REVIEW

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Concerning dissertation for awarding the Scientific Degree **"Doctor of Sciences"**
in scientific specialty **"Therapeutic Dental Medicine"**

Author: Prof. Dr. Neshka Atanasova Manchorova-Veleva, PhD
Topic: "AGE-DEPENDANT DENTINAL AND PULPAL CHANGES – STRUCTURAL, PHYSICO-CHEMICAL AND MOLECULAR-BIOLOGICAL CHARACTERISTICS"

General data

The review was prepared in implementation of Order No P-987/22.06.2021 of the Rector of MU-Plovdiv, based on a dissertation submitted on paper and electronically in a volume of 265 pages and 49 pages of bibliography. The theme, presentation and layout of the dissertation leave the overall impression of a complex, original conceived, conscientious, and consistently conducted study

The work is structured on a hybrid basis, tailored to the requirements for a dissertation preparation, but with an original approach in presenting own results. The main sections contain as follows: introduction – 1 p.; literary overview – 76 pp.; purpose and tasks – 2 pp.; summary - 6 pp., conclusions – 2 pp. and quoted literary sources on 49 pp. On 174 pp. each sub-purpose, the resulting tasks, the materials and methods used, the results obtained and the related discussions are figured out consistently.

Relevance of the topic

The choice of theme for dissertation work – changes in dentin and dental pulp related to age is justified and logical in view of the professional profile of the author as a dental doctor and researcher. Signaling and regulatory interactions between the non-collagen elements of dentin and odontoblasts in the dental pulp are interesting, multifactorial and unexplained in detail issues. Mineralization of intratubular dentin by secretory-independent route and phenotypic characteristics of odontoblasts in ageing are the focus of scientific pursuits of a number of teams. Apoptosis, autophagy, the involvement of lysosomes and mitochondria in the tissue remodeling of dentin and pulp with age provoke the scientific search of Prof.

Manchorova. The new methodological possibilities offered by modern cell and molecular biology and immunology tempt many researchers of different specialties. These modern techniques allow the study to explore the fine mechanisms, underlying various physiological and pathological processes and in therapeutic dental medicine.

It is in this direction that the research done by prof. Manchorova is addressed, which is large-scale and includes structural, physico-chemical and biological characteristics of the main object of research – dentin and dental pulp. The most significant advantage of the work is its interdisciplinary nature, which has allowed the author to greatly expand her knowledge in the field of fundamental science without losing the link to clinical practice. It is obvious that the driving force is scientific curiosity, which has led to the formulation of interesting hypotheses and the setting of high goals.

Knowledge of the problem

The literary review shows a thorough and diverse view of the problem. Detailed composition, structure, mechanisms for the formation and mineralization of dentin, as well as dentin types are considered. The composition, characteristics and components of the ECM are presented with possession and depth. The focus of the study are odontoblastic cells - their activity, phenotyping and age-dependant changes. Interesting new facts are described for autophagy-lysosomal system in the odontoblasts of the dental pulp at norm and pathology. Attention is paid to apoptosis as a fundamental process, incl. its impact in the dental pulp under physiological conditions and in ageing, as well as cellular protections against residual oxygen species. I believe that it would be more appropriate for the gene regulation of apoptosis to follow logically in the section dedicated to programmed cell death rather than in "Molecular cell tools for the realization of apoptosis". The author shows exceptional detailedness and awareness in the presentation of methods of ultrastructural, morphological, physico-chemical and biological examination of dentin and dental pulp. Modern and reliable technologies are included that allow electronic microscopy and immunohistochemical examination of dentin and dental pulp.

With regard to certain expressions in the review, as well as to the very title of the dissertation, I have the following recommendations: 1. The term "molecular-biological characteristic" implies the application of molecular and biological methods or the clarification of molecular and biological mechanisms. There are no such things in the work. Both methods and results are rather cellular-biological and immunological (methodical). For this reason, it would be more correct to talk only about biological characteristics. 2. Similarly, "molecular-cellular tools for the realization of apoptosis" does not sound exactly from a biological point of

view. It would be more acceptable to talk about factors and mechanisms for carrying out the process.

A very good impression makes the revealing the outstanding problems as a separate part of the overview. They systematize the data and are evidence of the author's research experience. Thus, the working hypothesis is perfectly logically formulated – intratubular dentin is formed by a secretory-independent mechanism – its genesis includes matrix-mediated, apoptosis-dependent and/or serum-guided intercellular diffusion of non-collagen components of ECM.

The purpose sounds clear and fully corresponds to the contents of the work. Breaking it down into sub-targets to some extent fragments the overall concept.

The **13 tasks** assigned are formulated specifically and as a level of complexity and volume correspond to those for the scientific degree "Doctor of Sciences".

Materials and methods of the study

They are presented to the relevant sub-purpose and not in a separate section. The units, time and characteristics of observation are correctly noted. It is a serious achievement of Prof. Manchorova obtaining the balance between different research methods and the assigned tasks. The diversity and interdisciplinary origin of the techniques used are an indication not only of well-conceived work, but also of exceptional energy and organization in the implementation of all analyses. They require precise coordination of specialists from different professional fields in the personal participation of the author.

The selected physico-chemical, ultrastructural, immunological and cellular-biological techniques allow for an adequate response to the tasks placed in the dissertation work. The statistical analyses were selected in accordance with the types of studies and the dependencies sought

Characteristics and evaluation of the dissertation work and contributions

The original scientific hypothesis of cellular-non-secretory formation of transparent dentin postulates studying of two mechanisms for dentinogenesis without the function of secretory odontoblasts: serum-mediated and apoptosis-related dentinogenesis. They were examined using rich methodical toolkits, analyzed 12 biologically active molecules in ageing and molecular mapping of the senescent odontoblasts with a molecular phenotype described was done. The results are illustrated with high quality microphotographs and original graphics. Data are presented to support the serum-mediated mechanism for the formation and mineralization of dentin without the participation of odontoblastic cells and a cellular independent route for dentinogenesis.

The significant own results are illustratively summarized in table 40, which systematizes the manifestations of ageing at tissue, cellular, organelle and molecular level.

I accept the **conclusions** of the dissertation work as contents and as a personal work of the author. In general, they correctly reflect the results of the dissertation. They sound specific and accurate.

The content of the **author's abstract of dissertation** corresponds to that of the dissertation itself. It is prepared according to the requirements for such work. The author's abstract of dissertation reflects the main results achieved in the dissertation.

Assessment of the publications and personal contribution of the author

Prof. Manchorova has submitted 8 publications related to the dissertation work in accordance with the requirements of the Regulations of MU-Plovdiv. On two of them, she is the first author. Four were printed in Int J of Science and Research. Two publications were published in journals with impact factor Bio-Medical Materials and Engineering with IF=0.7 and in Comptes Rendu of BAS with IF=0.284.

In the presence of a truly original scientific hypothesis and strong evidence supporting it, I expect much more serious publications in the international scientific journals, where it is desirable to see the Bulgarian contribution to the study of dentin. I believe that on the basis of the results in the dissertation, at least 4 more articles can be published, which will undoubtedly justify the long-standing and purposeful interest and work of Prof. Manchorova.

The citations also formally cover as a number the requirements of the Regulations of MU-Plovdiv, but most cite works directly not related to the current dissertation.

My personal impressions of Prof. Dr. N. Manchorova are related to her scientific, teaching and administrative activities, in which she has shown creativity and initiativeness. Impressive is the scientific curiosity, the search for new research niches and the enthusiasm in which she works and guides her young colleagues.

With regard to this study, I have no doubt that it is her personal work as a design and realization.

CONCLUSION

The dissertation work of Prof. Dr. Neshka Manchorova-Veleva, PhD contains scientific and applied results that represent an original contribution to science and meet the requirements of the Law on development of academic staff in the Republic of Bulgaria for the acquisition of the scientific degree "Doctor of Sciences" in the scientific specialty "Therapeutic Dental Medicine".

Due to the above, I give my positive assessment of the conducted study and propose to the members of the scientific jury to award the scientific degree "Doctor of Sciences" in therapeutic dental medicine, in the professional direction "Dental medicine" to Dr. Neshka Manchorova-Veleva.

30.08.2021

Reviewer:

Prof. Dr. Victoria Sarafian, DSc