

We thank the Editor and the Reviewer for their interesting observations and constructive comments. In the following, we report our answers (in bold face) to specific comments. On their basis we have amended the manuscript using changes track. We are confident that now the manuscript will encounter their satisfaction.

2 Peer-review report

Reviewer #1: The author's purpose of the investigation is interesting, also for scientists from related research fields. I would recommend the suggestions described below:

- 1) The title should be short and concise. According to recent studies that would favor future citations to the paper. What is really new in the paper? Is the title the mirror of all the paper information? Was the word cancer mentioned?

The title has been shortened; it refers to the main objective of our paper, which is to provide an overview of different ways to reduce pain due to electroporation-based treatments. Muscle contractions (thus, patient pain) is only related to the electroporation treatments, despite of its goal (cancer therapy or other), even if the main applications of clinical interests are for cancer treatments. Since healthy patients were also considered in some papers, we retain the word cancer should not be mentioned in the title.

- 2) Abstract should be quantitative as possible for rapid comparison with similar studies. Avoid imprecise terms such as:.... "shorter", but how much? lower, but how much? Quantitative values should be referred....After reading the paper information is missing in the abs. The abs should be a mirror of the paper and not a kind of intro, aims or approaches.

Modified.

- 3) Introduction should be less general and focuses in the main message of the paper. What are really the recent insights into ECT applications? At the end of the intro, it is also not clear what is the main message and relevant points of the paper that should be emphasize at this stage.

Modified.

- 4) I would suggest that the authors could eventually include a timeline for these studies on order to emphasized what was done and what was no done yet.

It is not possible to include a timeline for the analysed papers, since they consider the reduction of pain and muscle contractions from different points of view, taking into account different protocols, study setups, methods and applications. However, we inserted the information of the publication year in the Table 1.

- 5) A figure of the type and % of tumours study could be mention.

Since only 13 studies were included, we implemented the suggestion but describing how many studies were about what type of tumor. We added the following sentence: *four papers consider cutaneous and subcutaneous tumors [references], two papers consider sarcomas [references] and pancreatic tumors [references], and six studies were conducted on healthy subjects or phantoms [references].*

- 6) Criteria of exclusion/inclusion should be clear.

Criteria of exclusion/inclusion were clarified and adequately referred.

- 7) The results are not properly described. The authors should first describe in a quantitative manner the data before jump to conclusions. Avoid imprecise terms, such as lower and shorter

We added, if provided, (relevant) quantitative results from each paper.

- 8) Avoid jumping immediately to conclusion rather describe property the results, use quantitative values.

We added, if provided, (relevant) quantitative results from each paper.

- 9) The figures could be globally improved, as possible, once the Journal deserves high quality figures and with rigor would avoid lacking of interest for the data. Legends should be also as complete as possible.

Figures' dimensions were reduced in order to adapt them to the .docx page format. If necessary, we can separately provide original figures, so that the Journal can choose the adequate dimensions/resolutions. Figure 1 and figure 2 are in a .vsdx format (Visio software), as they are two flow-charts; figure 3 is in a .JPG format.

- 10) Discussion should be more assertive and concise and eventually be divided in sections with titles highlighting the major results.

We modified the exposition of discussion's contents, highlighting the main results. We also divided the discussion in subsections, as suggested, to clearly identify the main outcomes of the work. As a consequence, a new section (Conclusion) has been added and the content reworded.

- 11) Subsections for in vitro and in vivo studies could clarify the review.

We divided the papers based on the application used (ECT or IRE), since it was thought to be much more interesting in a clinical point of view; moreover, a clear identification of type of study (in vivo, in vitro or simulated) has been provided in table 1. We think that a split based on both the clinical application and the type of study might result in a less clear organization of the overview, because many papers performed both in vitro and in vivo simulations.

- 12) Globally after discussion, the conclusions should follow the order of presentation of the paper with partial conclusions first and then global conclusions.

We reworded the conclusion following the order of presentation of the analysis (relation of pulses frequency and shape, of sinusoidal pulses and electrode design, with pain reduction) including partial results.

- 13) A scheme for a take home message, could besides pedagogical helpful for understanding the aims.

We reworded the global conclusion in order to provide a take home message.

Reviewer #2: This is the first review dealing with pain associated with electroporation-based treatments. The review is thorough and deserves publication, since it covers all the aspects from ECT to IRE. I have only minor comment in abstract section.

- Rephrase or explain what is meant with "lower effective membrane permeability"...

Rephrased

- ... and "electric protocols with equivalent dose".

Rephrased/Explained