Review

By Prof. Dr. Biliana Pancheva Nikolova-Lefterova Member of the Scientific Jury (with order 98/21.02.2022 by the Director of IBFBMI-BAS)

Under a competition for the academic position of PROFESSOR in the field of higher education 4. "Natural Sciences, Mathematics and Informatics", professional field 4.3. "Biological Sciences", scientific specialty "Biophysics", with experience in modeling the interactions of biomaterials with cells, for the needs of the section "Electroinduced and adhesive properties" at the Institute, published in the "State Gazette", issue. 109/21.12.2021

With the only candidate who submitted documents for participation: Assoc. Prof. Dr. Natalia Alexandrova Krasteva.

From the reference made to the materials presented in the competition it is clear that the candidate covers and in some respects significantly exceeds both the minimum national requirements and the specific criteria of IBFBMI-BAS.

Group of indicators	Scientometric data of the candidate	Specific criteria of IBFBMI - BAS
Group A	50	50
Group B	-	-
Group C	135	100
Group D	259	220
Group E	486	120
Group F	440.82	150

Assoc. Prof. Natalia Krasteva graduated with a master's degree from the Faculty of Biology at Sofia University in 1994, with specialty in cell biology and embryology. Acquired the scientific and educational degree "Doctor" in the scientific specialty "Biophysics" in 2003, based on a dissertation on the topic: "Interaction of hepatocytes with synthetic membrane perspectives for the creation of an artificial liver", and in 2010 holds the academic position of "Associate Professor" in Institute of Biophysics.

The total number of scientific publications of Assoc. Prof. Natalia Krasteva is 40. Four of them are for obtaining the scientific-educational degree DOCTOR, 10 (+16 represented in NACID) are presented in a competition for the academic position of ASSISTANT PROFESOR and in this competition are presented 20 publications summarized as habilitation work (7) and 13 outside habilitation work.

In group of indicators B are presented 7 scientific publications distributed by quartiles as follows: Q1 4 articles, Q3 1, Q4 2 articles. In total, the publications bring 135 points out of

the required 100. The publications have been published for the period 2018-2021. The works are in the field of nanotechnologies, which are widely used in recent years.

In group of indicators C (scientific publications in publications that are referenced and indexed in world-famous databases with scientific information) are presented 13 scientific publications that carry 259 points instead of the required 220, 3 of them are published in journals with rank Q1, 4 in journals Q2, 3 in Q3 and 2 in Q4. Ten of the works have been published in journals with IF.

In group D (citations in scientific journals, monographs, collective volumes and patents) with a minimum national requirement of 120 points (60 citations of two points each) is presented a list of 32 scientific publications with a total of 243 citations (486 points), without auto-citations.

In group E the candidate presented a list of activities, which shows that the parameters included in this section are 440.82 points, with the required 150. Associate Professor Natalia Krasteva was: the head of a successfully defended PhD student - Milena Keremidarska (2016); she has participated in 7 national and 4 international research projects; led 3 national and three international projects funded by the NSF (the attracted funds are BGN 153,800).

The research interests of Assoc. Prof. N. Krasteva are mainly in research areas:

Cell-biomaterial interaction, cell adhesion, protein adsorption Reorganization of the extracellular matrix and expression of integrins in cancer cells and on the surface of biomaterials, development of collagen microcarriers for hepatocytes, tissue engineering, controlled engineering, controlled control/skeletons, Tissue compatibility of synthetic membranes, nanostructured biomemimetic surfaces for osteoblasts; nanoparticles as drug carriers, Cytotoxicity, Chemotoxicity, Genotoxicity, Mitotoxicity, Aging.

Assoc. Prof. Natalia Krasteva has participated in 43 international and national scientific forums. She was the research supervisor of four successfully defended the graduate and one doctoral student. She has participated in the practical training of students in the program "Student Internships". Her scientific-expert work consists of: participation in scientific juries and commissions, preparation of reviews for prestigious scientific journals, evaluator of project proposals to the Research Fund and guest editor of a special edition of Oxidative Medicine and Cellular Longevity, IF-6.543.

The presented scientific contributions of Assoc. Prof. Natalia Krasteva are grouped as follows:

I. Contributions from habilitation work (indicators group B)

II. Contributions of scientific papers by indicator Group D (scientific publications in publications that are referenced and indexed in world-famous databases).

Nano- and biomaterial surfaces, as well as their interaction with cells are the basis of the research of the scientific papers presented in the competition. Particular attention is paid to

graphene oxide nanoparticles and their possible use as a means of combating cancer. The toxic effects of nanoparticles have also been studied, gradually moving from in vitro studies to in vivo models of nematodes of the species *Caenorhabditis elegans*.

I. Contributions from habilitation work (indicators group B)

A series of in vitro studies have examined the effect of graphene oxide nanoparticle modifications on cancer cells.

The contributions from the habilitation work are divided into three groups:

A. Investigation of the biological effect of aminated graphene oxide on cancer cells.

B. Investigation of the biological effect of PEGylated graphene oxide on cancer cells.

C. Investigation of the synergistic effect of PEGylated graphene oxide in combination with NIR irradiation on colorectal cancer cells.

A quick and easy protocol for graphene oxide hydroxyliamine amination has been developed. A decrease in the size and zeta potential of the particles was found, but at the expense of this increase in their surface due to wrinkling. Higher and cell-specific toxicity of ammonia-aminated particles of GO was found compared to pure GO in an in vitro study with a set of cell lines (A549, Colon26 and Lep3). Concentration-dependent cyto- and mitotoxic effects of GO nanoparticles have been demonstrated, and no genotoxic effect of hydroxylamine-amplified graphene oxide (haGO-NH2) particles on hepatocellular cells of the HepG2 line has been demonstrated (papers B1, B2, B3, B4).

In order to reduce the toxicity of GO nanoparticles, they were treated with polyethylene glycol (PEG), studies based on literature data.

The method for pegylation of nanoparticles has been modified. It has been proven that PEGylation increases the size of the studied nanoparticles from 256.7 nm (pure GO) to 324.6 nm (GO-PEG), the zeta potential from -32.9 to -21.6 mV, wrinkles the surface of the nanosheets, increases absorption in the NIR region, improves the stability of nanoparticles in aqueous solution and their homogeneous distribution in cell culture medium with 10% fetal calf serum (FBS). Increased biocompatibility of GO after PEGylation was found in melanoma cells (A375) and in colorectal cancer cells with different invasive potential (Colon26 and HT29). PEGylation has been shown to have a cytostatic effect and enhance the DNA-damaging activity of GO against Colon26 and HT29 cells, depending on the duration of treatment and cell type, but improves the mitochondrial activity of cells (papers B5, B6 and B7).

The high photothermal reactivity of GO in combination with its improved physicochemical properties and cytotoxicity after PEGylation makes PEGylated graphene oxide a promising agent in synergistic photothermal cancer therapies.

Inhibition of the migratory capacity of cancer cells was found after combined treatment with PEGylated GO, activated by 5 minutes of irradiation with a laser with a power of 1.5 W / cm2.

The presented contributions have an original character and are essentially a personal work, as in all works she is the leading or last author.

II. Contributions of scientific papers by indicator Group D (scientific publications in publications that are referenced and indexed in world-famous databases).

Contributions outside of habilitation work are grouped into four sections.

A. Investigation of the chemocompatibility of graphene oxide and PEGylated graphene oxide nanoparticles. Due to the nature of the method of application of nanoparticles (GO and GO-PEG) as drug carriers, namely their intravenous introduction into the body, the assessment of their chemocompatibility is of great importance. The chemocompatibility of pure and GO-PEGylated nanoparticles at concentrations up to 50 μg/mL has been demonstrated. Concentrations above 100 μg/mL induce dose-dependent hemolysis in human blood, changes in red blood cell morphology, and platelet aggregation (G1).

B. In vivo study of the toxicity mechanisms of pure and PEGylated nanoparticles of graphene oxide (GO) and polystyrene nanoparticles in nematodes of *C. elegans*. An appropriate choice of model in vivo system - nematodes of the species *C. elegans* was made, explaining the advantages of this object. A strong negative effect of GO with concentrations above 50 mg/L on the movement of nematodes was found, which has been shown to be the result of suppression of the neuroligin nlg-1 after mutation (work G2). It was found that the genes act-5, erm-1, pkc-3 and hmp-2 are involved in the distribution and translocation of GO, and that pkc-3 is not involved in the storage of fat in nematodes. Two intestinal signaling cascades have been identified: PKC3-SEC8-WTS1 and PKC3-ISP1/SOD-3, which contribute to the effect and localization of GO nanoparticles (G3 work). CNC-2, NLP-29, and COL-19 have been shown to act as targets for BLI-1, but a knockdown of the genes that encode them does not induce ROS after treatment with GO-PEG. HBL-1 and LIN-41 (hunchback-like protein and protein lin-41) are direct targets for let-7 in controlling the time of passage of nematodes through the various stages of their development (papers G2, G3). Treatment of wild-type nematodes with GO-PEG has been shown not to affect the expression of mlt-7 (cuticle replacement heme peroxidase), while treatment of nematodes with mutations in the mlt-7 gene results in increased toxicity and translocation of nanoparticles (work G4). RNAi knockdown of epidermal bli-1 has been shown to increase let-7 microRNA expression, and the mutation in let-7 suppresses the sensitivity of bli-1 in nematodes to GO-PEG toxicity, i. impaired regulation in the let-7-mediated molecular cascade was observed to control the developmental time of GO-PEG in nematodes with epidermal barrier deficiency caused by the bli-1 mutation (work G5). The signaling cascade in the insulin signaling pathway in response to nanopolystyrene particles has been identified: DAF-2-AGE-1-AKT-1-DAF-16-SOD-3 / MTL-1/GPD-2. It has been found to act in the intestinal cells of worms to regulate the toxicity of nanopolystyrene particles (G6).

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

Composite coatings with different modulus of elasticity have been developed to control cell adhesion, growth and function and differentiation. A model system has been developed to study the effect of substrate elasticity on osteogenic differentiation of mesenchymal stem cells (MSCs) by depositing thin composite layers of polydimethylsiloxane and detonation nanodiamond, and it has been found that increasing from adipose tissue (G7, G13). New data have been obtained on the influence of substrate elasticity on myogenic differentiation of myoblast cells from the C2C12 cell line and murine primary myoblasts isolated from satellite cells (work G8). TiN / TiO2 coatings were found not to inhibit cell proliferation and did not affect the proliferative capacity of MG63 cells during 72-hour incubation. These data, along with the improved mechanical and antibacterial properties of TiN / TiO2 coated substrates, indicate that they can be used for surface strengthening of bone and dental implants (work G9). For the first time, a new approach based on the sol-gel technique was demonstrated for the functionalization of multi-walled carbon nanotubes (MWCNT) with the amino acids L-arginine, L (+) Arg and L-asparagine, L (+) Asp (Paper 10). Hybrid nanofibers of fibrinogen and polylactic acid (FBG / PLA) with different configuration (random and ordered) and dimension (2-D and 3-D) were created by the electrofibration method. The analysis of the results shows that the role of the arrangement of the fibers in the skeletons is an important factor for the differentiation of stem cells, which must always be taken into account (G11).

D. Study of changes in the organization of chromatin in connection with premature aging. It was found that altered chromatin structure during the chronological life of yeast cells with mutation in ARP4 (actin-like protein 4) and without the HHO1 gene for linker histone leads to strong changes in the gene expression profiles of a subset of genes involved in DNA repair and autophagy (RAD9, CDC28, ATG18) (G12).

The scientific contributions presented by the candidate correctly reflect the published results. I accept the contributions formulated in this way. Many of them are original in nature, representing new facts about science.

I have no critical remarks or recommendations to the candidate.

After a comprehensive analysis of the candidate's scientific output, her academic and administrative activities (publications and citations, reports of scientific forums, participation and leadership of scientific teams, teaching experience, specializations, participation in scientific juries and reviews of renowned scientific journals) my opinion is that the scientometric indicators presented above, the derived scientific contributions, as well as the overall work of Assoc. Prof. Natalia Krasteva fully cover, and in some indicators exceed the requirements for acquiring the academic position "Professor" set in the Law on Academic Development and the regulations for its implementation in IBFBMI, BAS.

My personal impressions of the candidate's work, as well as the duly presented documents on the competition give me reason to strongly recommend the scientific jury

to prepare a proposal to the Scientific Council of IBFBMI-BAS for the selection of Assoc. Prof. Natalia Alexandrova Krasteva for the academic position "PROFESSOR", professional direction 4.3 biological sciences, in the scientific specialty "biophysics".

21.04.2022

/prof. Biliana Nikolova-Lefterova/