Review

On a competition for the academic position of "Associate Professor", in the field of higher education 4. Natural Sciences, Mathematics and Informatics, Professional field: 4.3 Biological Sciences (Biophysics) for the needs of the section "Electroinduced and Adhesive Properties" at the Institute of Biophysics and Biomedical Engineering, BAS, announced in the State Gazette issue 58/18. 7. 2025 with the only candidate senior assistant, doctor Kamelia Hristova-Panusheva

Reviewer: Iana Hristova Tsoneva, DSc, Professor at the Institute of Biophysics and Biomedical Engineering, BAS, Sofia (member of the scientific jury according to order No. 74917.09.2025 of the Director of IBPhBME, BAS)

Education and career development

Dr. Hristova-Panusheva holds a bachelor's and master's degree from the Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia (1999-2005). In 2011 she defended her PhD in Biophysics, Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences with the thesis "Modulated Interaction of Osteoblasts with Hydroxyapatite Materials" supervised by Prof. Dr. Georgy Altunkov as a full-time doctoral student. ONE YEAR BEFORE DOCTORAL STUDIES SHE WORKED AS A SPECIALIST, AND SINCE 2012 SHE HAS BEEN A CHIEF ASSISTANT AT THE INSTITUTE OF BIOPHYSICS AND BIOMEDICAL ENGINEERING, BAS. SHE HAS SPECIALIZED 3 TIMES ABROAD IN CONNECTION WITH HER DISSERTATION WORK – (DOCTORAL SPECIALIZATION AT THE INSTITUTE OF BIOENGINEERING OF CATALONIA, BARCELONA, SPAIN - 2 TIMES, DOCTORAL SPECIALIZATION AT THE MARTIN LUTHER UNIVERSITY, HALLE, GERMANY, FINANCED BY THE GERMAN ACADEMIC EXCHANGE SERVICE DAAD). The candidate's total work experience at the IBPhBME, Bulgarian Academy of Sciences is 16 years and 3 months.

General characteristics of the submitted materials

The total number of points on the scientometric indicators, according to the report submitted by the candidate, is **494** points (Indicator A - 50, Indicator B - 125, Indicator D - 239, Indicator E - 96) with a requirement of **430** points as the minimum requirements for occupying the academic position of "Associate Professor".

The total number of scientific publications of Senior Assistant Professor Hristova-Panusheva is 23 (included in the dissertation for "doctor" - 3, and for the academic position of "Associate Professor" a total of 20).

The habilitation work (indicator B4) includes 5 publications, in 1 of which the candidate is the first author. The articles distributed by quartiles are in the following categories: B4: Q1 - 4 and - Q2 - 1. In total, the publications from this indicator give **125** points.

Apart from the habilitation work (Indicator G-7 and G-8), 15 works are included in the competition for "Associate Professor", distributed as follows by quartiles: in G7 - Q1 - 4, Q2 - 1, Q3 - 2, Q4 - 3, SJR - 4 articles, G8-chapter of a book 1 issue. (**Indicator G** - scientific publications in publications that are indexed in world-famous databases with scientific information - Web of Science and Scopus - a total of **239** points).

The works of Dr. Hristova-Panusheva are well cited in refereed and indexed publications - a total of 100 points. In fact, the citations in Scopus are more. Dr. Panusheva has a very active participation in scientific international and domestic forums with oral and poster presentations (15 in total). The candidate in the competition is the first author of 4 and second author of 5 articles. The total impact factor of the publications **(IF)** in the competition is **37.56**. The candidate participates in both national (3) and international (3) projects.

General characteristics of the research work

The main interests of the senior assistant professor Dr. Hristova-Panusheva are concentrated on the interactions between cells and biomaterial surfaces. In recent years, nanoparticles have also been included in this topic. The main area of research is focused on photothermal anticancer therapy using the synergistic effect of a laser with continuous irradiation in the near infrared (NIR) region, as well as a femtosecond pulsed laser, in combination with nanoparticles such as graphene oxide, pegylated graphene oxide, gold or silver nanoparticles. The aim of the research in in vitro models of cancer and non-cancer cell lines is to clarify the mechanisms of toxicity of nanoparticles regarding the biocompatibility of these new materials. The scope of the research is also extended to other areas such as monitoring the impact on cell adhesion of cancer and normal cells after exposure to substances such as the anesthetic halothane and the protease inhibitor cystatin; Bone tissue engineering; Mitochondrial ATPase activity of cancer cells towards aminated graphene oxide; Evaluation of the biocompatibility of ethanol extract of Haberlea rhodopensis towards normal skin cells with a view to therapeutic application in cosmetics.

I. Contributions related to habilitation work

1. Study of the effect of PEG-ylated graphene oxide nanoparticles and gold nanoparticles in combination with laser irradiation

New anticancer therapies using nanoparticles such as two-dimensional graphene oxide (GO) and its derivatives are the subject of intensive research. The advantage of GO as a new anticancer therapy is related to indicators such as the presence of a large surface area, which is easily loaded with various substances and has its own inhibitory effect. PEGylation of GO with polyethylene glycol (PEG) can reduce its cytotoxicity.

A. Study of the synergistic effect of PEG-ylated graphene oxide in combination with constant laser irradiation in the near infrared (NIR) region at the cellular, subcellular, and organ levels

The authors found that PEGylation of GO improves the biocompatibility of GO-PEG compared to GO in HepG2 and C2C12 cells [B4.4, B4.5]; NIR irradiation enhances the overall cytotoxic effects [B4.4., B4.5]; GO induces stronger arrhythmic contractions [B4.4]; GO does not decouple intact rat liver mitochondria and has little effect on ATPase activity [B4.4]. New data on the expression of five genes were obtained, showing that NIR irradiation upregulates genes related to apoptosis and cell cycle arrest, and that nGO shows upregulation of BECN1, a gene associated with activation of autophagy in response to stress [B4.5]. **The contributions of this section are mainly fundamental in nature [B4.4 and B4.5].**

B. In vitro study of the synergistic effect of PEGylated graphene oxide and gold nanoparticles in combination with femtosecond laser irradiation on normal kidney cells and hepatocellular cancer cells

The authors found that pegylated GO has better photothermal efficiency under femtosecond pulsed (Fs) lasers compared to GO at certain wavelengths [B4.2]. It was found that the higher power density of 0.2 W/cm2 and longer irradiation increased the temperature of the culture medium [B4.2].

For the first time, the effect of gold nanoparticles after irradiation with a femtosecond laser with a short wavelength and low intensity on cancer HepG2 and normal MDCK cells was studied and a synergistic photothermal effect was demonstrated at this low laser intensity. **I believe that the contributions in this paragraph have the character of obtaining new data for science [B4.2].**

C. A literature review [B4.3] of the current state and progress of targeted anti-tumor therapies for the needs of nanotechnology and the critical role of various nanoparticles (organic and inorganic) for personalized oncology medicine has been conducted. **This contribution and the analysis of the data on the progress of targeted anti-tumor therapies could be useful in clinical practice [B4.3].**

II. Contributions outside the habilitation work

A. Study of the effects of the inhalation anesthetic Halothane on the proteins of the focal adhesion contacts of lung A 549 cells

The authors found that inhalation halothane disrupts focal adhesion contacts in human lung adenocarcinoma A 549 cells. This effect is accompanied by suppression of the activity of focal adhesion kinase (FAK) and phosphorylation of paxillin, and not by proteolytic changes or inhibition of the expression of vinculin and paxillin. **This contribution could be useful in clinical practice [G7.1].**

B. Development and biological characterization of new materials and coatings for application in tissue engineering

B1. Development and optimization of plasma-polymerized coatings and thin layers of hexamethyldisiloxane (HMDS) for control of cell adhesion, growth, function and differentiation

A suitable method for modulating the surface hydrophilicity of plasma-polymerized films of hexamethyldisiloxane (PPHMDS) by treatment with ammonium plasma has been developed. The authors found that the proteins fibronectin (FN) and the plasma protein fibrinogen (FG) from the extracellular matrix adsorbed on all the plasma-polymerized films studied [G7.2].

New data on the biocompatibility of thin films of plasma-polymerized materials obtained by two different technological modes have been obtained. It has been established that plasma amination changes the surface hydrophilicity of the layer, which improves the proliferation of osteoblast cells of the MG63 line [G7.4]. A new composite material based on PPHMDS and detonation nanodiamond (DND) was created and it was shown that adding DND to the layer improves the efficiency of cell adhesion. It was found that by changing the type of DND filler, the chemical properties of hydrophobic PPHMDS films can be changed [G7.3]. It was found that the way of incorporating DND into the layers in PPHMDS also affects the surface properties of the composite materials and the behavior of mesenchymal stem (MSC) and MG63 cells. It was found that the way of incorporating DND into the layers in PPHMDS also affects the surface properties of the composite materials such as roughness and hydrophilicity. [G.8.1]. **Contributions in papers [G7.2, G7.3, G7.4 and G.8.1] are of fundamental nature.**

B2. Studying the influence of elasticity and stiffness of composite coatings and thin layers of HMDS/PDMS and DND on cell adhesion, growth, function and differentiation of myoblast cells

For the first time, thin composite layers of polydimethylsiloxane and detonation nanodiamond (DND/PDMS) with different elastic modulus were created and new information was obtained about the behavior and differentiation of mesenchymal stem cells (MSC) on the composite layers with different concentrations of DND particles and elastic modulus close to those of native bone tissue [G7.5, G7.11]. It was found that increasing the concentration of DND particles in the PPHMDS or PDMS matrix gives a clear increase in the elastic modulus of both types of composite layers and leads to improved osteogenic differentiation of MSCs [G7.5, G7.6]. It has been established that materials with an elastic modulus (2.5–4 MPa) higher than that of physiological skeletal muscle tissue do not disrupt the myogenic differentiation of both cell types, only temporarily restricting their proliferation [G7.11]. **The contributions of the papers are of fundamental and applied nature in medicine [G7.5, G7.6 and G7.11].**

C. Study of the biocompatibility of the two-layer TiN/TiO2 coatings deposited by DC magnetron sputtering on stainless steel

The biocompatibility of multilayer TiN/TiO2 coatings deposited on stainless steel by magnetron sputtering was confirmed in MG63 osteoblast cells. It was found that the TiN/TiO2 coatings did not suppress cell spreading and did not provoke a decrease in the proliferative capacity of MG63 cells. These data indicate that the substrates coated with TiN/TiO2 can be used for surface reinforcement of bone and dental implants. **The contribution has fundamental and applied potential in medicine [G7.9].**

G. Study of the biological effect of aminated graphene oxide on cancer cells and on the ATPase activity of rat liver mitochondria.

It has been shown that modification with ammonia changes the zeta potential from negative to positive. Higher and cell-specific toxicity of ammonia-aminated GO particles (GO-NH2) compared to pure GO was found in in vitro studies of different cell lines (A549, Colon26 and Lep3), due to the induction of greater ROS production and blocking of the cell

cycle in the G0-G1 phases. ROS production was also higher in mitochondria treated with GO-NH2. **The contributions are of a fundamental nature [G7.7, G7.8, G7.9 and G7.13].**

D. Study of the safety of a total ethanol extract of Haberlea rhodopensis in vitro culture (HRT) for therapeutic application in cosmetics

It was found that the interaction with the HRT extract at different concentrations did not cause the release of free radicals or genotoxicity for a long time after treatment. It was shown that the extract positively affects mitochondrial activity. An increase in mitochondrial membrane potential was observed, suggesting improved mitochondrial morphology resembling mitochondrial fission, which is a putative evidence of cellular rejuvenation. **The contribution is related to improved human health [G7.14].**

E. Study of the effect of a new plant cystatin on the adhesive behavior of normal and cancer cells

According to literature data, protease inhibitors such as cystatins can prevent the invasion and metastasis of cancer cells. It was found that the plant cystatin dgECP1, containing RGD instead of the HGD sequence characteristic of mammals, has a dual function: when pre-adsorbed on the substrate, it has adhesive properties and induces aggregation, mimicking the normal behavior of cancer cells. However, when added to the culture medium, the adhesion of cancer cells is suppressed. Its effect on normal cells is minimal. It has been suggested that both the mutant forms dgECP1 and dgECP1m1 are promising candidates for the modulation of cell adhesion and inhibition of cancer metastasis. **The contribution is of a fundamental nature [G7.12].**

I accept the contributions made and believe that they are the personal work of the candidate. They are of importance to science, practice, and reflect the candidate's work in the competition.

Conclusion:

After a comprehensive analysis of the candidate's scientific output, academic and professional activities: publications and citations, reports at international forums, participation in scientific teams and projects, I believe that Dr. Hristova-Panusheva meets all the requirements for the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for the Implementation of ADASRB of BAS and the specific

Regulations of IBPhBME, BAS for conditions and procedure of holding the academic position "Associate Professor".

The submitted materials for the competition, the indicated contributions and my personal impressions regarding the use of quite up-to-date experimental methodologies in the research activities of Dr. Kamelia Hristova-Panusheva give me grounds for a positive assessment of the candidate and to confidently recommend to the Scientific Jury to prepare a proposal to the Scientific Council of the IBPhBME the Bulgarian Academy of Sciences for her election to the academic position of "Associate Professor", professional field: 4.3 "Biological Sciences".

22. 10. 2025

Signature:

Prof. Iana Tsoneva, DSc