**Author’s response to reviews**

**Title:** The Effect of low Intensity Shockwave Treatment (Li-SWT) on human Myoblasts and mouse Skeletal Muscle

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**Author’s response to reviews:**
Response letter (2. round of review)

Dear James Mockridge

We hereby address, point-by-point, all reviewer comments to our manuscript, BMSD-D-16-00272 Please find below our comments provided in italics.

Yours sincerely,
Jeeva Sellathurai

Reviewer reports:
We would like to thank the reviewers for reading the manuscript and for their comments. We have addressed the comments below and find that the quality of the manuscript has been significantly improved.

Vinícius Ynoe de Moraes (Reviewer 1):

1. Abstract remains different from main text.
We have improved the abstract and there are no inconsistencies between abstract and manuscript now.

2. Study objectives should be more specific.
Answer: we have specified the study objectives and made the aim of the in vitro and in vivo studies clear (see Background section).

2. Statistical approach is not appropriate, otherwise should be justified.
Answer: the chosen statistical approach is commonly used in muscle lesion studies published in several papers. As our studies include n=3, we assume that the data are normally distributed, as this can not be shown when n=3. We have discussed the statistical approach with a statistician, who approved our methods.

3. Quality of written English is poor
Answer: the language has been edited and improved.

Tomasz Halski (Reviewer 2):

This original study design is an in vivo study in mice and an in vitro study. As Authors was signalise that there are no published results in the indexed literature on the effect of Li-SWT on myoblast and skeletal muscle undergoing regeneration after acute injury.

The first impression after reading this paper is more than positive. The topic of this manuscript falls within the scope of the BMC Musculoskeletal Disorders subject and has a significant scientific value.

There are three things that need explanation:

1. Page 9 line 1: You wrote: "The viability of myoblasts was tested, and within the range of 300 to 1500 impulses the energy application was well tolerated. For further in vitro studies a dose of 300 impulses of 0.1 mJ/mm2 was used." - the objective of this study was assessment the effects
of Li-SWT on regenerating skeletal muscle not only safety of dose 300 impulses of 0.1 mJ/mm2, so could you explain, why did you choose 300 impulses in vitro.

Answer: we wanted to make sure that the Li-SWT was not harmful to the myoblast even when the cells received a high number of impulses. The 300 impulses were chosen to be close to the number of impulses used by others in a published study showing increases expression of angiogenic cytokines (Zhang X. et al, 2014).

Page 9 line 31 and in conclusion :
Could you add how was the dose of total energy calculated.
Answer: the total amount of energy was displayed by the Duolith SD1, after the equipment was set to the chosen parameters.

Page 14:
Could you write if difference between cell death treated and control was significant.
Answer: we found no difference in cell death between treated and control, and this is now added in the manuscript.

Pierpaolo P. Iodice (Reviewer 3):

In the present manuscript, Hansen and coworkers examined the effect of low intensity shockwave therapy (Li-SWT) on the tissue regeneration and vascularization in skeletal muscle and myoblasts. The authors report significant increase in expression of apoptotic, angiogenic and myogenic genes in vivo. Li-SWT did not induce significant changes in vitro cell survival, proliferation and apoptosis of myoblasts. The authors conclude that Li-SWT induced cellular changes that could promote muscle regeneration.

This article addresses an important clinical topic in treatment with Li-SWT and is well-conducted. However, I have reservations with the statistical analysis implemented.

Below, I have provided a constructive feedback to the authors in an attempt to help them strengthen their manuscript.

General:
1. I advise the authors get editing help from someone with full professional proficiency in English.
Answer: the language has been edited and improved.

2. At the end of the Introduction, please make sure to specify more clearly the hypotheses of the study.
Answer: we have specified the study objectives and made the aim of the in vitro and in vivo studies clear (see Background section).

3. A crucial flaw is the statistical analysis, authors generally report the statistic method used but we do not found out numerical data in results, discussion or in Figures. The authors have to specify the dependent factor for each statistical test and effect sizes as complement of statistical hypothesis testing.
Answer: As the numerical data for the gene expression studies are hard to read from the graphs, we have now included the data from all the gene expression experiments in Supplemental material (S3). The statistical tests were made to test the difference between Li-SWT treated and control, which is made clear in the Methods and throughout the results section.

4. Please justify your choice for using a paired t-test rather than a Friedman Test. Please note that the Friedman Test is the non-parametric equivalent of a Repeated-Measures ANOVA within a parametric paradigm. To investigate effects of Li-SWT treatment at different days after injury a Repeated-Measures ANOVA analysis should have been more appropriate for the scope.
Answer: as mentioned before, the chosen statistical approach is commonly used in published muscle lesion studies. As our studies include n=3, we assume that the data are normally distributed, as this cannot be shown when n=3. As the reviewer suggests, it is not possible to conduct analysis with repeated measures statistics, as we do not use the same animals at different time points. We have discussed the statistical approach with a statistician, who approved our methods.

5. A more in-depth description of the Results should be provided. Specifically, comment on the magnitude of the observed differences for the non-parametric rank comparison.
Answer: we believe that the results section adhere to the submission guidelines. The magnitude of the observed differences can be seen in the figures (and supplemental S3) and further specification of the magnitude of the observed differences for e.g. ALL genes in the results section would affect the reader-friendliness and take focus away from the overall assessment of how several genes are expressed in Li-SWT treated and control samples.

6. Provide clear avenues for clinical procedure and future research. Specifically, expand on the theoretical and applied importance and novelty of results.
Answer: as suggested, we have now provided a more clear description of clinical avenues and underlined the novelty of the results. The Li-SWT research community is small with limited number of papers published, but we have included all relevant literature published on this subject.

Specific Comments:
1. A space is sometimes placed between numbers and units, but not always (i.e., 10 cm and 10cm). Usage should be consistent.
2. The Authors used female mice, this choice has to be argued, a reference should be included.
Answer: female mice were chosen, as female mice are considered to regenerate better than males.
3. Page 9 Line 39. ".was repeated every third day OF THE PERIOD"
4. Page 10 Line 34 " The data WERE analysed"
5. Page 11 Line 36 " Powervision...WAS used"
6. Page 22 Line 8-16. This sentence should be rephrasing.
7. Fig 3 caption: the tense needs to be consistent here
8. Frequently in manuscript the tense switches between past and present - please make this consistent
Answer: the corrections suggested above (point 1-8) have all been included in the manuscript.

9. The manuscript (including the abstract) contains several small typos that could benefit from proof-reading.
Answer: the language has been edited and improved.