

REVIEW

Considering the competition for the academic position "Professor" in the field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological Sciences, scientific specialty "Biophysics" announced in the State gazette issue 63 dated July 30, 2021, for the needs of the Institute of biophysics and biomedical engineering, Bulgarian Academy of Sciences, department of "Photoexcitable membranes".

with candidate in the competition **Assoc. Prof. Dr. Biliana Pancheva Nikolova-Lefterova**

by **Prof. DSc Stefka Germanova Taneva**, Institute of biophysics and biomedical engineering, Bulgarian Academy of Sciences

Education and career development

Assoc. Prof. Dr. Assoc. Prof. Nikolova has a master's degree in biochemistry and microbiology, acquired in 1992 at the Faculty of Biology, Sofia University "St. Kliment Ohridski".

Her scientific career began in 1992 as a specialist at Institute of Biophysics - BAS. During the period 1994-2001 she was a doctoral student, and defended a dissertation on "Electrical transmission of DNA. Role of adsorption and mechanism of electroporation in low-frequency low-amplitude pulses". From 2001 to 2005 she was a research associate II degree, and from 2005 to 2013 a senior assistant; in 2013 she was habilitated as an associate professor at the Institute in Biophysics and Biomedical Engineering - BAS.

Presentation of the materials received for review

Assoc. Prof. Dr. Biliana Nikolova from IBFBMI - BAS, section "Electroinduced and adhesive properties" is the only candidate in the competition for professor. The materials presented by the candidate are in accordance with the Regulations for the scientific development of the academic staff of IBFBMI-BAS and the criteria for holding the academic position "Professor". All submitted publications are in the scientific field of the announced competition and were not presented in previous competitions for obtaining the educational and scientific degree "Doctor", and for the academic position "Associate Professor"

According to the submitted documents by the applicant, the total number of points on the scientometric indicators is 983 points (indicator A - 50, indicator B - 115, indicator G - 247, indicator D - 326 and indicator E - 245), while 640 points according to the minimum national requirement and according to the regulations of ZRAS - IBFBMI-BAS for occupying the academic position "Professor".

Scientific indicators

The total number of publications according to the publication list presented by Assoc. Prof. Nikolova is 47, of which 27 are in journals with impact factor and Q rank (9 with Q1, 11 with Q2, 6 with Q3 and 1 with Q4) and 5 with SJR (Q3). 5 of the publications are included in the dissertation of Assoc. Prof. Nikolova and 16 in the competition for the academic position of Associate Professor.

A list of 163 citations is presented in the reference for the minimum requirements (indicator D). According to the Scopus database, the independent citations in peer-reviewed journals and indexed editions of Assoc. Prof. Nikolova's publications are 260; h-index 8 (according to Scopus database, November 24, 2021).

Assoc. Prof. Nikolova has a very active participation in scientific forums (43), of which 28 international and 15 national.

In the competition Assoc. Prof. Nikolova participated with 25 scientific papers, 21 of which are in peer-reviewed specialized scientific journals with Q rank (Q1 - 3, Q2 - 9, Q3 - 6, Q4 - 3).

The habilitation work (Indicator B) included 6 of the publications with rank Q1 - 1, with Q2 - 4 и with Q3 - 1.

The publications outside the habilitation work (Indicator G) are 19 (2 with Q1, 5 with Q2, 4 with Q3, and 4 with Q4).

Assoc. Prof. Nikolova is the first author of 9 of the peer-reviewed publications and corresponding author of 5 of the publications, that highlights her contribution to the published works.

Assoc. Prof. Nikolova has coordinated 1 project and 1 COST-Action funded by FNI. She was scientific adviser of 3 projects, and participant in 17 projects: 9 international, of which 3 COST-Action, and 8 projects at NSF.

She has specialized in IPBS, SNRS, Toulouse, France (November 21-December 21, 2005; June 1-30, 2006; June 1-30, 2007) and in MIG, NIRS, Chiba, Japan (July-October 2014; January 17-February 17, 2018), and participated in a course in theory and practice of "Molecular Genetics of Yeast", ICGEB, Trieste, Italy, 3-20 May, 1993.

She is a member of the Union of Scientists in Bulgaria

General description / characteristics of the candidate's research activity

The scientific interests of Assoc. Prof. Biliiana Nikolova, reflected in the presented scientific papers, are directed in the following research areas: (1) application of electrochemotherapy, a method based on the formation of temporary pores in the cell membrane when applying electrical impulses (electroporation), for treatment of patients with clinical and cytological diagnosis of skin tumors; (2) development of effective therapeutic strategies and new nanomaterials (polymersomes and nanohydrogels) for theranostics - carriers of therapeutics for targeted drug delivery and internalization in tumors; synthesis and characterization of new antitumor substances, and investigation of their antitumor effect. The optimization of the electrical parameters and the local delivery of antitumor agents (visualization of penetration, localization and pharmacokinetics of long-circulating fluorescently labeled nanoparticles in tumors) were also explored.

Major Scientific Contributions

The habilitation work includes 6 publications that include research work on the development of new pharmaceutical agents - non-toxic bioactive substances with potential for anticancer drugs, and exploring their antitumor action alone and combined with electroporation that is a subject of active research in the field of targeted drug delivery.

Dose and time dependent changes in the morphology, adhesion, viability, migration and possibility of colony formation in low and high metastatic breast cancer cell lines induced after trehalose treatment, isolated from the strain *Rhodococcus wratislaviensis*, have been identified. A mechanism of the trehalose action is proposed, related to the asymmetry between the outer and inner monolayer of the membrane bilayer leading to the formation of endosome.

Anti-cancer properties of polysaccharides, secondary metabolites isolated from red microalgae, *Porphyridium sordidum* and *Rhodella reticulata*, have been demonstrated - induced reduction in the viability of breast cancer tumor cells and changes in cell morphology, while the morphology and viability of the non-tumor control cells were maintained normal. The heteropolysaccharide from *Rhodella reticulata* red microalgae applied by electroporation drastically reduces the specific viability of a cancer cell line in a dose-dependent manner in contrast to the effect on non-tumor cells. These data suggest potential of the polysaccharide as a new anticancer drug.

The combined action of the alkylphospholipid (ALP) miltefosine and electroporation induces destruction of the cell cytoskeleton and increases the permeability of cell monolayers due to disruption of cell-cell contacts, and reduces the viability of keratinocyte cells.

Non-habilitation publications

The publications not included in the habilitation work summarize results on the combined action of chemotherapeutics and electroporation, and the application of the electrochemotherapy, based on the formation of temporary pores in the cell membrane when applying electrical pulses, for the treatment of patients with clinical and cytological diagnosed skin tumors; development of nanoparticles (polymersomes and nanohydrogels labeled with nanodots) for drug delivery, as well as the synthesis of new bioactive drug agents and the study of possible side effects.

The combined use of SN38 chemotherapy and electroporation increases the sensitivity of cancer cells to conventional SN38 anticancer therapy, affects cell viability and cytoskeletal integrity, leads to changes in the redox homeostasis of cancer cells and results in cell death by apoptosis and / or ferroptosis. This effect is accompanied by increased damage to DNA, production of intracellular superoxide and hydroperoxides, and changes in the expression of membrane ABC transporters and retention of the chemotherapy agent in the cells. The study showed an increase in the sensitivity of cancer cells to anti-cancer therapy with SN38 when combined with electroporation.

Analysis of the effect of combination of the two chemotherapeutic drugs SN38 / EF24 and their combination with melatonin, SN38 / EF24 / melatonin, on the ratio of "oncogenic" / "oncosuppressive" reactive oxygen species (ROS), that is a factor in triggering carcinogenesis, shows that both combinations significantly inhibit colon tumor growth in experimental animal models, and that the anti-cancer effect

of the triple combination EF24 / SN38 / melatonin is accompanied by a decrease in "oncogenic" and an increase in "onco-suppressive" ROS and almost complete destruction of the tumor.

Electrochemotherapy has been successfully applied for the treatment of patients with skin tumors. It has been shown that the method is effective, gentle, and suitable for the treatment of lesions and scars; the effect of electrotherapy being followed by measurement of autofluorescent spectra of the lesions and the skin around it.

A method has been developed for the construction of chemically modified chitosan-based polymersomes (polyionic hollow vesicles) labeled with quantum dots and nanohydrogels loaded with quantum dots and / or manganese, that allows their visualization in the bloodstream and in solid tumors.

Evidences are presented that polymersomes (polyionic complex hollow vesicles based on chemically modified chitosan) labeled with fluorescent contrast and conventional organic dyes are suitable for lymph node mapping and for the development of drug delivery nanoplatfoms.

The redistribution and stability of nanoparticles in tumor tissue of experimental colon graft cancer models *in vivo* after electroporation was studied and the appropriate conditions to avoid nanoparticle accumulation in surrounding non-cancerous tissues and artifacts when electroporation was applied to treat solid tumors were determined. Quantum dot-labeled polymersomes have been found to be predominantly delivered to the tumor.

The electroassisted delivery of size-controlled long-circulating quantum dot-labeled polymersomes and fluorescently labeled multimodal model nanohydrogels have been shown to be a promising therapeutic strategies for the treatment of solid tumors.

Analysis of the redox-modulating properties and anti-cancer effect of vitamin K and the redox system vitamin K&C, which prevents mitochondrial dysfunction and restores oxidative phosphorylation, revealed that vitamin K&C can sensitize cancer cells to conventional chemotherapy and achieve effect upon the application of lower effective dose of the drug.

A new series of 9 biologically active thienopyrimidines, derived from 1,3-disubstituted benzimidazoles, was synthesized and their cytotoxicity against breast cancer cell lines with high and low metastatic potential was evaluated; the compound with the highest cytotoxicity was identified.

Possible side effects of the natural biosurfactant, trehalose from *Nocardia farcinica* strain, and mistletoe plant lectins (*Viscum album* L., VA) with anti-cancer activity were investigated. Reduced viability of cancer cells and no effect of trehalose on isometric contractions of rat mesenteric arteries *in vitro*, and weak effect of lectins on the contractility of human vasculature, suggesting their use in anticancer therapy without vascular side effects was demonstrated.

Expert activities

Assoc. Professor Nikolova was:

- supervisor of one doctoral student who successfully defended doctoral Thesis (2017);
- supervisor of three successfully defended master's theses;

- member of the Scientific Council of the IBPhBME - BAS and head of the section: "Electroinduced and adhesive properties" IBFBMI-BAS since 2019 so far;
- member of scientific juries for awarding the scientific and educational degree "Doctor"; for holding the academic positions of "Research Assistant" and "Professor" in procedures and competitions at the Institute of Solid State Physics, BAS, the Institute of Biophysics and Biomedical Engineering, BAS and Sofia University "St. Kliment Ohridski", MU-Sofia;
- guest Editor of the magazine "Separations".
- scientific adviser of students in the OPERATIONAL PROGRAM "HUMAN RESOURCES DEVELOPMENT" 2007 - 2013 and 2016 Invest in your future! BG051PO001 - 3.3.07-0002 "STUDENT PRACTICES"

CONCLUSION

The presented scientific production and the scientific indicators of Assoc. Prof. Biliana Nikolova-Lefterova fully meet and exceed the recommended requirements for occupying the academic position of "Professor" according to the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for the Application of ADASRB in BAS and the specific requirements of IBPhBME-BAS.

The contribution of Assoc. Prof. Biliana Nikolova-Lefterova in the field of electrochemotherapy and the development of therapeutic strategies, new pharmaceutical agents with potential for anticancer drugs and novel nanoplateforms for drug delivery allows me to express my positive opinion on her candidacy for the academic position "Professor" and to recommend to the Scientific Jury to vote positively and to recommend the Scientific Board of IBPhBME-BAS to elect Assoc. Prof. Biliana Pencheva Nikolova-Lefterova for the academic position "Professor" in the field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological Sciences, scientific specialty "Biophysics".

Sofia
24.11.2021

/Prof. DSc Stefka Germanova Taneva/