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DOCTORAL DISSERTATION REVIEW
of BASSEEM RADWAN, M.Pharm.
entitled

“ Spectroscopic molecular probes of endothelial function and dysfunction in *in vitro* models and their potential in *ex vivo* applications ”

The reviewed doctoral dissertation of **BASSEEM RADWAN, M.Pharm.** was conducted at the Faculty of Chemistry and Jagiellonian Centre for Experimental Therapeutics, Jagiellonian University in Krakow under the supervision of Prof. Małgorzata Barańska.

The topic undertaken by the doctoral student is part of a very important scientific trend in the field of implementation of spectroscopic imaging methodologies to study endothelial cells (ECs) functionality and endothelial dysfunction (ED). The doctoral student presented in an interesting way novel Raman probes vs label free approach for lipidic subcellular structures investigation in the *in vitro* and *ex vivo* applications.

The Ph.D. student's reviewed dissertation was composed in accordance with the rules adopted for experimental work. It is written in correct English and is clearly designed graphically. The work contains 154 pages, and consists of the following parts: Abstract, List of abbreviations, Figures, Tables and Introduction section which is Theoretical part. The second part of the thesis manuscript is Experimental Section which is divided into 5 chapters each one finalized with the conclusion section. The end of the thesis are summary and final conclusion, limitations and future directions. The dissertation consist of 34 figures and 2 tables. Appropriately selected references, consisting of 179 mostly come from recent years, are used and quoted correctly. Its use in the work, both in the introduction and in the discussion, proves the ability to use the scientific literature resources and a good subject understanding.

The theoretical part of this doctoral thesis is a well written introduction about Endothelial cells, its' dysfunction problems, nucleus, mitochondria, lysosomes and current method to study endothelial cells. This chapter present basis of fluorescence microscopy, transient absorption microscopy, raman microscopy, confocal raman imaging and all

methodology about label free raman imaging vs novel raman probes. It's worth to emphasise that not only theoretical introduction is well presented but also in each paragraph the advantages and limitations were mentioned and discussed. Reading this part of the manuscript you can see that it is nicely planned and gives the reader good feedback guidelines. This confirms a very good way of conveying the knowledge and scientific maturity of the writer.

The experimental part of this thesis was divided into four stages to verify the following four main hypotheses.

Chapter 1 was dedicated to introducing a novel labelled Raman imaging-based method to study ECs subcellular lipidic structures relying on AXT as a new Raman probe for intracellular lipids.

Chapter 2 showed establishing the properties of AXT as a lipids biomarker in ECs of various origins, and its excited state dynamics in live cells.

Chapter 3 is describing investigating the uptake and anti-inflammatory effects of free and encapsulated AXT in ECs using label-free and labelled Raman, and fluorescence imaging techniques.

Chapter 4 presented introduction of a novel labelled Raman imaging-based approach to study ECs proliferation and *ex vivo* regeneration, complemented with fluorescence imaging.

All of those hypotheses were confirmed by the experiments performed within this doctoral research plan.

At the end of the thesis manuscript there is a chapter about the future direction and the limitations of the application of the novel label free raman techniques which is very interesting. This chapter presents the reliable approach and predicts difficulties in optimizing selected processes.

The doctoral thesis submitted for evaluation is a logically related series of seven publications. It is noteworthy that the Ph.D. student is the first author in two of these articles (and one more submitted to prestigious journal), which indicates his leading role. These are the listed publications:

Publications related to the PhD thesis:

(1) “**Radwan, B.**; Prabhakaran, A.; Rocchetti, S.; Matuszyk, E.; Keyes, T. E.; Baranska, M. Uptake and anti-inflammatory effect of liposomal astaxanthin on endothelial cells studied by Raman imaging” (Submitted to Chemical Communications, Elsevier) IF=6.065, MEiN=200

(2) “**Radwan, B.**; Rocchetti, S.; Matuszyk, E.; Sternak, M.; Stodulski, M.; Pawlowski, R.; Mlynarski, J.; Brzozowski, K.; Chlopicki, S.; Baranska, M. EdU Sensing: The Raman Way of Following Endothelial Cell Proliferation in Vitro and Ex Vivo. *Biosens. Bioelectron.* 2022, 216 (August), 114624. <https://doi.org/10.1016/j.bios.2022.114624>.” IF=12.545, MEiN =200

(3) “**Radwan, B.**; Adamczyk, A.; Tott, S.; Czamara, K.; Kaminska, K.; Matuszyk, E.; Baranska, M. Labeled vs. Label-Free Raman Imaging of Lipids in Endothelial Cells of Various Origins. *Molecules* 2020, 25 (23), 5752. <https://doi.org/10.3390/molecules25235752>.” IF=4.927, MEiN =140

(4) “Czamara, K.; Adamczyk, A.; Stojak, M.; **Radwan, B.**; Baranska, M. Astaxanthin as a New Raman Probe for Biosensing of Specific Subcellular Lipidic Structures: Can We Detect

Lipids in Cells under Resonance Conditions? *Cell. Mol. Life Sci.* 2021, 78 (7), 3477–3484. <https://doi.org/10.1007/s00018-020-03718-1>.” IF=10.001, MEiN =140

(5) “Yang, T.; Chettri, A.; **Radwan, B.**; Matuszyk, E.; Baranska, M.; Dietzek, B. Monitoring Excited-State Relaxation in a Molecular Marker in Live Cells—a Case Study on Astaxanthin. *Chem. Commun.* 2021, 57 (52), 6392–6395. <https://doi.org/10.1039/D1CC01907D>.” IF=6.065, MEiN =200

(6) “Adamczyk, A.; Matuszyk, E.; **Radwan, B.**; Rocchetti, S.; Chlopicki, S.; Baranska, M. Toward Raman Subcellular Imaging of Endothelial Dysfunction. *J. Med. Chem.* 2021, 64 (8), 4396–4409. <https://doi.org/10.1021/acs.jmedchem.1c00051>.” IF=8.039, MEiN =200

(7) “Matuszyk, E.; Adamczyk, A.; **Radwan, B.**; Pieczara, A.; Szcześniak, P.; Mlynarski, J.; Kamińska, K.; Baranska, M. Multiplex Raman Imaging of Organelles in Endothelial Cells. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* 2021, 255, 119658. <https://doi.org/10.1016/j.saa.2021.119658>.” IF=4.831, MEiN =140

It is worth to emphasize that conducted research was financed as part of two scientific projects, where Mr Basseen Radawan was involved:

- 1) European Union’s Horizon 2020 Research and Innovation Programme under Marie Skłodowska-Curie [Grant Agreement 813920 (LogicLab ITN)],
- 2) National Science Center Poland (NCN) (Opus15 no. UMO-2018/29/B/ST4/00335).

Besides the abovementioned list the Ph.D. Candidate was also a co-author of 3 more manuscript and some conference proceedings.

The methodological aspect of the work deserves special praise. The dissertation shows different approaches in lipids analysis’s using label free-, raman probes and combined click chemistry raman probe + fluorescent tag in the cells analysis.

After reading the whole dissertation, I have a few questions. I hope that Author will have an opportunity to comment on them during the public defense of this doctorate thesis.

- 1) In experimental part - Chapter 1 about using astaxantin (AXT) as sensitive Raman probe - what was the process to control laser power during the experiment? Could Author describe the optimization process of control laser power which explain how to control the undergo bleaching process? Was there any limits in this process?
- 2) Which of the described in the thesis methods, would be used, as the most promising method of choice within next 10 years as a standard approach for cellular lipids analysis? Label free raman techniques or using e.g. raman probes or “click chemistry” with a fluorescent azide?
- 3) Editorial comments e.g. on page 105 and 106 in the figures 13.2 and 13.3 the image size scale bar is not described.

- 4) On page 112 – there are described different concentrations of DOX 0.1 μM and 1 μM used, and claimed that this could be used to control the process of altered cell proliferation – where there any intermediate concentrations checked?

The substantive value of this doctoral dissertation is high. Mr. **Basseem Radwan**, mastered the ability to plan scientific research, and then successively carried out the project. He showed a good understanding of theoretical issues in the field of the subject, knowledge of the research methods used and is able to summarize and critically discuss his own results in relation to the publications of other authors.

The doctoral dissertation presented to me for evaluation meets the formal and substantive requirements set out in the Act on Academic Degrees and Academic Title and Degrees and Title in accordance with the requirements of Law on Higher Education and Science (Journal of Laws of 2018, item 85, as amended).

The novel results obtained during realization of this Ph.D. thesis will certainly contribute to the extension of designing spectroscopic approach in lipids analysis.

On this basis, I am applying to **the High Council of the Discipline of Chemical Sciences of the Jagiellonian University in Krakow** to accept this manuscript thesis and admit **BASSEEM RADWAN, M.Pharm.**, to further stages of his doctoral dissertation. Based on the presented results of an innovative approach using novel Raman probes (“click chemistry” with tags or label free) taking into account the scientific achievement in accordance **with the rules of the Doctoral School of the Jagiellonian University in Krakow, I would like to recommend this doctoral dissertation for distinction.**

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