

REVIEW

official opponent: Associate Professor of the Faculty of Chemical Engineering and Technology of the Cracow University of Technology, Doctor of Philosophy in Technical Sciences, Engineer (prof. PK, dr hab. inż.)

Marek Piątkowski

on PhD thesis

Kateryna Diedkova

"Regeneration of the myocardium using new MXene - containing electrically conductive polymer scaffolds obtained by electrospinning", which was submitted for the defense to the Specialized Academic Council of Sumy State University of the Ministry of Education and Science of Ukraine for the degree of Doctor of Philosophy in the field of knowledge 22 "Health Care",
specialty 222 "Medicine"

Relevance of the topic of the PhD thesis

According to the World Health Organization, cardiovascular diseases claim approximately 17.9 million lives annually, accounting for 32% of all deaths worldwide. Standard methods of treatment, including medication, surgical intervention, and preventive measures, have improved the situation with cardiovascular diseases. However, there is still an urgent need for more advanced and long-term solutions to regenerate the heart muscle and conductive tissues, which cannot be achieved with standard treatments.

Tissue engineering methods are being actively researched and developed to create alternatives to organ transplantation and restore lost tissue functionality. Tissue engineering allows to create a personalized heart transplants while minimizing the risk of immune rejection and optimizing compatibility.

One of the main areas of tissue engineering is the creation and development of leading scaffolds, which must match the recipient tissue in terms of their structural and

physicochemical properties and have the ability to proliferate cells. In addition to the basic requirements for a scaffold: porosity, biodegradability, and biocompatibility, scaffolds must conduct electrical impulses to maintain myocardial contractile function. According to the literature, conductive gold or graphene nanoparticles are often added to scaffolds to impart conductive properties. Unfortunately, long-term adverse reactions and instability of nerve impulse conduction limit their use in clinical practice.

Given the above factors, an urgent task is to improve the technology of creating three-dimensional conductive polymer scaffolds suitable for the regeneration of conductive tissues and to conduct a comprehensive study of their structural, physicochemical, and biological properties.

Linking of the PhD thesis to state or industry research programs

The PhD thesis was carried out in accordance with the research plan of the Educational and Research Medical Institute of Sumy State University within the framework of the research project on reimbursement mechanisms for contributions under the Horizon 2020 program "Determination of biocompatibility and general toxicity of different groups of nanomaterials for biomedical purposes" (state registration number 0123U102756), Horizon Europe MSCA-2021-SE-01 of the MX-MAP project "Towards MXenes' biomedical applications by high-dimensional immune MAPping" (№ 101086184) and the project funded by the Ministry of Education and Science of Ukraine "Establishing mechanisms of interaction of MXene with biological systems" (0122U000784). The PhD thesis was the executor of all projects.

Degree of validity and reliability of scientific provisions, conclusions and recommendations formulated in the PhD thesis

The statistical analysis of the data is comprehensive, which ensures maximum reliability. The results were tested for normal distribution using the Kolmogorov-Smirnov test. Students' t-tests and one-way analyses of variance with

Bonferroni correction were used to compare independent data. The statistical significance threshold was considered to be more than 95% ($p < 0.05$). Statistical analysis was performed using Microsoft 365 Excel software with Analysis ToolPak and GraphPad Prism v. 8.0.1.244.

The PhD thesis was carried out at a high methodological level using modern research methods. The main scientific provisions and conclusions formulated in the PhD thesis are based on the obtained results. They are sufficiently substantiated and consistent with the purpose and objectives of the research.

Scientific novelty research and received results

The novelty of the PhD thesis is confirmed by the main provisions:

1. For the first time, a comparison of different methods of treatment of polycaprolactone electrospun membranes to impart hydrophilic properties while maintaining three-dimensional structuring and subsequent evaluation for cytotoxicity has been carried out.
2. $Ti_3C_2T_x$ MXene on a three-dimensional electrospun polycaprolactone membrane by deep coating was highlighted, with further characterization of the structural, physicochemical and biological properties of the composite material.
3. Never before, a tissue-engineering structure has been created that supports cell proliferation and has the ability to conduct electricity, which is important for the conduction of nerve impulses for the regeneration of heart tissue.

Theoretical and practical significance of the research results

A fibrous membrane based on a synthetic polymer was created by the method of electrospinning and the optimal parameters of its preparation were selected. The three-dimensional tissue-engineering structures with randomly

oriented submicron fibers promoted cell proliferation and adhesion, which can be used to develop and create scaffolds to regenerate conductive tissues.

This PhD thesis study demonstrates the structural, physicochemical, and biological properties of the PCL-MXene nanocomposite after chemical and oxygen-plasma treatment. A new method for immobilizing two-dimensional nanolaminates by immersion in a suspension of MXene was presented to impart conductive properties to scaffolds and to determine the conductivity range. The data obtained provide insight into the mechanisms of deposition of two-dimensional nanolaminates on polymeric scaffolds, opening the way for developing functional biomaterials for use in tissue engineering and regenerative medicine.

The results of the experimental research have been implemented in the educational and scientific process at the Department of Pathological Anatomy and Forensic Medicine of Poltava State Medical University, the Department of Human Anatomy named after M.G. Turkevych of Bukovinian State Medical University, the Department of Normal and Pathological Physiology of the National University of Pharmacy, the Department of Postgraduate Dentistry of Zaporizhzhia State Medical and Pharmaceutical University, and the Department of Pathological Physiology of the National University of Pharmacy.

Approbation of the results of the PhD thesis, completeness of presentation of the main provisions, conclusions and recommendations

Based on the materials of the PhD thesis, 10 scientific papers were published, including 6 articles indexed by the Scopus scientometric database and 4 abstracts in the proceedings of scientific and practical conferences. The aggregate of all publications reflects the research results presented in the PhD thesis, which meets the requirements of clauses 8, 9 of the requirements for awarding the degree of Doctor of Philosophy "Procedure for awarding the degree of Doctor of Philosophy and revocation of the decision of a one-time specialized academic

council of a higher education institution, scientific institution to award the degree of Doctor of Philosophy", approved by the Resolution of the Cabinet of Ministers of Ukraine of January 12, 2022, No. 44.

Absence (presence) of violation of academic integrity

The verification of the PhD thesis work of Kateryna Diedkova on the topic "Regeneration of the myocardium using new MXene - containing electrically conductive polymer scaffolds obtained by electrospinning" revealed no signs of academic plagiarism, all references for textual and illustrative borrowings to primary sources were set correctly; no intentional distortions were found (protocol of verification of the work by the StrikePlagiarism system of Sumy State University dated 12/14/2023). Accordingly, it can be concluded that there are no violations of academic integrity.

Assessment of the content, design and scope of the PhD thesis, comments on design

PhD thesis responds to the requirements of design theses, in accordance with the Resolution of the Cabinet of Ministers of Ukraine from 12th January 2022, No 44, "The Procedure for awarding the degree of Doctor of Philosophy and cancellation of the decision of a One-time Specialized Academic Council of a Higher Education Institution, Scientific Institution to award the degree of Doctor of Philosophy".

The PhD thesis is written in Ukrainian on 174 pages. The work consists of an abstract, introduction, literature review, research materials and methods, 5 chapters of the author's own research, analysis and synthesis of the research results, conclusions, a list of references and appendices. The PhD thesis contains a list of references, which includes 241 titles (1 in Cyrillic and 240 in Latin), 53 figures and 1 table.

The PhD thesis begins with an abstract, keywords, and a list of publications

on the topic of the PhD thesis, which are written in the state language, as well as in English. The abstract briefly presents the research's main results, indicating a combination of scientific novelty and practical significance.

In the introduction, the Author comprehensively substantiates the relevance of the PhD thesis topic, indicates the novelty and practical significance, and formulates the purpose and objectives of the research.

Chapter 1, "Literature Review," consists of 3 subsections.

The first one consistently highlights the relevance and prevalence of cardiovascular diseases, describes standard treatments used in medicine and tissue engineering methods for cardiac tissue regeneration. The second one demonstrates and describes the prospects of researching conductive materials to regenerate conductive tissues and shows unexplored data. The literature review ends with a generalization that justifies the choice of the PhD thesis topic, the formulation of the goal and objectives.

In Chapter 2, "Materials and Methods of Research," the author describes the research methods used in the experimental research work, and all the techniques used are described in detail and thoroughly.

Chapter 3, "Results of our own research" consists of subsections.

In the first subsection, the structural properties of the PCL membrane without treatment were studied using Scanning Electron Microscopy.

The second subsection is devoted to the study of the structural properties of the MXene suspension using Transmission Electron Microscopy and Scanning Electron Microscopy with X-ray Diffraction.

The third subsection demonstrates the study of the physicochemical and structural properties of PCL-MXene membranes after sulfuric acid treatment.

The fourth subsection is devoted to studying the structural properties of PCL-MXene composites after treatment with sodium hydroxide.

The last subsection investigates the structural and biological features of the composite material after oxygen plasma treatment.

In Chapter 4, "Analysis and Generalization of the Research Results," the Applicant thoroughly analyzed the research results, discussed them, and compared them with current data obtained by other researchers.

The Applicant has formulated 8 conclusions that logically follow the results obtained, are scientifically sound, and meet the research objectives.

The list of references covers 29 pages of the PhD thesis, formed in the order of references in the text and formatted according to the established standard. The manuscript is completed with appendices containing a list of published works on the topic of the PhD thesis, the results of the PhD thesis, and 4 implementation acts.

Comments on the content and design of the PhD thesis

The work was generally performed and written at a high methodological level, and there are no fundamental comments. The main comments are:

1. The biological properties of the composites should also be tested using e.g. cardiomyocytes.
2. The biodegradability of the composites should be improved. According to OECD 301/310 norm only materials, which degrade at least in 60% after 28 days can be considered as biodegradable.

These remarks are not fundamental to the essence of the work and relate to the interpretation of the results or the design of the work, and do not reduce the scientific value of the PhD thesis as a whole.

Questions to the PhD candidate

1. Do you plan to test the composites with other human cells?
2. Do you plan to modify the composites to improve the biodegradability? What are the possibilities?
3. Did you perform FTIR analysis of PCL membrane before treatment with NaOH/H₂SO₄ and after this procedure?



Conclusions on the compliance of the PhD thesis with the established requirements

The PhD thesis of Kateryna Diedkova on "Regeneration of the myocardium using new MXene - containing electrically conductive polymer scaffolds obtained by electrospinning" fully complies with the requirements of the Resolution of the Cabinet of Ministers of Ukraine No. 44 of January 12th, 2022 "Procedure for awarding the degree of Doctor of Philosophy and revocation of the decision of the One-Time Specialized Academic Council of a Higher Education Institution, Scientific Institution on awarding the degree of Doctor of Philosophy", and Kateryna Diedkova, taking into account the full implementation of the educational component of the educational and scientific program and the individual research plan, deserves to be awarded the degree of Doctor of Philosophy in the field of knowledge 22 "Health Care", specialty 222 "Medicine".

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