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Date	**10. 8. 2020**
Name Evaluator	**Doc. RNDr. Marcela Buchtová, Ph.D.**
Affiliation	**Institute of Animal Physiology and Genetics, Brno, Czech Republic**
Name PhD candidate	**Anna Zavaďáková**
Title PhD thesis	**Role of fibroblasts in regulation of wound healing**

Evaluation of the scientific quality of the thesis

The candidate, Anna Zavaďáková, presents in her PhD thesis the results of experimental research undertaken to study the response of dermal fibroblasts in wound healing with focus on key stress factors in 2D cultures as well as 3D collagen hydrogel cultures. This combination represents very useful complex approach to study different aspects of cellular behavior including their responses to soluble factors secreted by bacteria. The main topic of her work is very actual and attractive.

Some of the main findings was already included in manuscripts. Anna Zavaďáková is the first author of one already published manuscript and one paper submitted to the journal Scientific Reports. Moreover, she is the co-author of two published papers focused on wound healing and the usage of collagen hydrogels. One more manuscript about the role of fibronectin in venous diseases is submitted to the journal Vascular Medicine. In PhD thesis, there are also included numerous unpublished data, which are planned to embed into manuscripts in preparation.

The introduction part is well written and contains sufficient levels of details for a reader to follow the experiments presented in the main body of the thesis. The chapters cover general aspects of processes during wound healing and associated diseases; further it includes key characteristics of dermal fibroblasts and possible in vitro models to study questions related to cellular response during healing progress.

The methodology is presented in detail for individual approaches. Large spectrum of methods was used including general in vitro culturing, treatment of cells, characterization of cells morphology as well as analyses of responses of cells on stress including their proliferation, metabolic activity or migration. Moreover, the quantification of interleukins was performed by ELISA and MMPs level by zymography.

Main results are arranged into three chapters focused on individual experimental approaches. It covers careful analyses of the cellular responses of dermal fibroblasts to experimentally induced stress in 2D and 3D conditions. Valuable is especially the last chapter where author focused on the optimization analytical approaches to determine cell number.

The discussion parts are well balanced mixture of interpretations of data based on previously published literature and more daring speculations as well as proposals of new experiments to prove mentioned hypothesis.

In conclusion, the PhD thesis as a whole is well presented, and it was a joy to read it. The introduced information in terms of understanding, citation of relevant literature and style of writing is on respectable level. This is continued into the main sections, which demonstrated deep analysis of the data and a notable consideration of the possible conclusions in the light of the extensive literature in these fields.

In summary, I can only repeat one more time that I find the work comprising the thesis of Anna Zavaďáková of great extension of recent knowledge covering all different aspects of wound healing. I thereby declare that I support this thesis for the public defense and further procedure.

My comments/questions for the thesis disputation are following:

Introduction:

- In Chapter 1.3.1. you mentioned the origin of dermal fibroblasts, but their embryonic origin is not really described. Could you more discuss this topic? Are there some region-specific differences? Are there some functional consequences?
- On page 15, there is comments that MSC cells do growth in lack of nutrition only for 2 passages. However, in your experiments with NHDF from patients, you used 3rd-5th passage. Could you comment how you would explain better survival of your cells?

Methods:

- From how many patients skin biopsy was taken? Were they healthy donors? How you selected them – which criteria you used?
- Have you observed some variability between sample response? Did you get enough cells to perform all analyses on cultures established from one patient?

Results and discussion:

- How the experimental condition with low nutrition corresponds to wound healing process? It is not really clear, which stage of healing are you modeling?
- You hypothesize that low nutrition-LPS would provide more impaired cultures than just low nutrition condition. What you would propose as treatment to observe rescue effect?
- Could you propose some method, which would be useful to study cell morphology closer?
- Quantification of collagen type 1 synthesis can be tricky especially by fluorescent labeling? Could you discuss further other possible methods how you could confirm your findings?
- How you explain cell morphology alterations after bacterial treatment? It is only connected to cell death or some other factors could play a role in this shape changes? Have you tried to quantify cell dead?
- The level of interleukins is differently changed between SA and PA treated cultures (page 59-62). Have you expected such large differences in fibroblast response? What is the base of this differential production? Can you discuss which soluble factors can be produced differentially by these bacteria?
- Collagen hydrogel contraction and change in its color is significant in fibroblast 3D cultures. Is the composition of collagen hydrogel altered by fibroblast? Has anybody analyzed this aspect in previous studies? Can this aspect of increasing hydrogel stiffness be directly connected to the inhibition of fibroblast proliferation in 3D cultures?
- Page 68: proliferation of dermal fibroblasts seems to be growing in 2D cultures, which is in contrast to stagnation of growth in 3D cultures. This result does not correspond to observed alteration of metabolic activity. Could you discuss this difference more? Is there some explanation based on molecular level of this aspect in published papers?

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