally afflicted media (white arrowheads). Structural alterations in the aortic wall are demonstrated by the MT-stained samples (Figure 8B.3, C.3, D.3, E.3, F.3, and G.3), where compacted and homogenized collagens (yellow arrows) reflect thermally damaged adventitial tissue and the shrunken muscle cells (yellow arrowheads) indicate thermally damaged media. The respective mean ± SD acute BS and the SEM analysis are presented in Figure 9A and Figure 10, respectively.

Fifty-second SSCL raised the s-t temperature to 84.7 ± 5.2 °C (Figure 8B) and produced an acute BS of 355.1 ± 52.6 N/cm^2 (Figure 9) that coincided with full thickness thermal damage (Figure 8B.1-3). A ~20 °C temperature gradient was created between the center and lateral sides of s-t interface during irradiation. Figure S4 depicts the temperature gradient and the corresponding thermal damage in both regions.

With respect to SSPL, the first pulse was terminated after 25s (in all single spot pulsing groups) on the basis of the observed color change. The mean ± SD s-t temperature after the first pulse was 80.7 ± 6.8 °C in all multiple pulse regimens. After a 5-, 10-, or 15-s cooling interval, the administration of a second pulsed raised the s-t temperature to 92.5 ± 4.9 °C, 85.7 ± 7.1 °C, and 82.9 ± 5.1 °C, respectively. The two-pulse regimens inflicted thermal damage up to 1/3 of the aortic wall (Figure 8C.1-3, D.1-3, and E.1-3). A third single spot pulse of 10 s or 15 s, following a 10-s cooling interval, induced thermal damage up to 2/3 of the aortic wall (Figure 8F.1-3 and G.1-3).

With respect to acute BS, only the 25-|10|-15 s and 25-|10|-15-|10|-10 s regimens obtained BSs that were comparable to the control group, namely 292.3 ± 59.3 N/cm^2 and 323.4 ± 25.2 N/cm^2, respectively (Figure 9A). However, SEM analysis revealed that solid solder/scaffold coagula were only achieved with the control and the 25-|10|-15-|10|-10 s regimens (Figure 10A and F, red circles). The s-t temperatures of the 25-|10|-15-|10|-10 s regimen were 79.7 ± 4.1 °C|45.8 ± 2.4 °C|78.9 ± 4.5 °C|46.6 ± 2.2 °C|74.6 ± 3.5 °C, respectively.

Hydration experiments were performed on the SSPL-subjected samples in which welding strengths were achieved that were comparable to the 50-s irradiation group (i.e., 25-|10|-15 s and 25-|10|-15-|10|-10 s) and in which the least thermal damage was found (i.e., 25-|15|-15 s). After 1-d of hydration, the BS in the control, 25-|10|-15 s, 25-|15|-15 s, and 25-|10|-15-|10|-10 s groups had decreased by 27%, 43%, 32%, and 21%, respectively (Figure 9B-E). Following 14d of hydration, welding strengths of the 25-|10|-15 s and 25-|15|-15 s groups had become considerably lower than the control group (Figure 9C vs.B), whereas the 25-|10|-15-|10|-10 s modality obtained comparable post-hydration BS relative to the control group (Figure 9E vs. B).

Figure 9. (A) Mean ± SD acute BS following PLGA ssLAVW plotted as a function of irradiation regimen. The post-hydration mean ± SD BS of 50 s (B), 25-|10|-15 s (C), 25-|15|-15 s (D), and 25-|10|-15-|10|-10 s (E) are also plotted as a function of hydration period. (*) designates the level of significance versus 0-d hydration and (#) indicates the level of significance of each time point versus the same time point of the control group.

Figure 10. SEM images of the cross-sectional areas of PLGA ssLAVWed samples irradiated in 50-s continuous (A) and pulsed single spot regimens comprising 25-|5|-15 s (B), 25-|10|-15 s (C), 25-|15|-15 s (D), 25-|10|-10-|10|-10 s (E), and 25-|10|-15-|10|-10 s (F). White arrows point to the solder (S)–tissue (T) interface, black arrows indicate the gaps at the s-t interface, yellow circles designate the solid solder/scaffold coagulum, and red circles highlight the reticular segments in the solder/scaffold coagulum.

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4. Discussion

The aim of this research was to optimize the previously described ssLAVW modality [21]. The study revealed the importance of intact fibers for the scaffolds' mechanical strength and acute BS, but not for maintaining post-hydration BS. PLGA ssLAVW with BSA-HPMC solder produced higher acute BS than ssLAVW with PCL. However, the welds made with PLGA exhibited a reduction in strength with increasing hydration time, which in turn was ameliorated by the addition of genipin to the BSA-HPMC solder. As a result, ssLAVW with PLGA + BSA-HPMC-genipin yielded comparable post-hydration welding strength as PCL ssLAVW. Finally, it was shown that SSPL (versus SSCL) minimized collateral thermal damage and retained acute and post-hydration BS.

The thermo-mechanical properties of a biomaterial in part dictate the suitability of the biomaterial for a given application, particularly if the application is associated with the generation of high temperatures such as during (ss)LAVW. Due to the relatively low melting point of PCL (62 °C) versus PLGA (148 °C), it was expected that thermal denaturation of PCL fibers would compromise post-LAVW welding strength. Indeed, mechanical tests on PCL and PLGA scaffolds confirmed that PLGA was stronger than PCL after irradiation, both as free solder-impregnated scaffold and as a scaffold imposed on a vascular segment during ssLAVW. With respect to the latter, ssLAVW with PCL yielded an acute BS of 248.0 ± 54.0 N/cm^2, which was significantly lower than the 408.6 ± 78.8 N/cm^2 produced by PLGA ssLAVW. This corresponds to a breaking force of 1.4 ± 0.3 N for PCL ssLAVW and 2.2 ± 0.4 N for PLGA ssLAVW. Earlier studies on ssLAVW with solvent casted particulate leached PLGA scaffolds reported breaking forces of 1.31 N [16], 1.5 ± 0.6 N [17], and 1.1 ± 0.2 N [18], which were all lower than the breaking force achieved in this study with comparable scaffold material. On the other hand, the breaking force of PCL ssLAVW achieved in this study was higher than an earlier report on PCL ssLAVW by Alfieri et al. (1.1 ± 0.2 N) [23] but lower than the 1.9 ± 0.4 N achieved in the study by Bregy et al. [22].

Despite the higher acute BS, aortas welded with PLGA + BSA-HPMC solder exhibited deterioration of welding strength under quasi-physiological conditions. Similar results have been reported by Sorg and Welch [16]. Although the exact mechanism responsible for the weld deterioration in PBS is currently elusive, the increase in the number of clefts in the scaffold/solder coagulum and the considerable separation at the s-t interface suggest water-induced retrogradation of cohesive and adhesive bonding. The fact that PCL is more hydrophobic [25] than PLGA may partly explain the relative absence of these phenomena in PCL-LAVWed coaptations subjected to 14-d hydration. The hydrophobic character of PCL apparently renders the scaffold material less amenable to water-induced loosening of cohesive and adhesive bonds. On the basis of these considerations and the post-irradiation yield strength of PCL and PLGA scaffolds, it can be concluded that (intact) PLGA fibers give rise to stronger cohesive bonding whereas (thermally denatured) PCL scaffolds yield more stable welds.

The improvement in post-hydration welding strength in PLGA ssLAVW and the corollary enhancement in adhesive bonding by the addition of genipin reflect the important role of genipin in promoting cross-linking between albumin and tissue collagen [26]. However, the reticulated solder/scaffold coagula observed in all BSA-genipin groups suggest excessive heat development in the solder/scaffold coagulum. The addition of HPMC to BSA-genipin solder produced lower s-t temperature and resulted in a more solid and stable solder/scaffold coagulum, yielding slightly higher acute and post-hydration BS. Ultimately, welds created with PLGA + BSA-HPMC-genipin produced post-hydration welding strengths that were comparable to those achieved with PCL ssLAVW. In a recently published study by our group [31], however, it was demonstrated that end-to-end anastomoses (porcine carotid arteries with an external diameter of 4.3-5.9 mm) that had been welded along the entire coapted circumference with BSA-HPMC-genipin PLGA scaffolds were more resilient in a 24-h pulsatile pressure test than welds made with BSA-HPMC PCL scaffolds. Despite the similar welding strengths achieved in this study with both types of hydration-subjected scaffolds, the 24-h pulsatile pressure test data [31] suggest that LAVA/R with BSA-HPMC-genipin PLGA scaffolds constitutes the most optimal combination. Nevertheless, ssLAVA experiments comparing the utility of BSA-HPMC-genipin PLGA scaffolds to BSA-HPMC PCL scaffolds must be performed in an in vivo proof-of-concept setting to arrive at a definitive conclusion, particularly since the weld degradation kinetics in vivo may differ from those in a quasi-physiological environment.

To determine the relationship between heat build-up at the s-t interface and welding strength as well as thermal damage, the temperature profiles during ssLAVW were determined for different welding regimens. The s-t temperature during a 50-s single spot laser pulse, namely 80-85 °C, was similar to values reported by Sorg and Welch [16]. This regimen, however, produced more extensive thermal damage than sLAVW, i.e., in the absence of scaffold [17]. Histology results showed considerable reduction of thermal damage using SSPL instead of SSCL. The cooling time allows tissue to thermally relax before the subsequent irradiation. In comparison to a 5- and 15-s cooling interval, the10-s cooling interval produced the most optimal results in terms of welding strength. Furthermore, despite the comparable acute BS achieved between the 25-|10|-15 s and control regimens, the two-SSPL regimen failed to retain post-hydration welding strength. Consequently, a third pulse was required to solidify the solder/scaffold coagulum and to increase the stability of the welds. The 25-|10|-15-|10|-10 s regimen reduced thermal damage to 2/3 of the aortic wall and produced comparable acute and post-hydration BS relative to SSCL (control). Moreover, the acute breaking forces obtained by 25-|10|-15-|10|-10 s and

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25-|10|-15 s regimens, namely 1.7 ± 0.2 N and 1.5 ± 0.3 N (corresponding to a BS of 323.4 ± 25.2 N/cm^2 and 292.3 ± 59.3 N/cm^2, respectively), were considerably higher than the breaking force reported by Alfieri et al. for the 15-|15|-15 s regimen, namely 0.6 ± 0.04 N [23].

5. Conclusions and future outlook

In the final analysis, the previously described ssLAVW modality was optimized, but neither of the modalities proved to be superior over the other regarding post-hydration BS, the most important parameter for in vivo LAVW. Whereas PLGA ssLAVW yielded better acute cohesive bonding, PCL ssLAVW produced more stable welds. The quality of each modality should therefore be tested in ex vivo or in vivo models for further evaluation. Nevertheless, it is compelling to argue that a dual-layer scaffold composed of an inner PCL layer and an outer PLGA layer may provide more stable and stronger welds than ssLAVW with either biomaterial alone. Electrospinning allows the fabrication of a double-layered scaffold [32]. Also, genipin should be further explored as a weld-stabilizing agent, particularly when PLGA is used. An additional advantage of genipin is that it reduces an inflammatory response [33, 34], and may therefore be promising for clinical application especially when PLGA is used. Finally, the possibility of decreasing thermal damage while maintaining acute and post-hydration welding strength increases the potential of in vivo and clinical application of ssLAVW. However, application of end-to-end or end-to-side anastomosis requires modification of the laser beam to allow a 360° simultaneous and homogeneous irradiation. This type of lasing could be achieved by means of intraluminal irradiation with an isotropic beam [13] or external irradiation with several laser beams attached to a clip probe.

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