OPINION

by Prof. Anelia G. Dobrikova, PhD
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Member of the Scientific Jury according to Order No. 98/21.02.2022
of the Director of IBPhBME-BAS

Regarding the competition for the academic position "Professor" in the professional field 4.3. "Biological Sciences", scientific specialty "Biophysics", announced in the State Gazette issue No.109/21.12.2021, for the needs of the Department "Electroinduced and Adhesive Properties" at the Institute of Biophysics and Biomedical Engineering – BAS

For participation in the announced competition for the academic position "Professor", only one candidate has submitted documents: **Assoc. Prof. Natalia Alexandrova Krasteva, PhD**. The documents presented by the applicant for participation in the competition are in full accordance with the requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADASRB) and the Regulations for its implementation at IBPhBME-BAS.

Short biography of the applicant

Natalia Krasteva received her master's degree in Cell Biology and Embryology from the Biology Faculty at the Sofia University "St. Kliment Ohridski" in 1994. In 2003, she defended her doctoral dissertation on theme: "Interaction of hepatocytes with synthetic membranes – perspectives for creating biohybrid liver", for the educational and scientific degree "doctor" (PhD) in the scientific specialty "Biophysics" at the Institute of Biophysics, now IBPhBME-BAS. From 2004 to 2010, Dr. Natalia Krasteva was a research associate at the same institute; she also worked at the Bank for Cell and Tissue Cultures "Cytonet", Sofia (2004-2005). In 2005-2006, she was a postdoctoral fellow for one year at the Institute für Polymer-Forschung, Teltow, Germany (the Helmholtz-DAAD fellowship). In 2010, she won a competition for the academic position of "Associate Professor" in the Department "Electroinduced and Adhesive Properties" at IBPhBME-BAS. Between 2015 and 2019, she served as head of the Department. Dr. Natalia Krasteva has been a visiting scientist at a number of universities and institutes like: Hannover Medical University, Germany; Institute of Pharmaceutical Technology and Biopharmacy, the Martin Luther University, Halle, Germany; Institute for Bioengineering of Catalunya (IBEC), Barcelona, Spain; Medical University, Innsbruck, Tirol, Austria; University of Warsaw, Faculty of Biology, Department of Cytology, Poland; University of Ljubljana, Faculty of Electrical Engineering, Slovenia, etc.

Research activities of the applicant

Assoc. Prof. Natalia Krasteva has a total of 49 scientific publications, including 45 referred in Scopus (36 of them are in impact factor journals) and 3 book chapters (Elsevier, Springer and IntechOpen). According to Scopus (April 2022), they are cited more than 410 times with *h*-index: 13 (without self-citations). A list of 43 participations in scientific forums is also presented.

In the announced competition, she participates with a total of 20 scientific publications in the period 2015-2021, 15 of which are in peer-reviewed journals with IF (total IF: 63,98), 4 with SJR and 1 book chapter (Elsevier); 11 of them are with rank Q1, 1 with Q2, 2 with Q3 and 4 with Q4 (according to Scopus, www.scimagojr.com). Assoc. Prof. Natalia Krasteva is a leading author in 12 scientific publications. A reference list with a total of 243 citations after

2011 (database Scopus) is presented for the competition. All this suggests for the high quality of scientific research and publications.

All publications presented for the competition reflect significant interdisciplinary research in the following areas: biochemistry, molecular biology, biophysics, tissue engineering, nanomaterials, pharmacology and medicine, which fully correspond to the needs and topics developed in the Department "Electroinduced and Adhesive Properties", which announced the competition. They also show that Dr. Natalia Krasteva uses a large set of methods for physico-chemical and biological characterization of bio- and nanomaterials, including the study of their cytotoxicity, chemotoxicity, genotoxicity, mitotoxicity, as well as to study the mechanisms of cellular interactions with bio- and nanomaterials *in vitro* and *in vivo*. The research skills and infrastructure of a number of national research groups and international partner organizations have been used effectively, which defines the candidate as an enterprising and communicative scientist.

According to the submitted documents, Dr. Natalia Krasteva has participated in a total of 7 national (NSF) and 4 international research projects, and she has been a leader of 3 projects with NSF and 3 international projects with Germany, China and Egypt. Assoc. Prof. Natalia Krasteva has been a research supervisor of a successfully defended PhD student (Milena Keremidarska, 2016) and is currently a research supervisor of one part-time PhD student (Trayana Kamenska). She has also been a supervisor of three and a consultant of one diploma theses of students from UCTM and Sofia University.

These scientific activities fully meet the requirements of the ADASRB and the regulations for its implementation at IBPhBME-BAS for the academic position "Professo"", and significantly exceed the minimum requirements for all groups of indicators as follows: C – 135, D – 259, E – 486 and F – 440,8.

Scientific topics and contributions of the publications:

The main scientific topics in the publications of Assoc. Prof. Natalia Krasteva, presented for the competition, are in the current and promising scientific field related to the study of interactions of normal and cancer cell lines with nanoparticles and biomaterial surfaces. A parallel with their biocompatibility has been sought and efforts have been made to improve it, which has been the subject of increasing research interest in recent years and is fully consistent with the topic of the announced competition.

The scientific results and original contributions of the presented publications are described very well and in detail in the Extended Habilitation Report. They are of great interest for both the fundamental science and the practical application in the development of therapeutic approaches for various diseases.

The most significant scientific contributions from the publications in the Habilitation work (No. C1-C7) can be related to the research of graphene oxide (GO) nanoparticles and its modifications, which not only function as drug carriers, but also have a potential to exercise their own inhibitory effect on tumour cells and may also serve as photosensitizers. All these studies are important for the development of new anti-cancer therapies. The main contributions can be summarized in two groups:

1) The cytotoxicity of aminated GO (in two different ways) has been studied on a set of normal and cancer cell lines (No. C1-C4). The aminated GO particles were found to have the highest toxicity for colon cancer cells (Colon26), while embryonic cells (Lep3) and lung cancer cells (A549) were less sensitive to aminated GO, but more serious DNA damage was observed (No. C1-C3). Concentration-dependent cyto- and mitotoxic effects, as well as a lack of genotoxic effect of hydroxylamine-aminated GO particles (haGO-NH2) on human hepatocellular cancer cells of the HepG2 line were also found (No. C4). It has been shown

that the amination of GO with hydroxylamine reduces the size and negative zeta potential of the particles, thus providing easier penetration through the cell membrane of liver cancer cells, and their increased surface area favors loading of drugs and other biologically active molecules to facilitate targeting to cancer cells.

2) It has been shown that the modification of GO with polyethylene glycol (PEG) increases the size of nanoparticles, reduces their negative zeta potential, increases absorption in the NIR (near infrared) region and improves their stability in aqueous solution (No. C5-C7). The inhibitory effects of PEGylated GO nanoparticles on A375 melanoma cells, normal kidney MDCK cells (No. C6) and colorectal carcinoma cells (No. C5, C7) have been investigated. For the first time, the synergistic effect of the PEGylation of GO in combination with NIR laser irradiation on low and highly invasive colorectal cancer cells (HT29 and Colon26) has been studied (No. C5, C7).

The scientific contributions of the other publications (No. D1-D13) can be summarized as follows:

1) The haemocompatibility of the newly synthesized nanoparticles of pure and PEGylated GO has been established for their future use as drug carriers and their intravenous introduction into the body (No. D1). The increased haemocompatibility of PEGylated nanoparticles opens new possibilities for their safe application in various medical practices. These findings provide important information on the mechanisms by which PEGylation increases the compatibility of GO with human blood cells. The data obtained are crucial for the molecular design and biomedical application of PEGylated GO nanomaterials in the future.

2) The mechanisms of toxicity of pure and PEGylated GO nanoparticles, and polystyrene nanoparticles in nematodes of *C. elegans* have been studied *in vivo.* A strong negative effect of GO with concentrations above 50 mg/L on the movement of nematodes was found (No. D2). Four genes, required for the function of intestinal barrier against the toxicity of GO, have been identified (No. D3). The effect of deficiencies in the epidermal barrier on the translocation and toxicity of PEGylated GO in *C. elegans* has been also studied (No. D4). The data provide the molecular basis for the role of the epidermal barrier against toxicity and translocation of nanomaterials in organisms. The profile of microRNA molecules that have altered expression induced by PEG-GO exposure by knockdown of intestinal-specific genes has been identified (No. D5). The signalling cascade in the insulin signalling pathway in response to polystyrene nanoparticles has been identified and found to act in the intestinal cells to regulate the toxicity of nanopolystyrene particles (No. D6). Data from these studies are important for elucidating the molecular mechanisms of toxicity of various nanomaterials in living organisms, in particular in response to GO and nanopolystyrene particles.

3) Development and biological characterization of new materials with potential application in bone tissue engineering. Composite coatings based on detonation nanodiamond particles and polydimethylsiloxane with different modulus of elasticity have been developed to control cell adhesion, cell function and differentiation (No. D7, D8, D13). The biocompatibility of multilayer TiN/TiO_2 coatings deposited by DC magnetron sputtering on stainless steel with osteoblast cells has been also studied (No. D9). Along with the improved mechanical and antibacterial properties of TiN/TiO_2 coated substrates, it has been shown that they can be used for surface strengthening of bone and dental implants.

4) For the first time, a new approach based on the sol-gel technique has been demonstrated for the functionalization of multi-walled carbon nanotubes (MWCNT) with the amino acids L-Arginine and L-Aspargine. The results confirm the important role of these amino acids in the development of MWCNT-based composites for use in bone tissue engineering (No. D10). Hybrid nanofibers with different configurations and dimensionality have been developed to promote osteogenic differentiation of mesenchymal stem cells (No. D11). The role of

nanofibre arrangement in the cytoskeleton has been shown to be an important factor in stem cell differentiation. Changes in the chromatin organization in the processes of cellular aging have also been established (No. D12).

Conclusion

The materials presented in the competition and the scientometric indicators of Assoc. Prof. Natalia Krasteva meet all the requirements and even significantly exceed the minimum requirements for all groups of indicators of the ADASRB and the regulations for its implementation at IBPhBME-BAS. I confidently believe that she is an established, internationally recognized scientist, who, with her competence and scientific output, is a very suitable candidate for the academic position "Professor" in the Department "Electroinduced and Adhesive Properties" at IBPhBME-BAS.

All of the above allows me to give my positive assessment and strongly recommend to the Scientific Jury to prepare a proposal to the Scientific Council of IBPhBME-BAS for the election of **Assoc. Prof. Natalia Alexandrova Krasteva, PhD,** for the academic position "Professor" in the professional field 4.3. "Biological Sciences" and scientific specialty "Biophysics".

Date: 05.05.2022 Prof. Anelia Dobrikova, PhD