



MEDICAL UNIVERSITY OF LUBLIN
Faculty of Biomedical Sciences
Independent Unit of Spectroscopy and Chemical Imaging

ul. Chodźki 4a, 20-093 LUBLIN, POLAND
e-mail: anna.sroka@umlub.pl

Lublin, 19.12.2022 r

Anna Sroka-Bartnicka, Ph.D, D.Sc
Head of Independent Unit of Spectroscopy and Chemical Imaging
Medical University of Lublin

DOCTORAL DISSERTATION REVIEW
of FATIH CELAL ALÇIÇEK, M.D.
entitled

“Insight into the links between sex- and age-related mechanical and biochemical alterations of erythrocytes and their ATP profile in ApoE/LDLR double-deficient mice model”

The reviewed doctoral dissertation of FATIH CELAL ALÇIÇEK, M.D. was conducted at Jagiellonian Centre for Experimental Therapeutics, Jagiellonian University in Krakow under the supervision of Asst. Prof. Katarzyna M. Marzec, Ph.D., D.Sc.

The topic undertaken by the doctoral student is part of a very important scientific trend in the field of red blood cells (RBCs) analysis and its biochemical, mechanical, and functional properties. The doctoral student presented in an interesting way the advantages of a multimodal approach including conventional and spectroscopy techniques to characterize various properties of RBCs in healthy animals as well as in a model of atherosclerosis in mice.

This dissertation presented the comprehensive characterization of the alterations in various mechanical, biochemical, and functional properties of RBCs acquired from female and male, 8- and 24-week-old, C57BL/6J and ApoE/LDLR^{-/-} mice and their controls. The findings were correlated among the investigated parameters. Moreover, studying the ATP release feature of RBCs in a mouse model of atherosclerosis provided novel and promising knowledge that could lead to developing new treatment strategies in atherosclerosis disease, as well as in various diseases that exhibit hypercholesterolemia. Furthermore, fully understanding the ATP release mechanism including its components may lead to manipulating this pathway pharmacologically in RBCs to regulate the microcirculation to increase blood flow and oxygen transport to tissues. Such an approach could be used in variety of diseases that include failure of microcirculation, as well as in critically ill patients in intensive care units.

The research plan was divided into four stages to verify the following four main hypotheses:
1) Basal intracellular and extracellular ATP levels are correlated with specific alterations of RBCs observed for male and female ApoE/LDLR^{-/-} mice along with the progression of atherosclerosis.

- 2) The irreversible alterations of the secondary structure of proteins in RBCs cytosol in ApoE/LDLR-/- mice have an impact on changes of 2,3-DPG levels and changes in ATP profile.
- 3) RBCs cytosol and membrane alterations have an impact on ATP release and therefore both depend on the stage of the disease.
- 4) Hypoxia-induced ATP release from RBCs in ApoE/LDLR-/- mice is not impaired, may even occurs greater and could contribute to a mechanism that resulted in the previously reported higher exercise capacity.

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

Thus far, the author has presented and discussed comprehensively the disease-, sex-, and age-related alterations in RBCs acquired from female and male, 8- and 24-week-old, C57BL/6J and ApoE/LDLR-/- mice by applying conventional techniques, as well as vibrational spectroscopy methods. The abovementioned alterations in another important feature of RBCs, i.e., ATP release, are characterized in response to different stimulators, and their relations are investigated with previously reported alterations of RBCs.

The innovative potential of using an approach based on applying the spectroscopy techniques such as FTIR and Raman was proved and very well presented. It was demonstrated that hypercholesterolemia and atherosclerosis disease have an impact on RBC features and functions. Moreover, the sex and age of mice were indicated as factors that influence some of the exhibited biochemical and mechanical alterations of RBCs. Changes in the lipid profile of the RBC membrane and the Hb structure were revealed by the application of vibrational spectroscopy techniques: FTIR and Raman spectroscopy.

The doctoral thesis submitted for evaluation is a logically related series of three prestigious publications and one patent. It is noteworthy that the Ph.D. student is the first author in each of these articles, which indicates his leading role. These are the listed publications and patent application:

- 1) **Alcicek FC**, Mohaissen T, Bulat K, et al. Sex-Specific Differences of Adenosine Triphosphate Levels in Red Blood Cells Isolated From ApoE/LDLR Double-Deficient Mice. *Front Physiol.* 2022;13:223. doi:10.3389/fphys.2022.839323
- 2) **Alcicek FC**, Blat A, Rutkowska W, et al. Secondary structure alterations of RBC assessed by FTIR-ATR in correlation to 2,3-DPG levels in ApoE/LDLR-/- Mice. *Spectrochim Acta Part A Mol Biomol Spectrosc.* 2023;284:121819. doi:10.1016/J.SAA.2022.121819
- 3) Dybas J, **Alcicek FC**, Wajda A, et al. Trends in biomedical analysis of red blood cells – Raman spectroscopy against other spectroscopic, microscopic and classical techniques. *TrAC - Trends Anal Chem.* 2022;146:116481. doi:10.1016/j.trac.2021.116481
- 4) Polish Patent Application PL441216 submitted on May 18, 2022 to Polish Patent office - **Alcicek FC**, Mohaissen T, Marzec KM. “Device for the evaluation of deformation-induced ATP release from red blood cells and a method for assessing the ATP release potential from red blood cells.”

It is worth emphasis that conducted research was financed as part of three scientific projects, where **FATİH CELAL ALÇIÇEK, M.D.** was in the role of the main contractor:

- 1) Polish National Science Centre, the OPUS grant No. UMO 2016/23/B/ST4/00795,
- 2) Centre for Technology Transfer CITTRU at the Jagiellonian University in Krakow for the project “Method for the measurement of the deformation-induced ATP release from RBCs based on a prototype of the deformation-induced ATP releaser (DIAR)” as the Principal Investigator.
- 3) NAWA STER Mobility Grant, from Doctoral School of Exact and Natural Sciences at Jagiellonian University, as the Principal Investigator

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 169 pages, and consists of the following parts: Summary, Abstract, List of abbreviations, Theoretical part, Aim of the work, Experimental part, Results and discussion, Summary, The most important results and conclusions, and Bibliography. The dissertation consists of 1 table and 26 figures. Appropriately selected references, consisting of 271 mostly come from recent years, are used and quoted correctly. It is mainly professional, English-language literature, published in well-known, important journals. 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 thematic understanding.

The methodological aspect of the work deserves special praise. The dissertation shows how effective and powerful applying advanced spectroscopic techniques such as infrared and Raman spectroscopy is. Both methods were used in a qualitative and quantitative way. Lipids to proteins ratio was investigated on the basis of the FTIR spectra. E.g. esterified lipids were analysed and expressed by the ratio of 1740 to 1004 cm^{-1} bands, assigned to the C-O stretching and phenylalanine vibration modes. The unsaturated lipid content of RBCs membranes was expressed by the ratio of the 1668 cm^{-1} band, corresponding to the C=C stretching vibrations, to the 1451 cm^{-1} band attributed to scissoring modes of the CH₂/CH₃ groups.

The reviewer's duty is also to make a critical comment on the evaluated work. 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) On page 45 in the theoretical part the doctoral candidate claims “The detailed analysis of such spectra as well as their differentiation can be analyzed using chemometric methods” which of those methods were used to analyse spectra. Is there any potential to furtherly improve the differentiation method of the spectral analysis?
- 2) Some of the results were not published yet. Will the doctoral student take further steps to publish other interesting remaining results?
- 3) The Author conducted experiments using an animal model ApoE/LDLR double-knockout (ApoE/LDLR^{-/-}) mice. What was the reason for choosing this specific group in case of atherosclerosis in humans?

The substantive value of this doctoral dissertation is high. Mr. **FATİH CELAL ALÇIÇEK, M.D.**, 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 art. 187 of the Act of July 20, 2018. Law on higher education and science (Journal of Laws of 2018, item 1668, as amended).

On this basis, I am applying to **the High Council of the Discipline of Biological Sciences of the Jagiellonian University in Krakow** to accept this dissertation and admit **FATİH CELAL ALÇIÇEK, M.D.**, to further stages of his doctoral dissertation. Based on the presented results of an innovative approach using several advanced analytical methods and 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.**

Anna Sroka-Bartnicka, Ph.D, D.Sc.
University Research Professor