

## STATEMENT

By Assoc. Prof. Georgi Tomchev Tomov, PhD

Medical University of Plovdiv

Faculty of dental medicine, Department of periodontology and oral mucosa diseases

**on a thesis for the acquisition of the scientific degree "Doctor of Sciences"**  
in Medical University - Plovdiv, Faculty of dental medicine, Department of operative dentistry  
and endodontics

**Higher education field:** 7. Healthcare and sports

**Professional field:** 7.2. Dentistry

**Scientific specialty:** Therapeutic dental medicine

**Author:** Professor Neshka Atanasova Manchorova-Veleva, PhD

**Topic:** Age-dependent changes in the dentin and the dental pulp – structural, physico-chemical and molecular-biological characteristic.

### 1. General presentation of the procedure and the candidate

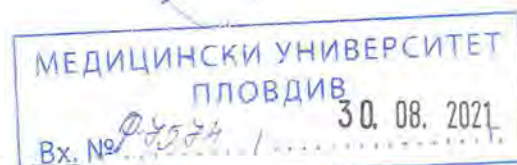
The set of materials presented to me on paper and electronic media is in accordance with Article 123 (1) of the Procedure for the Acquisition of the Scientific degree "Doctor of Science" in MU - Plovdiv, The Rules and Regulations of MU-Plovdiv from 06.11.2014 and includes all required documents.

Copies of 8 articles are presented on the topic of the dissertation published in international and Bulgarian peer-reviewed scientific journals. There are 6 scientific reports, of which 5 participations in scientific forums abroad and 1 participation in scientific forums in our country.

The scientific indicators of Prof. Manchorova are in accordance with the national requirements by groups of indicators for the academic degree "Doctor of Sciences" in Professional field 7.1. Medicine (under Art. 2b. para. 2 and 3 of Law for the development of the academic staff in the Republic of Bulgaria - promulgated SG, amended and supplemented, issue 30 of 03.04.2018, amended. SG No. 17 of 26 February 2019 and the Regulations for the application of Law for the development of the academic staff in the Republic of Bulgaria - amended and supplemented, SG No. 56 of 6 July 2018. Amended and supplemented, SG No. 15 of 19 February 2019)

### 2. Topicality of the thesis

Despite the intensive research in the recent years, many aspects of aging of the dentin and dental pulp remain unclear and debatable. Moreover, the accumulation of new evidence changes our understanding for pulpo-dentin homeostasis in the context of age. More and more



attention is paid to non-collagen complexes of dentin (more than 800 molecules), which show a signaling and regulatory role to odontoblasts, as well as the mechanisms for formation and mineralization of intratubular dentin. In this sense, the tasks set by Prof. Manchorova in this paper are both relevant and open a wide prospective for future research in the field of dental biology.

In search of answers for these questions and after a comprehensive examination of the world achievements of the molecular biology and proteomics in the field of dentin and pulp biology, Prof. Neshka Manchorova builds up a scientific hypothesis for cell-non-secretory formation of transparent dentin by two mechanisms - serum-mediated and apoptosis-related. The design, methodology and results in the present doctoral thesis present arguments in support of the formulated scientific hypothesis.

The original results of the dissertation are published in Bulgarian and international scientific journals.

### **3. Knowledge and understanding of the problem**

The candidate demonstrates good knowledge on the problem. The literature review includes the impressive 881 sources, of which 9 are in Cyrillic and 872 in Latin. It presents current data on dentin and pulp biology, as well as data on age-dependent changes at the tissue, cell, organ and molecular levels.

The review reveals that there is a lack of consensus in the scientific community about the mechanisms and manifestations of dentin and dental pulp aging. Moreover, although a remarkable number of bioactive molecules, mediators, modulators have been found, signaling pathways and regulatory mechanisms have been identified, however much of the function of biomarkers remains unknown or incompletely studied.

### **4. Aim and method of research**

The aim and tasks of the thesis are expertly set.

The dissertation aims to establish the morphological, ultrastructural, physicochemical and molecular changes in the dentin and odontoblasts of the dental pulp during physiological aging, as well as to study the relationship between age and pulpo-dentin homeostasis.

For the implementation of the set goal Prof. Manchorova formulates 5 sub-goals with different number of tasks to them. The selected design of the study is presented in detail, the



author groups the research methods into **4 large groups** - **microscopic techniques** (light microscopy with polarization microscope, SEM, light microscopy of paraffin sections), **crystallographic** (powder X-ray diffractometry, differential thermal mass analysis with gas - spectrometry, infrared spectrometry), **Nano-mechanical studies** (nanoindentation and atomic force microscopy) and **molecular biological methods** (immunohistochemistry of paraffin sections of dentin and dental pulp), which allow successful performance of specific tasks and ensure the achievement of the main goal.

The object of the performed analyzes are natural human teeth, extracted according to different indications, grouped in three age groups in response to the sub-goals of the research.

Various and modern laboratory and immunological methods have been used, which contribute to the solution of the research tasks.

The results are statistically processed with appropriate methods of analysis and are illustrated with a sufficient number of figures and tables.

## **5. Characteristics and evaluation of the dissertation**

The dissertation is written on 321 pages and is formed according to the generally accepted academic form. It is illustrated with 40 tables, 30 diagrams and 177 figures. The literature includes 881 sources, 9 of which are in Cyrillic.

The results of each task of the study are presented in a comprehensive volume and are discussed competently, with an in-depth analysis and specific conclusions.

The analysis of the results confirms that with the aging of the dentin there are changes in the mineral phase, collagen, bound and free water. These changes are characterized by tubular reorganization and sclerosis of the dentinal tubules, reduction of the amount of hydroxylapatite in the dentin, qualitative changes in its composition (non-apatite impurities), new mineral phases with fine crystalline characteristics, hard and inelastic dentin with altered fracture mechanics. According to the author, the described intratubular formation of transparent dentin in tissue aging cannot be explained by the classical concept of cell-secretory dentinogenesis. This gives the author reason to study two new mechanisms - serum-mediated and apoptosis-related, which are the basis of the formulated scientific hypothesis. The results of the performed immunohistochemically studies show that with the aging of the dental pulp its vascularization is not reduced, but the so-called adaptive angiogenesis. The interpretation of the variable

immunoreactivity of the coronal and root pulp and dentin in the three age groups compared to DMP-1, OPN (dentin) and BID-CASP8, JAK1-SAT3, NFkB, MAP LCII3 and COX2 (pulp) showed that with the aging of the dentin and the pulp include fundamental biological processes that form the so-called. "Molecular map of old age", which includes expression of non-collagenous proteins of the SIBLINGs family (DMP-1 and OPN), impaired mitophagia, active autophagy, active cytoprotection, several alternative pathways for apoptosis and sex determinism for OPN and JAK1-SAT3. This important conclusion is based on correlation analysis, multidimensional scaling and cluster analysis of the obtained results.

The obtained results are appropriately illustrated, which makes their perception easy and convincing.

The summary and argumentation of the scientific hypothesis, as well as the conclusions of the dissertation are well formulated and reflect the essence of the obtained results.

Prof. Machorova presented 18 final conclusions, which are derived from the specific results of the study. They substantiate the scientific hypothesis formulated by the author and help for a more detailed understanding of the biology and homeostasis of the pulpo-dentin complex in the different phases of the aging process. Last but not least, guidelines for future research in the field of dental medical molecular biology are provided, with targeted biomarkers and as yet unclear hypotheses.

The dissertation is composed precisely and I have no significant remarks on the methodology, presentation of the results and their analysis. However, the presented work would gain more if the interpretation of the results obtained for the group "old pulp and dentin" takes into account the fact that these samples originate from teeth extracted by periodontal indications (such are II to III degree of mobility and deep periodontal pockets with the presence of biofilm!!!), which contributes to changes in the pulpo-dentin complex. This may provide a logical explanation for the fact that the dentin of these otherwise "intact" teeth is statistically more immunopositive for OPN.

#### **6. Assessment of the candidate's contributions, publications and personal contribution**

I accept the formulated main contributions and believe that they objectively reflect the real results of the research.

I consider the following contributions to be more significant:



- Pulp vascularization (and angiogenesis) was analyzed in view of the dynamics of serum-mediated dentinogenesis in the conditions of aging of the dental pulp.

- Some of the mechanisms, signal transduction and control of apoptosis of pulp odontoblasts with age have been studied, in view of its participation in the formation of physiological transparent intratubular dentin.

- The links between cellular aging, autophagy and apoptosis are presented.

- The manifestations of aging at the tissue, cellular, organ and molecular level and the "molecular map of old age" of the odontoblasts in the dental pulp are presented.

In connection with the dissertation Prof. Manchorova has published 8 articles (in two of them she is the first author) in international and Bulgarian peer-reviewed scientific journals, 2 of which with IF.

There are 6 scientific communications (of which she is the first author), of which 5 participations in scientific forums abroad and one participation in a scientific forum in Bulgaria.

The number and the quality of the publications related to the dissertation meet the requirements for recognition of the Scientific degree "Doctor of Sciences" at MU - Plovdiv.

## 7. **Author's summary**

The summary is written on 96 pages and is prepared according to the requirements, reflecting the dissertation research, results and conclusions in a precise manner.

## **CONCLUSION**

In conclusion, I would like to summarize that the presented thesis of Professor Neshka Atanasova Manchorova-Veleva, PhD on "Age-dependant changes in the dentin and the dental pulp – structural, physico-chemical and molecular-biological characteristic" is a scientific study on a topical issue with a well-planned and implemented design. Original results of significant scientific and applied value have been obtained.

The dissertation fully meets all the requirements of the Law for the development of the academic staff in the Republic of Bulgaria; The Regulations for the application of the same law and the respective Rules and Regulations of MU - Plovdiv.

All of the above gives me reason to give a positive assessment of the research and I

propose to the members of the esteemed scientific committee that Professor Neshka Atanasova Manchorova-Veleva, PhD be awarded the scientific degree "Doctor of Science" in the Scientific specialty "Therapeutic dental medicine ", Professional field: Dentistry.

A handwritten signature in blue ink, appearing to read 'G. Tomov', written over a horizontal dotted line.

27.08.2021

Assoc. Prof. Georgi T. Tomov, PhD