



Department

Second Faculty of Medicine, Charles University and Motol University Hospital

Head: Title Name Surname

Opponent's review of the dissertation

The submitted dissertation "**Functionalized nanofiber membrane for ultrasensitive multifunctional bionanosensor**" by **Leontýna Varvařovská** is the result of her postgraduate studies in the Doctoral Study Program in Biomedical Engineering. Her supervisor was RNDr. Taťána Jarošíková, CSc., and her co-supervisor was Prof. RNDr. Evžen Amler, CSc. The dissertation was prepared at the Department of Natural Sciences, Faculty of Biomedical Engineering, Czech Technical University in Prague (CTU).

1. The topicality of the dissertation topic:

The topic of the dissertation falls within the field of nanomedicine. This area of medicine is undergoing intensive development at both the basic and applied research levels. The development of new and/or more advanced nanomaterials is the basis for accurate and rapid diagnosis and targeted therapy.

This work deals with the development of nanofibers combined with specific surface functionalization of the nanofiber membrane using specific antibodies. This system, based on polyacrylonitrile (PAN) microfibers, is suitable for filtering liquids and air and enables the detection of bacteria in situ. In today's world, the development of sensitive biosensors has broader significance in the protection of public health.

2. Achievement of dissertation objectives and 3. Methods and procedures for resolution:

Three objectives were set in the dissertation. 1. To find a suitable polymer material for preparing a nanofiber subassembly for specific functionalization and to determine the appropriate surface density to ensure its effectiveness for the final application, specifically filtration. 2. To develop a nanofiber biosensor for the detection of pathogenic organisms in the air, and to design a method for controlled air filtration. 3. To develop a nanofiber biosensor for detection of pathogenic organisms in body fluids or liquid samples. 4. To test the developed biosensor in laboratory conditions.

Overall, it can be said that Ing. L. Varvařovská worked on all four tasks. The work is methodological in nature. Her research is focused on the best nanomaterials with the required properties for air and liquid filtration and on fiber functionalization for sensitive pathogen detection.

Methods and procedures for resolution are therefore described in detail in several subchapters of the methodological part of the thesis (Nanofiber membranes; Carbon dots; Biosensor for analyte detection from air; Biosensor for analyte detection from liquid samples). The methods and procedures are adequate, are at the current scientific level, and correspond to the objectives of the dissertation.

4. The results of the dissertation and the specific contributions of the dissertation author:

The results of work related to the optimization of nanomaterial properties and the development of biosensors are presented in four articles in journals. In three articles, each with an impact factor (between 0.6 and 5.6), Ing. Varvařovská is the first author. The results of the experimental work are described in 20 pages of the dissertation. The results show that it was possible to optimize a suitable nanomaterial for filtering air or liquid samples and to develop an effective immunosensor for microorganisms, *Escherichia coli* and *Staphylococcus aureus*, representing G- and G+ bacteria, respectively.

As the author herself states, the results are also discussed in the Results chapter. Given the methodological focus of the work, this is partly understandable. However, I do not consider this to be

entirely optimal in this part of the work (see below). The Results section also lacks an overview of statistical methods.

The large number of results shows that the author is capable of performing various types of experiments with nanomaterials, functionalizing them, and measuring their various parameters using spectrometric and other methods, particularly biophysical methods. She is also capable of working with biological material, i.e., microorganism. The student has been conducting long-term research into nanomaterials (master's thesis).

5. Significance for practice and development of the field of study Biomedical and Clinical Engineering.

The pre-clinical results achieved by the student in her experimental work with nanofibers have significant translational potential. Ing. L. Varvařovská developed a biosensor with excellent optical properties. This sensitive system, moreover with short-time detection, surpasses other methods for pathogen detection and is innovative in design and function. Research into nanomaterials and biomedical engineering is fully in line with the field of study Biomedical and Clinical Engineering.

6. Formal layout of the dissertation and its linguistic level:

The overall formal layout of the dissertation is good. The thesis is written in English.

The work contains the standard chapters Introduction – State-of-the-Art, Aims, Methodology, Results, Discussion, Conclusion. This is followed by the chapters Contribution to Biomedical Engineering, List of Publications, References (121 articles) and Appendices A and B. The work has a total of 96 pages. State-of-the-Art is very well written. However, I have a few formal comments on the chapter Results, Discussion and Conclusion, or rather on the content of individual chapters, see below and other minor details.

7. Comments and final evaluation of the dissertation:

- The abbreviation PAN is not explained in the Abstract (abbreviations should not be used or explained in the abstract).
- The list of abbreviations is not in alphabetical order, which makes it difficult for readers to navigate the text. What are HGF cells? (p. 36)
- Page 14: Figure 2.2: The legend says A, B, C, but not inside the figure.

Results chapter:

- Page 50: Paragraph Other SEM.... Appendix B. Appendix B on pp. 91-96 is labeled Fig. A1-A6, not B1 – B6.
- Page 51: What does "a significant difference" was observed... mean? How is this quantified? Next sentence beginning: Although the sizes... by those three methods - what three methods? How can I find this in Figure 5.3?
- Page 55: paragraph Lastly, ... to enhance cell viability ... - how is viability increased? Was there anything toxic? In addition, the figure is missing (in the dissertation). This paragraph could be in the Discussion.
- Some passages from Results could be in Methodology, e.g., on page 51: Paragraph The diameters... A measurement of DLS - if it is described in the Methodology there is no point in repeating it all. Similarly, on p. 52, paragraph To visualize CDs... it would be more appropriate to put this in the Methodology.
- Insufficient description of Figures: for fluorescence measurements, the excitation and emission wavelengths are missing.

Discussion chapter:

- Compared to the other chapters, it is very short (4¼ pages) and vague. On the contrary, some sentences or paragraphs from the Results chapter should be included in the discussion (e.g., respiratory filters FFP1 and FFP2, p. 64). The Discussion should include a more in-depth comparison with other, less effective biomaterials/biosensors and a comparison of specific measured parameters, to highlight the significance of the work compared to other researchers. There is no discussion of whether other authors have attempted to develop an immunosensor for pathogens or other biomarkers and with what results. Among other things, it would be useful to mention in the Discussion a comparison with so-called "green" carbon dots, which are only mentioned in Chapter 8 (Contribution to biomedical engineering).
- Page 72 presents statistics probably related to filter capability and Table 5.2. on page 64. Why are the statistics not included in the Results and only appear at the end of the Discussion?

Conclusion chapter:

- For clarity, it should focus more on the four objectives (Aims) set out. Some passages could have been part of the discussion (e.g., the paragraph beginning with "One of the most significant...", "to overcome this...", etc.).
- I did not find any detection time of microorganism in the Results section, but surprisingly, a comment appears in the Conclusion chapter (p. 73).

Next comment /questions

- The text uses the term "filtration efficiency (of PAN)," e.g., on pages 64, 71, and 72. Elsewhere in the text, the term "filtration effectiveness" is used (p. 64). Is there any difference between these two terms?
- Explain Figures 5.12 and 5.13. What is taken as 100% or 0%?
- Assign individual aims of the thesis to publications.

Conclusion: Based on the above facts and despite shortcomings of a predominantly formal nature, I conclude that the submitted thesis "**Functionalized nanofiber membrane for ultrasensitive multifunctional bionanosensor**" by ing. LeontýnaVarvařovská **meets the requirements** for a doctoral dissertation in the given field and demonstrates the author's ability to work independently at an appropriate scientific level. I **therefore recommend** the aforementioned dissertation for defense on the basis of which the academic title of Doctor (Ph.D.) may be awarded in accordance with § 47 of the Higher Education Act No. 111/98 Coll.

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