Review of the Ph.D. thesis by Mgr. Petra Vahalová „Ultra-weak photon emission from biological samples“

The thesis of Petra Vahalová deals with the oxidative stress in biological samples and its consequences in the form of light emission referred as biological autoluminiscence (BAL). It is based on three articles, one review, and two experimental papers where Petra is the first author of all three of them with significant contribution. These publications document very good quality of the research as well as author´s deep knowledge of the topic. Furthermore, accepted chapter “Selected biophysical methods for enhancing biological autoluminescence” from currently prepared book “Ultra-Weak Photon Emission from Biological Systems: Endogenous Biophotonics and Intrinsic Bioluminescence”co-authored by the student is a part of the appendix of the thesis.

In the introductory part, the author briefly summarises the topic of reactive oxygen species (ROS) in biological systems and its consequences including antioxidants, formation of oxidative products from biomolecules, and electronically excited species like triplet excited carbonyls or singlet oxygen, which are able to emit light. The last part of the introduction is devoted to the explanation of the motives behind the introduction of the term BAL rather than using one of many already existing terms that describe the phenomena of light emission from living systems.

State of the art chapter is short and the author missed the opportunity to explain the difference between commonly known bioluminescence (enzyme + substrate reaction) and biological autoluminescence (ROS related reaction) in chapter 2.5 Applications employing chemiluminescence.

The introductory part ends with goals of the thesis which are clearly stated.
The second part consists of the three articles published by Petra.

The first article reviews methods for monitoring oxidation in biochemical systems, cells, and tissue cultures in its first part, and later on it is focused on BAL and its correlation with various aspects. The authors tried to include too many topics for one review and due to that, not all the aspects are discussed in proper detail. I would suggest prolonging the text, add more detailed information especially in the chapter "Methods for monitoring oxidative/free radical processes in cell cultures" and publish it is a form of monography.

The second article deals with the correlation between BAL from yeast *Sacharomyces cervisiae* and different endogenous and exogenous parameters such as glucose concentration, oxygen partial pressure, pH, ROS or antioxidant added exogenously.

The third article shows the usage of BAL for monitoring the formation of ROS generated by pulsed electric field. Bovine serum albumin was used as a standard chemical system.
Both experimental articles bring new information about the correlation of BAL with various parameters and they are taking us one step closer to widely usage of BAL as non-invasive technique for monitoring of oxidative stress from various biological samples.
The last part of the thesis consists of a brief description of the obtained results and their contribution to the current state of the art.
The Appendix contains the accepted chapter for the book in preparation and supplementary information for the 2nd and 3rd article.

Concerning the formal aspects of the thesis, it is written in good English with almost no spelling errors.

I have a few questions to the author:
1. There is no uniform definition of ROS in the scientific community, how do you define it? Please include explanation why do you rank alkyl radical as ROS, and why is hydrogen peroxide marked as ROS precursor.
2. You have shown the importance of Fenton reagents ratio in both experimental articles, yet you decided not to stick with fixed ratio throughout the experiments, e.g. OD measurements in 2nd article. Can you explain why?
3. In 2nd article Trypan blue assay showed higher effect of Fenton reagent at the highest concentration out of 3 different tested, yet microscopic evaluation of the this concentration haven´t been done. Why?

4. In the 3rd article it is proposed that hydroxyl radical formed via Fenton reaction oxidize the BSA in a different way than hydroxyl formed by pulsed electric field based on the measurement of tyrosine and tryptophan fluorescence. Which seems unlikely to me. Did you consider and test other possible explanations for the fluorescence decrease like BSA interaction with Fe^{3+} described in Xu et al., Journal of Fluorescence, 2007?

In summary, the review and results presented in this thesis reflect the ability of Petra Vahalová to perform high quality and internationally recognized scientific work, therefore, in the case of successful defense, I recommend her for a Ph.D. degree.

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