INSTITUTE OF ANIMAL PHYSIOLOGY AND GENETICS
Czech Academy of Sciences
Assoc. Prof. RNDr. Marcela Buchtová, Ph.D.

Date	**20. 5. 2024**
Name Evaluator	**Doc. RNDr. Marcela Buchtová, Ph.D.**
Affiliation	**Laboratory of Molecular Morphogenesis, Institute of Animal Physiology and Genetics, Brno, Czech Republic**
Name PhD candidate	**Mgr. Barbora Echalar**
Title PhD thesis	**Impact of nanomaterials on mesenchymal stem cells and tissue regeneration**

Evaluation of the scientific quality of the thesis

The candidate, Barbora Echalar, aims to study the impact of metal nanoparticles on main characteristics and functional properties of mouse mesenchymal stem cells. She also evaluated the effect of nanoparticles on their metabolic activity, differentiation potential, gene expression of immunoregulatory molecules and production of cytokines or growth factors. PhD thesis represents a compact story around one central topic. Since metal nanoparticles can act as effective antimicrobials to promote wound healing, there is significant potential for their broader applications.

The introduction part is well written, and chapters cover general aspects related to mesenchymal stem cells, metal nanoparticles and wound healing, summarize mechanisms of stem cell action and their immunomodulatory properties. Figures, which are taken from published manuscripts, well accompany the introductory text. Some of them however display diminished quality (such as Fig. 2, 4) and labels are difficult to read. On the other hand, I appreciated small amount of typos through the text.

Used methodology are not included in thesis but they are presented in detail in all attached manuscripts. Different cellular processes were tested as the response on nanoparticles treatment, such as evaluation of reactive oxygen species production, lipid peroxidation, DNA fragmentation and oxidation, the frequency of micronuclei, apoptosis or cell cycle. Therefore, large spectrum of methods was used including classical analytical approaches such as flow cytometry, gene expression analyses by QPCR, WST-1 metabolic activity assay and many others. In some cases, there is not very clear by which methods exactly Barbora Echalar contributed to individual manuscripts, please provide more information during PhD defence.

The main findings of PhD thesis are included in three already published manuscripts and Barbora Echalar is the first author on one of them. She also participated on four other impacted manuscripts not directly related to the main PhD topic, which indicates a great ability of candidate to be involved in other projects. The first manuscript is focused on the understanding of the impact of four commonly used metal nanoparticles such as ZnO, Ag, CuO and TiO_2 on metabolic activity of mouse adipose-derived mesenchymal stem cells. She proved that the metabolic activity was downregulated in a dose-dependent manner after treatment with all of the tested nanoparticles and adipogenic differentiation was suppressed by some types of nanoparticles. On other hand, metal nanoparticles were able to support osteogenic differentiation of mesenchymal stem cells. Therefore, metal nanoparticles with antimicrobial properties were found to exhibit some negative impact on function properties of stem cells and their regeneration potential was decreased.

The second manuscript is focused on the effects of nanoparticles on the ability of mesenchymal stem cells to stimulate secretion of macrophages. Heat inactivated stem cells were found to be unable to produce cytokines or growth factors, while macrophages when cultivated in the presence of heat-inactivated stem cells were able to express significantly more immunomodulatory molecules. Moreover, nanoparticles displayed a direct negative impact on the production of cytokines and growth factors by mesenchymal stem cells in a dose-dependent manner.

In the third paper, nanoparticles with antimicrobial properties were tested for their possible negative effect on various cells including mesenchymal stem cells. Nanoparticles were found to increase the production of ROS in stem cells, lipid peroxidation, induced DNA fragmentation, enhanced oxidation and the apoptosis of mesenchymal stem cells was induced by nanoparticles treatment.

Text of discussion is rather short and not well organized and it would be useful to split it into several subchapters regarding of main topics. In particular, the first part of the discussion could be better structured. It repeats multiple pieces of information from the results section, and the implications of the results are unclear because they are neither discussed nor compared with the literature. Conversely, some paragraphs merely summarize recent knowledge without connecting it to the results of the presented experiments. However, this can be more discussed during PhD defence, I included some questions below.

In summary, the main aims of the PhD project to better understand the effect of nanoparticles on mesenchymal stem cell action were fulfilled and they are supported by published manuscripts. Results obtained by PhD project of Barbora Echalar are the extension of recent knowledge of processes regulating wound healing. I thereby declare that I support this thesis for the public defense and further procedure.

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My comments/questions for the thesis disputation are following:

Can you briefly summarize what you believe is the most significant contribution of your work to the field?

You mentioned some contradictory findings regarding the effect of Ag nanoparticles on mesenchymal stem cells. Can you discuss potential pitfalls of these experiments in more detail? What would you propose to overcome these obstacles?

Elevated ROS production was observed following CuO NP treatment. How could you further test the proposed association with insufficient antioxidant mechanism activity?

You suggested that additional studies are necessary for validation of the adequacy of metal nanoparticles for the usage in wound healing. What experimental approaches would you propose to further confirm the potential of nanoparticles for future applications?

Recommendations with respect to public defence

☒ **Approved to proceed with no or minor changes to be made to the final version of the thesis, with no new evaluation by the examination committee required;**

☐ **Approved to proceed but with major changes to be made, requiring a new evaluation by the examination committee before the public defence can take place;**

☐ **Not approved to proceed with the public defence, where a new predefence may be warranted.**

Signature