Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: Andrea Hatmanstorfer

Thesis title: Volume Scanning Electron Microscopy and Stereology for Investigation of SARS-CoV-2 Infected Murine Lung Tissues

Supervisor: Marie Vancová, Ph.D.
Co-supervisor: Jiří Týč, Ph.D.

Referee: Martin Selinger, Ph.D.
Referee`s affiliation: Faculty of Science, University of South Bohemia, Czech Republic & Faculty of Medicine, Umeå University, Sweden

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages)**, balanced length of the thesis parts** (recommended length of the theoretical part is max. 1⁄3 of the total length)**, logical structure of the thesis**	0-3	3
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	2.5
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	3
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	3
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	3
Formal requirements – points in total		19.5
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	3
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	3

[1] Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	3
Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	3
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements – points in total		25.5
POINTS IN TOTAL (MAX/AWARDED)	**48**	45

Comments of the reviewer on the student and the thesis:

The bachelor thesis of Andrea Hatmanstorfer focused on elucidating the alterations in lung anatomy and cellular composition of SARS-CoV-2-infected transgenic mice expressing human orthologue of ACE2 receptor. The thesis is very well written and I greatly appreciate its coherence and structure. The length of 49 pages (including the list of references and appendix) is more than satisfactory. The thesis is written in an excellent English with just a few typos. Additionally, I would like to emphasize the overall high quality of the thesis, especially the Discussion chapter, which was reaching the level of a scientific paper.

The topic of ultrastructural changes in lung morphology upon SARS-CoV-2 infection is very up-to-date, notably in combination with the infection kinetics (2 and 5 DPI) and documented changes in cell type composition. Observed alterations were in agreement with previously described cases and included the reduction of empty alveolar space, increased migration of immune cells, and increased amount of necrotic tissue. Despite the limited time and thus also a limited amount of analyzed mice individuals, I find the thesis as extremely interesting and important for the potential follow-up experiments.

My comments to the formal part of thesis are:

1) As a person, who is not familiar with the anatomy of murine/human lungs, I would greatly appreciate a graphical overview describing the alveolar structure with all cell types mentioned in the text.
2) The same applies for SBF-SEM method or Volume Microscopy methods in general.
3) I am also lacking information about the mice in terms of SARS-CoV-2 infection, i.e. how were the mice infected, was the viral burden somehow analyzed, were there any observable symptoms?

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

1) Given the method used, do you think you should have also observed viral particles in 2 DPI and 5 DPI samples? If so, what could have been the reason behind unsuccessful detection?

2) This might be a naïve (and possibly also stupid) question, but what is considered a sufficient area size in terms of statistical relevance? Is there any "golden rule" for this?
3) If the imaging was not done randomly in the case of this thesis, how the proper sampling should have theoretically looked like?
4) If I understood the principle of SBF-SEM correctly, the SBF-SEM image series should also generate a 3D structure. Can you please explain why none of these have been shown?
5) What is the advantage of SBF-SEM in comparison to other methods generating 3D visualizations? For example the electron tomography?
6) How come there is a necrotic tissue in 0 DPI control sample? (p. 12)
7) In appendix tables, I have noticed an increase of feature entitled "fibroblast in alveolus" in 5 DPI specimen (6.93%) in comparison to 0 DPI (0%) and 2 DPI (0%). Is this the marker of lung fibrosis? Can you comment on this phenomenon, please?

Conclusion:

In conclusion, I **recommend** the thesis for the defense and I suggest the grade **1 (excellent)**. However, the final grade will depend on the performance of the candidate during the defense and her answers to my questions

In Umeå, Sweden, 9.6. 2023

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