

Вх. № 890.КН / 19.09.2022.

REVIEW

Considering the competition for **the academic position "Associate Professor"** in the field of higher education 4. "Natural Sciences, Mathematics and Informatics", professional direction 4.3. "Biological Sciences", scientific specialty "Biophysics", for the needs for Department "Lipid-Protein Interactions" at the Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, announced in "State Gazette", issue 32, publish on April 26, 2022

Reviewer: Prof. Dr. Emilia Lyubomirova Apostolova, Institute of Biophysics and Biomedical Engineering – Bulgarian Academy of Sciences, member of the Scientific Jury according to order No. 304/30.05.2022 of the Director of IBPhBME - BAS.

Candidate: Senior Assist. Prof. Anelia Stefanova Kostadinova, PhD

For participation in the announced competition for **the academic position, "Associate Professor"** one candidate has submitted documents: Senior Assist. Prof. Dr. Anelia Stefanova Kostadinova, PhD. The candidate has attached all the necessary documents in accordance with the requirements of the Act on Development of the Academic Staff in the Republic of Bulgaria as well as the Regulations for its application approved by BAS and IBPhBME-BAS. The materials presented by the candidate are precisely prepared and well arranged.

Education and career development

Dr. Anelia Kostadinova graduated with a master's degree in the specialty "Cell Biology and Developmental Biology" at the Faculty of Biology of the Sofia University "Sn. Kliment Ohridski" in 1993. She defended a dissertation and receive the educational and scientific degree "doctor" in 2011. The title of her dissertation is "Modulation of the interaction of cells with polymer surfaces and membranes". Scientific career of Dr. Kostadinova began at the Institute of Biophysics (now IBPhBME) in 1995 and she was a specialist-biologist, assistant and senior assistant professor. She currently holds the academic position of senior assistant professor in Department "Lipid-Protein Interactions" at IBPhBME-BAS. The candidate has many years of experience in scientific research (26 years and 9 months). Dr. Kostadinova was on specializations in Germany for a total of 19 months in the period from 1996 to 2005.

Research activity

The scientific researches of Dr. Kostadinova are in the field of nanotechnology. The interdisciplinarity of her research determines the cooperation with a number of national and

international scientific groups from the Faculty of Biology of SU "Sn. Kliment Ohridski", the Faculty of Chemistry of Burgas University, University of Chemical Technology and Metallurgy - Sofia, Luxembourg Institute of Science and Technology and Nanotechnology and Advanced Materials Central Lab. and Agriculture Research Center, Egypt. The total publication activity of the candidate includes 33 publications, 5 of which are related to the dissertation for receiving the educational and scientific degree " Doctor".

For the participation in the competition for **the academic position, "Associate Professor"** Dr. Kostadinova has presented 33 scientific publications 17 with IF or SJR (Q1 - 5, Q2 - 4, Q3 - 8), one chapter of a book and 15 publications without IF or SJR. The total impact factor of the publications is 25.253. The habilitation reference (group indicator B of the presented documents) includes 5 publications with IF (Q1 – 2, Q2 – 2, Q3 – 1). According to the report submitted by the candidate, the total number of points in indicators is 454 points (indicator A - 50, indicator B - 105, indicator D - 235, indicator D - 64) with a requirement of 430 points. The candidate exceeds the minimum national requirements and the regulations for their application approved by the BAS and IBPhBME-BAS. The candidate also presents 32 citations from foreign authors.

Dr. Kostadinova has presented materials from her research at 27 scientific forums, including 6 abroad. The candidate has participated in the development of 20 research projects, on one of which she is the leader. Doctor Kostadinova is the supervisor of a four students from the Faculty of Biology of the Sofia University "St. Kliment Ohridski". From 2004 to 2022, she also led lectures and practical exercises at the Faculty of Biology of the Sofia University "St. Kliment Ohridski".

Scientific activity of the candidate

The scientific investigations included in the extended habilitation report are divided into three main thematic areas:

- Interaction of different types of cell lines with synthetic and natural materials as well as nanoparticles. Biocompatibility was evaluated by considering their cytotoxicity and changes in cell adhesion phenotype and cell contacts. As a result of these studies, the changes occurring in the cell membrane and cell physiology have been established. The research may find application in modern biomedicine.
- Interaction of cell membranes with amphiphilic molecules of natural and synthetic origin - research is aimed to reveal their influence on cell morphology, cell-cell and cell-extracellular matrix contacts. Research are aimed at investigating the antitumor effect of these molecules in in vitro model systems. Also of interest are the applied techniques that facilitate the entry of these molecules into the cells. Research in this field has a scientifically applied nature.
- Investigation of lipid-protein and lipid-lipid interactions in the cell revealed the influence of lipid oxidation on lipid arrangement and membrane organization.

In the bibliography of the extended habilitation reference, 23 publications are included (Q1-5, Q2-4, Q3-8 and 6 without IF or SJR), and the main scientific contributions are divided into two groups:

I. Fundamental investigations - the interaction of human cells with synthetic or natural material, as well as with nanoparticles.

Influence of surface characteristics (hydrophilicity, hydrophobicity, roughness). Establishing the relationship between the composition, structure and properties of the substance and the possibilities for its application is essential for obtaining new materials with predetermined properties. Based on studies performed on the influence of fibroblasts of different characteristics with GKSS Berlin Teltow synthetic membranes and coatings, the hydrophobicity of the surfaces has been shown to influence the organization of integrin receptors and focal adhesive contact formation by fibroblasts. An important contribution to these studies is the demonstrated organization of αV integrin on adherent cells with hydrophobic materials. The applied research techniques allow for a complex evaluation in the study of the tissue compatibility of newly synthesized materials and can be useful in the development of new biomaterials with precisely defined composition and properties.

Application of natural and synthetic polymer surfaces. It was established for the first time that the interaction of cells with modified polyethylene glycol surfaces depends on the length, structure and density of the polymer chains, as well as the presence of COOH groups bound to polydimethylsiloxane. PDMS-b-PAA (polydimethylsiloxane-block-polyacrylic acid) surfaces were obtained, characterized by very good stability, continuous fibers and uniform width, which favor the attachment and proliferation of epithelial cells.

Research on nanoparticles and nanomaterials. New collagen/zinc titanate antimicrobial biomaterials have been developed that show significant antimicrobial activity and moderate cytotoxicity and cell-specific response in *in vitro* studies with human cells of different origins – keratinocytes, fibroblasts and osteoblasts. It was found that chitosan-based nanoparticles can influence the lipid ordering in large unilamellar vesicles composed of various lipids and their mixtures, mimicking the lipid architecture of mammalian plasma membranes. Studies with graphene oxide show that collagen materials with incorporated graphene oxide and added silver and silicon compounds have increased antibacterial activity and lead to specific and adjustable activity towards eukaryotic cells.

II. Scientific-applied investigations - the interaction of cell membranes with amphiphilic molecules of natural or synthetic origin.

Studies of the biological activity of amphiphilic molecules of natural origin. The studies of this group included extracts from the Bulgarian endemic *Haberlea rhodopensis*, which is rich in amphiphilic biologically active substances, such as antioxidants, polyphenols and other specific secondary metabolites.

- It has been established that extracts of *Haberlea rhodopensis* may be a good candidate for use in the complex treatment of pathological dermatological conditions.
- Studies of the phenylglucoside myconoside extracted from *Haberlea rhodopensis* affect the membrane lipid ordering and the cytoskeleton of lung adenocarcinoma A594, and these changes are likely to be related to cell viability.
- Using biomimetic membranes, myconoside has been shown to reorganize membrane lipids by changing the fraction of enriched swingomyelin-cholesterol domains. A mechanism of action of myconoside on plasma membrane lipids of adenocarcinoma A594 as well as on the filaments has been proposed to explain its cytotoxic effect at high concentrations.
- For the first time, the inclusion of the specific fluorescent probes (Laurdan and Di-4-ANEPPDHQ), was applied to evaluate the lipid arrangement in cell membranes of living cells and monolayers by means of the GP (general polarization) parameter.

Studies of the biological activity of amphiphilic molecules of synthetic origin.

- Electroporation of NaCaT cells in combination with rifampicin was found to induce cytoskeleton disruption and an increase the permeability of the cell monolayer. The application of this new combined approach in the treatment of psoriasis will allow to avoid the side effects of conventional chemotherapy.
- In the combined treatment of NaCaT keratocyte cells with an electric pulse (200-500V/cm) and miltefosine, destruction of the cytoskeleton and an increase in the permeability of the cell monolayer is observed, which leads to a decrease in the vitality of the cells. The results of this research can be used in the treatment of skin cancer and other pathological conditions affecting the integrity of the skin.
- When applying the combined action of an electric pulse with miltefosine in the treatment of A549 (cancer alveolar epithelial line) and MDCK (kidney cell line) cells, a different effect on the two types of cells was found. Cancer cell line was found to be more sensitive than non-cancerous cells.
- It has established an increase in the activity of the synthetic medicinal preparations miltefusin and rimfamycin when they are applied topically at the same time as an electric field.

In the extended habilitation report, Dr. Kostadinova also indicates her future creative plans. Her future research is a natural continuation of what has been carried out so far, in which she will use the accumulated knowledge, experience and acquired methods, as well as are included new topics in the field of nanotechnology. The candidate divides her future investigations into three groups:

The first group of research will be related to the development of new 3D biocompatible nanocomposites with a matrix of reduced graphene oxide (RGO), which would have applications in biomedicine and tissue engineering.

The second group of studies is related to the use of nanomaterials with optical properties that have high anticancer and antibacterial activity. The candidate plans to continue her cooperation with colleagues from University of Chemical Technology and Metallurgy - Sofia and Burgas University "Prof. Dr. Asen Zlatarov". As a result of this collaboration, it is planned to investigate the action of newly synthesized compounds of double selenate salts on biomimetic membranes.

In the third group of studies, fundamental studies related to the structural and functional characteristics of nanocomposites for their application in photonics and biomedicine are foreseen.

Conclusion

The investigations of Dr. Kostadinova provide new knowledge characterizing the interactions of human cells with nanoparticles, modified polyethylene glycol surfaces or with synthetic and natural materials as well as the development of new antimicrobial biomaterials from collagen/zinc titanate. A significant part of her research has a scientific-applied nature, and it should be noted the importance of the established increase in the activity of amphiphilic molecules in the interaction with cell membranes when they are applied simultaneously with an electric field. From the submitted documents for the competition, it is clear that science indicators of Dr. Kostadinova fully meet and even exceed the recommended requirements for occupying the academic position of "associate professor" according to the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Implementing Regulations of the law in BAS and the specific requirements of IBFBMI-BAS.

Based on the above, I allow myself to recommend to the members of the Scientific Jury to propose to the Scientific Council of IBFBMI-BANS to elect **Dr. Anelia Kostadinova for the academic position " Associate Professor "** in professional direction 4.3. "Biological Sciences", scientific specialty "Biophysics".

19.09.2022

Sofia

/Prof. Emilia Apostolova, PhD/