REVIEW

by Prof.. Stefan Panaiotov, PhD, DSc from the National Center for Infectious and Parasitic Diseases on the dissertation work for the award of the educational and scientific degree "Doctor" (PhD) in the field of 4.3 Natural Sciences, specility in "Microbiology" on the topic:
"New Photosensitizers and Carbon Composites as Agents with Antimicrobial Action"

Author: Marieta Belcheva, Ph.D. candidate at the Institute of Microbiology – BAS

Scientific Supervisors:
Prof. Lyudmila Kabaivanova, PhD and Prof. Boyko Tsintsarski, PhD

The submitted documents of the candidate comply with the requirements of the internal regulations of the Institute of Microbiology of BAS for applying the Law on the Development of the Academic Staff in the Republic of Bulgaria.
I declare that I have no conflict of interest under Article 4, paragraph 5 of the Law for the Development of the Academic Staff.

Career development of the candidate:

Marieta Belcheva graduated in Medicine in 2001 from the Medical University – Sofia.
She worked as an assistant professor at the Medical University and Head of the microbiological laboratory at the SofiaMed hospital.
She has had several med-term specializations abroad:

- 2005; 3 months specialisation at the University of Zurich, Switzerland;
- 2013; 3 months specialisation at the University Medical Center Groningen, Netherlands;
- 2023-2024; 5 months specialisation at the University Clinic Muenster, Germany.
  She speaks fluent English, German, and Russian.

She has published 6 articles in Bulgarian scientific journals and 6 articles in international journals. She has presented 13 scientific reports or posters at national and international congresses.

Relevance of the topic:

The topic of the dissertation is the study of the inactivation effect of new metal-carbon composites on clinically relevant microorganisms. Antimicrobial resistance is a global public health problem. Scientific research into the development of new therapeutic agents, methods and techniques with mechanisms of action different from those of widely used antibiotics and chemotherapeutics is becoming increasingly important. The thesis investigated the effect of photodynamic inactivation of new metal-containing phthalocyanine photosensitizers against some of the most common microorganisms: *Staphylococcus aureus, Enterococcus faecalis, Candida albicans,*

Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, and *Prevotella intermedia*. Additionally, other compounds with antibacterial potential, namely metal nanoparticles and their oxides, have been studied. The antimicrobial activity of metals is due to their ions' ability to inhibit enzymes, damage cell membranes, and prevent the uptake of essential trace elements by microbes. In addition, some metals can exhibit direct cytotoxic activity. Activated carbon is widely used in water and air purification. Metal-containing carbon composites combine the properties of metals and activated carbon, thus enhancing antibacterial properties. A significant part of the research by the candidate is focused on the inactivation effect of these new metal-carbon composites on clinically significant microorganisms.

The topic is timely, innovative and interesting for research.

General description of the dissertation:

The dissertation consists of 145 pages, 56 figures, and 4 tables. The bibliography includes 304 references. The dissertation is structured in a standard format, including Introduction, Literature Review, Objectives and Tasks, Materials and Methods, Results and Discussion, Conclusion, and Contributions. The dissertation is written very competently and is visualized by artistic figures.

The literature review describes in detail the state of research on the problem, including data from scientific literature on the microbiological characteristics of bacteria, their clinical manifestations in the human body, particularly in the oral and maxillofacial region. Key resistance mechanisms, the essence and achievements of photodynamic therapy, are also described.

Objectives and tasks:

The main objective of the dissertation is clearly formulated – to test the antimicrobial activity of newly synthesized metal-containing phthalocyanine photosensitizers and carbon composites on microorganisms significant to human health, applicable as alternatives in the fight against hard-to-treat infections in the oral and maxillofacial region and as antibacterial agents in air purification, particularly in medical masks. The objectives are divided into two main tasks, each with four subtasks.

Materials and Methods:

A wide range of methods are applied and described in detail, making the research multidisciplinary. The description reflects the candidate's high level of laboratory competence.

Results and Discussion:

Extensive research was carried out and significant results were obtained. The results and discussions of the experiments with the new metal-phthalocyanine photosensitizers relate to reference strains, clinical isolates, and biofilms. The results and discussions of the experiments

with the new metal-carbon composites concern reference strains and clinical isolates. The results are thoroughly described and well-illustrated with figures and photographs.

Conclusions and contributions:

The candidate has formulated 12 conclusions and 6 contributions. The most significant are:

- The best antimicrobial effect among the tested photosensitizers was observed with gallium phthalocyanine, methylpyridyloxy zinc phthalocyanine, and p-tetra-mercaptopyridine zinc phthalocyanine.
- Zinc phthalocyanine is the most effective in treating reference yeast cultures.
- Aerobic bacterial isolates are most affected by zinc phthalocyanine.
- In biofilm experiments, the best effect was observed with silicon phthalocyanine on 48-hour biofilms of *E. faecalis*.
- The newly synthesised copper composite shows good potential as an antibacterial agent.

Contributions:

- The application of photodynamic inactivation with metal-containing phthalocyanine photosensitizers is a new optimized alternative to currently used methods in the fight against bacterial infections in the oral cavity and their prevention.

Some of the contributions formulated have a wishful tone. In the final version of the thesis, the text has been edited.

On the basis of the presented results of the thesis it can be concluded that the doctoral candidate Marieta Belcheva worked diligently and correctly interpreted the obtained results. The objectives and tasks of the dissertation were achieved. I have no comments on the planning, execution and results achieved. The recommendations made during the internal defence were taken into account. I have no comments on the formatting and text of the thesis.

Publications and personal contributions of the candidate:

The PhD candidate Marieta Belcheva presents 6 publications related to the goals and results of the dissertation.

The presented results and contributions are original and not borrowed from studies and publications in which the candidate is not involved. The credit points accumulated during the doctoral program exceed the required minimum.

It is evident that the PhD candidate Marieta Belcheva made a significant contribution to the development of the dissertation. She put special effort into mastering and applying innovative methods for solving scientific tasks.

Conclusion:

The PhD candidate Marieta Belcheva has achieved her goals and set tasks. The dissertation is innovative and has scientific and practical significance. The candidate's publications and other documents meet all scientific criteria according to the Law on the Development of the Academic Staff for the defense of a dissertation for the educational and scientific degree "Doctor (PhD)"

I give my positive evaluation of the conducted research, the results, and the prepared dissertation, and I recommend the Scientific Council of the Institute of Microbiology – BAS to award the educational and scientific degree "Doctor (PhD)" in the field of 4.3 Natural Sciences, speciality in "Microbiology," to Marieta Belcheva.

Sofia, 19/09/2024

Reviewer: /Prof. Stefan Panaiotov, DSc/