Journal of Clinical and Translational Research
Peer review process file 20150101

Laser-assisted vascular welding: optimization of acute and post-hydration welding strength

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Review timeline:

Received: 21 April, 2015
Editorial decision: 9 June, 2015
Revision received: 18 June, 2015
Editorial decision: 21 June, 2015
Published online ahead of print: 21 June, 2015

1st editorial decision:

Date: 9-Jun-2015

Ref.: Laser-assisted vascular welding: optimization of acute and post-hydration welding strength

Dear Dr. Heger:

There is interest in your manuscript, Laser-assisted vascular welding: optimization of acute and post-hydration welding strength, which you submitted to Journal of Clinical and Translational Research. As you can see from the review below, before the manuscript can be found acceptable, it will be necessary for you to undertake revisions in accordance with the comments of your reviewer and for the paper to possibly undergo further review. Before you resubmit your paper, please carefully proof-read the manuscript to minimize typographical, grammatical, and bibliographical errors. In addition, check to make sure that all abbreviations are defined. Please include a cover letter which indicates in detail the changes you have made and why. Also, indicate which of the suggested changes, if any, you have elected not to make and your reasons. I will contact you as soon as possible with a final editorial decision.
When you are ready, complete the resubmission of your manuscript in its entirety by sending it to choib@uci.edu. I look forward to receiving your revised manuscript.

Sincerely,

Bernard Choi, Ph.D.
Editorial Board Member, Journal of Clinical and Translational Research

*****Reviewer comments*****

Reviewer 1

The authors present a thorough and detailed study exploring the optimization of scaffold-enhanced solder-mediated laser assisted vessel welding (ssLAVW). The study explores, in aorta specimens, how variations in numerous different parameters (solder composition, scaffold materials and dimensions, laser parameters, etc.) can be altered to yield an optimal breaking strength, both acute and after hydration periods. The results add some new information to the field and also confirm previous reports in the literature regarding biomaterial scaffold enhancement of solder breaking strength.

There are a number of issues that should be addressed prior to publication:

1. Statistical analysis: The authors mention that Mann-Whitney U tests, Kruskal-Wallis tests, Student's t tests, and ANOVA tests were performed for statistical analysis. However, it appears that in groups with more than two treatments that the authors may have used multiple t tests (or non-parametric equivalent) for testing differences between the various treatments. If this is the case, then this can lead to a high experimental error rate (high probability of a Type I error). A more appropriate method for multiple comparisons testing would be to first use ANOVA (or non-parametric equivalent) and if differences are found, then do a post-hoc multiple comparisons test like the Bonferroni Test, Newman-Keuls post-hoc analysis, or the non-parametric Wilcoxon Rank Sum Test. Additionally, if a comparison is only being made between various treatments to a single control group, something like Dunnet's test can be used. The authors should repeat the statistical analysis throughout the paper with appropriate multiple comparisons tests.

2. A number of different chromophores have been added to solder for selective absorption by a laser for coagulation, including MB as used in this paper. Please elaborate on the rationale for the choice of MB over others available and used previously in the literature, especially if there is some particular reason it was chosen that relates to breaking strength, in vivo compatibility, etc. or if it was simply compatible with the particular laser used in this study.

3. The biomaterials used for the scaffold in this study were optimized and selected mostly in terms of breaking strength. However, they may have different degradation rates in vivo, and this may affect the strength of the material over time (the degradation rate in vivo may not necessarily be the same as that in PBS). Was this considered at all in the selection of biomaterials for the scaffolds? Please comment on this.

4. For the breaking strength measurements, please comment on whether the cross-sectional area of the solder was measured before or after the breaking strength measurement was performed since it is possible that in some cases the cross sectional area of the solder changed irreversibly after heating or while under stress.

5. Figure 8 – The microscopy images should have a scale bar so dimensions can be easily assessed.

6. Regarding the thermal damage measurements in Figure 8, do the authors have quantitative data measurements of the depth of thermal damage produced? This should be reported (mean +/- standard deviation). Also, the thermal damage experiments were performed on pieces of aorta. How does the depth of thermal damage in the aorta specimens compare to the vessel wall thickness of vessels where ssLAVW would likely be applied (e.g., if the depth of thermal damage is about 30% of the aorta specimen thickness, would full thickness damage be expected in the walls of vessels where ssLAVW would be applied)?

7. Figure 9 – Panel A is very confusing to view in the way the breaking strength comparisons are depicted; for clarity it may make more sense to arrange the groups in order of ascending or descending magnitude of measured breaking strength. Also, I expect that some of the comparisons in the Figure 9 A-E plots may change in terms of statistical significance when a post-hoc multiple comparisons test is performed (see comment #1).

Authors' rebuttal:

Reviewer 1

The authors present a thorough and detailed study exploring the optimization of scaffold-enhanced solder-mediated laser assisted vessel welding (ssLAVW). The study explores, in aorta specimens, how variations in numerous different parameters (solder composition, scaffold materials and dimensions, laser parameters, etc.) can be altered to yield an optimal breaking strength, both acute and after hydration periods. The results add some new information to the field and also confirm previous reports in the literature regarding biomaterial scaffold enhancement of solder breaking strength.

Dear reviewer, thank you very much for the thorough critical appraisal of our manuscript. Your suggestions, which have been implemented to the fullest extent, have resulted in a significantly improved paper.

There are a number of issues that should be addressed prior to publication:

1. Statistical analysis: The authors mention that Mann-Whitney U tests, Kruskal-Wallis tests, Student's t tests, and ANOVA tests were performed for statistical analysis. However, it appears that in groups with more than two treatments that the authors may have used multiple t tests (or non-parametric equivalent) for testing differences between the various treatments. If this is the case, then this can lead to a high experimental error rate (high probability of a Type I error). A more appropriate method for multiple comparisons testing would be to first use ANOVA (or non-parametric equivalent) and if differences are found, then do a post-hoc multiple comparisons

test like the Bonferroni Test, Newman-Keuls post-hoc analysis, or the non-parametric Wilcoxon Rank Sum Test. Additionally, if a comparison is only being made between various treatments to a single control group, something like Dunnet's test can be used. The authors should repeat the statistical analysis throughout the paper with appropriate multiple comparisons tests.

The statistical analysis has been reperformed according to your suggestions.

For the intragroup analysis, we had employed ANOVA (or a Kruskal-Wallis test for non-parametric data sets) followed by a Bonferonni or Dunn's post-hoc test in the original submission. In the revised version we recalculated the statistical significance using a Dunnet's post-hoc test when comparisons were made to the control group (i.e., native scaffold, 0 d hydration, or 50 s single spot continuous lasing).

For intergroup comparison (for example: the comparison between PCL and PLGA scaffold with the same solder composition or the same hydration time or the comparison between the e-modulus of PCL and PLGA scaffolds) a student's t-test was used since the comparison was made between 2 groups at a time.

The following changes have been implemented in the text (section 2.9):

"When differences were found in the multiple comparison tests, a Bonferroni (ANOVA) or Dunn's (Kruskal-Wallis) post-hoc test was performed. When comparing various treatment regimens to a control group (i.e., native scaffold, 0-d hydration group, or 50-s single spot continuous lasing) a Dunnet's post hoc multiple comparison test was employed."

The figures (statistical symbols) have been modified where applicable.

2. A number of different chromophores have been added to solder for selective absorption by a laser for coagulation, including MB as used in this paper. Please elaborate on the rationale for the choice of MB over others available and used previously in the literature, especially if there is some particular reason it was chosen that relates to breaking strength, in vivo compatibility, etc. or if it was simply compatible with the particular laser used in this study.

The following text has been added to section 2.3 to explain the reason for selecting methylene blue as chromophore:

"Of note, MB has the advantage of turning white upon heating (i.e., undergo a transition to its leucoform), which switches off MB-mediated heat production and hence deters extensive overheating during irradiation. The leuco-form transition property of MB and its beneficial implications on peri-irradiation thermodynamics were the reasons for choosing MB over other available chromophores."

3. The biomaterials used for the scaffold in this study were optimized and selected mostly in terms of breaking strength. However, they may have different degradation rates in vivo, and this may affect the strength of the material over time (the degradation rate in vivo may not necessarily be the same as that in PBS). Was this considered at all in the selection of biomaterials for the scaffolds? Please comment on this.

In addition to their mechanical and thermal properties as well as in vivo safety profile, the biomaterials used in this study were selected on the basis of the material's biodegradability. We wanted to compare the stability of the rapidly degrading PLGA to the slowly degrading PCL scaffolds (Li WJ et al., Acta Biomat 2007). Accordingly, hydration experiments were performed to investigate the effect of scaffold degradation on welding strength. Indeed, in vivo degradation kinetics may not necessarily mirror those in PBS. However, this test provided an approximate indication of what to expect in future animal studies.

While performing this study we also conducted an ex-vivo study where we subjected end-to-end ssLAVAed porcine arteries to a 24-h pulsatile pressure test. This study was recently published in the Journal of Vascular Surgery (J Vasc Surg 2015;62:200-9) and serves as the last step before in vivo proof-of-concept studies. SsLAVA with the BSA-HPMC-genipin solder produced the most durable welds.

To address these data in light of your valid remarks regarding the potentially different degradation kinetics in vivo, we included the following text in the Discussion:

"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 4.3-5.9mm) 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."

4. For the breaking strength measurements, please comment on whether the cross-sectional area of the solder was measured before or after the breaking strength measurement was performed since it is possible that in some cases the cross sectional area of the solder changed irreversibly after heating or while under stress.

The cross-sectional area of the solder was measured right before the breaking strength (BS) measurement was performed (after welding and after hydration period). This information was

added to section 2.6.

5. Figure 8 – The microscopy images should have a scale bar so dimensions can be easily assessed.

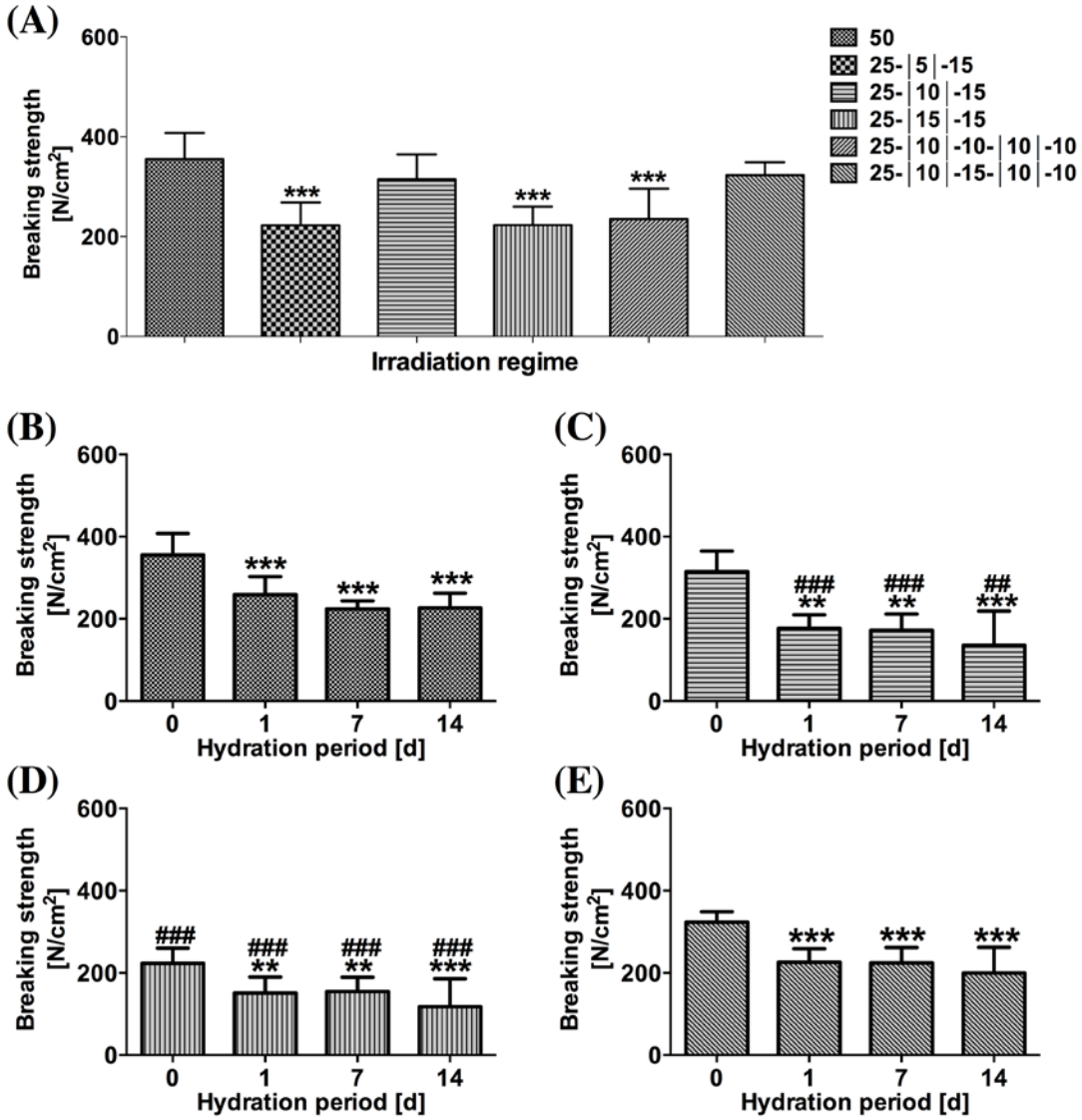
A scale bar has been added to Figure 8.

6. Regarding the thermal damage measurements in Figure 8, do the authors have quantitative data measurements of the depth of thermal damage produced? This should be reported (mean +/- standard deviation). Also, the thermal damage experiments were performed on pieces of aorta. How does the depth of thermal damage in the aorta specimens compare to the vessel wall thickness of vessels where ssLAVW would likely be applied (e.g., if the depth of thermal damage is about 30% of the aorta specimen thickness, would full thickness damage be expected in the walls of vessels where ssLAVW would be applied)?

We do not have quantitative data on the depth of the thermal damage because the sample size was too small to perform robust quantitative analysis. At this point we cannot stipulate with certainty how the thermal damage profiles achieved in our specimens are representative of thermal damage profiles in other types of vessels. Naturally, it is expected that smaller-diameter vessels will experience more extensive thermal damage at the same lasing parameters as employed in our study because the thermodynamics will not be too different for comparable tissue compositions. However, rather than speculating on this in the discussion, an in vivo proof-of-concept study should be performed first to ascertain which regimen produces the strongest welds. Subsequently, an in vivo study should be performed to determine which lasing parameters should be employed for a specific range of vessel diameters. We have addressed this in the Discussion and Conclusions sections.

7. Figure 9 – Panel A is very confusing to view in the way the breaking strength comparisons are depicted; for clarity it may make more sense to arrange the groups in order of ascending or descending magnitude of measured breaking strength. Also, I expect that some of the comparisons in the Figure 9 A-E plots may change in terms of statistical significance when a post-hoc multiple comparisons test is performed (see comment #1).

We have simplified Figure 9A as shown below. (#) indicates the level of significance to the control group (50 s single spot continuous lasing). The statistical analysis in Figure 9A-E has also been modified as explained in the answer to the reviewer's first point.



2nd editorial decision:

Date: 21-Jun-2015

Ref: Laser-assisted vascular welding: optimization of acute and post-hydration welding strength

Dear Dr. Heger:

I am pleased to inform you that the above referenced manuscript has been accepted for publication. Prior to publication, we will prepare a set of proofs for your review.
Thank you for your contribution to Journal of Clinical and Translational Research.

Sincerely,

Bernard Choi, Ph.D.
Editorial Board Member, Journal of Clinical and Translational Research