Scientific and Technical Report

Analysis of the interactions between nanoparticles and biomolecules to understand the role of nano-bio-interface in predicting nanoparticle behavior and effects on biological systems

Project duration: 24 months

Execution phase nr. 3/2020

Project director,

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Objectives of the project

Objective 1. Purchasing and characterization of nanoparticles with different properties. Analysis Time evolution analysis of the NPs-protein corona formation and dynamics – part I. Data correlation according to nanoparticles' characteristics – part I

Activity 1.1. Selecting and purchasing the NPs

Activity 1.2. Measurement of the macromolecules' influence on the NP size, dispersion and stability state

Activity 1.3. Evaluation of morpho-structural and conformational changes on proteins after the interaction with NPs

Activity 1.4. Data analysis and correlation of experimental results obtained on NP-biomolecule interactions

Objective 2. Analysis of the evolution in time of the formation and dynamics of the protein corona of the nanoparticles - part II. Analysis of interactions between membrane models and nanoparticles with different characteristics. Investigating the interactions between nanoparticles and nucleic acids - part I. Data correlation according to the characteristics of nanoparticles - part II

Activity 2.1. Measurement of the kinetics of binding affinity and desorption of proteins on the nanoparticle surface over time

Activity 2.2. Establishing the composition of the protein corona on the nanoparticle surface

Activity 3.3. Measurement of the effects induced by nanoparticles on the organization of Langmuir-Blodgett films as a model of cell membrane

Activity 2.4. Determination of changes induced by nanoparticles on lipids

Activity 2.5. Evaluation of the effects induced by nanoparticles on DNA-protein complexes

Activity 2.6. Data analysis and correlation of experimental results obtained on nanoparticle-biomolecule interactions

Objective 3. Investigation of the interactions established between nanoparticles and nucleic acids - part II. Data correlation according to the characteristics of the nanoparticles - part III

Activity 3.1. Evaluation of chromosome structure after interaction with nanoparticles

Activity 3.2. Data analysis and correlation of the experimental results obtained on the nanoparticles-biomolecules interactions

Abstract of project phase

Different types of nanoparticles were purchased from specialized manufacturers or were obtained from Romanian Institutes in order to test them within the project (graphene quantum dots (G-QDs), quantum dots of silicon (Si QDs, silicon nanoparticles, zinc oxide nanoparticles, titanium dioxide nanoparticles of different sizes and functionalities, carbon nanotubes and iron oxide nanoparticles (Fe_3O_4 - magnetite). Incubation of the silicon QDs with fetal bovine serum resulted in a rapid increase in zeta potential compared to particles suspended only in water, suggesting that the proteins affected the stability of QDs due to their rapid and continuous interaction with nanoparticles. The time-dynamic analysis of the Si/SiO_2 QDs hydrodynamic diameter after the incubation with human hemoglobin showed that particle size increased due to protein adsorption on nanoparticle surface. Regarding the incubation of G-QDs with different proteins for 30 minutes or 24 hours, it was found that albumin was the best protein to ensure the dispersion of particles. FTIR analysis showed changes in the BSA secondary structure after incubation with silica QDs, such as the loss of α-helix structure by 30% and increased β-turn content after the first 5 minutes of incubation. Subsequently, the percentage of α-helix increased during the next hours, when the secondary structure resembled to that of native BSA. Also, a dynamic interaction of BSA with magnetite particles in time was revealed, resulting in an important adsorption followed by desorption.

The analysis of protein corona dynamics and of the interactions between membrane models and nanoparticles with different characteristics, as well as the investigation of the interactions between the nanoparticles and nucleic acids were performed. Measurement of the time kinetics of proteins binding affinity and desorption from the surface of silica, titanium dioxide and zinc oxide nanoparticles showed more significant changes after the incubation with bovine serum albumin (BSA) as compared to human serum albumin (HSA). The highest amount of protein adsorbed on the surface of iron oxide nanoparticles was revealed in the case of fibrinogen (FBN), followed by BSA and HSA. Characterization of the protein corona composition formed on the surface of Si quantum dots (Si QDs) showed that these rapidly developed a complex corona from bovine serum. Further, in order to understand the effect of nanoparticles on biological membranes, the interactions between these and the phospholipid monolayer were analyzed by measuring pressure isotherms using Langmuir-Blodgett trough. This analysis showed that the particles coated with the protein corona interacted strongly with phosphatidyl choline and were incorporated between the lipids, increasing the monolayer rigidity. Phase contrast images showed that the nanoparticles became an integral part of the monolayer, forming complex structures with serum

proteins and phospholipids. QDs also exert a strong effect on the physical state of the phospholipid monolayer leading to its expansion and fluidization, but the presence of serum proteins contributes to the formation of a protein corona responsible for attenuating the disruptive effect on this model system. In addition, only silicon QDs induced significant changes in the cholesterol structure over time, which may affect the lipid structure and cell membranes, and induce lipid peroxidation with malondialdehyde formation. Exposure to the tested nanoparticles (photocatalytic TiO_2, graphene or silicon QDs) did not induce genomic DNA fragmentation. Regarding the effect induced by the exposure to Si QDs, no significant changes in the length of telomeric terminal restriction fragments were observed throughout the study, the values obtained remaining close to the control.

Also, within this project, the chromosomes' structure was evaluated after the interaction with nanoparticles. After 72 hours of incubation with the nanoparticles selected for this study, there was a significant increase in the percentage of nuclear DNA content found in the comets' tail, indicating the formation of DNA breaks in the chromosome structure. The concentration of 25 µg/mL nanoparticles increased the level of 8-OHdG after all the studied time intervals, highlighting a high level of oxidative stress that changed the nucleotides' structure. An induction of Bax and p53 genes transcription was observed after 24 hours of incubation with nanoparticles compared to control cells, followed by their overexpression after 72 hours, regardless of the concentration tested. The deregulation of gene expression indicated an accumulation of genetic defects that can have significant consequences on normal cell function and proliferation.

The obtained results were processed and disseminated by presenting at four specialized conferences, and publishing seven scientific articles, one book chapter and 2 review papers. In conclusion, all of the activities associated with Stages 1, 2 and 3 of the project activity planification were accomplished.

The results were analyzed and corelated in order to conclude the main effects of the interactions between nanoparticles and biomolecules. These were disseminated as:

<u>- oral presentations and posters at national and international conferences</u>

• Stan M.S., Nica I.C., Miu B., Dinischiotu A. "*Are TiO2 nanoparticles genotoxic? Their influence on the cell cycle of human pulmonary fibroblasts*", Al XII-lea Congres National de Citometrie, 30-31 Mai, 2019, Bucuresti, Romania. (Poster)

• Stan M.S., Mernea M., Cristian R.E., Mohammad I.J., Lambert C., Dinischiotu A. "*Evaluation of titanium dioxide nanoparticles effect on phagocytic activity of granulocytes*", Al XII-lea Congres National de Citometrie, 30-31 Mai, 2019, Bucuresti, Romania. (Oral presentation)

• Stan M.S., Cristian R.E., Mohammad I.J., Mernea M., Sbarcea B.G., Trica B., Dinischiotu A. "*Analyzing the interaction between different types of nanoparticles and serum albumin*", 21st Romanian International Conference on Chemistry and Chemical Engineering, September 4-7, 2019, Constanta-Mamaia, Romania. (Oral presentation)

• Nica I.C., Dinischiotu A. Stan M.S. *"Genotoxic potential of TiO_2 nanoparticles with enhanced photocatalytic properties investigated by Comet assay in human lung cells".* 13th International Comet Assay Workshop, 24-27 June, Pushchino, Moscow, Russia, p. 65 (ICAW 2019). (Poster)

• Strugari A., Stan M.S. *Nanoparticle intestinal transport characterization using in vitro co-culture models.* Proceedings of 1st International Online-Conference on Nanomaterials (01.09-15.09.2018), DOI 10.3390/IOCN_2018-1-05480. (Poster)

- research articles published in ISI journals

1) Stan MS, Mernea M, Cristian RE, Mohammad IJ, Sbarcea BG, Trica B, Dinischiotu A. Correlating the serum albumin corona of zinc oxide nanoparticles with their physico-chemical properties. *Rom. Rep. Phys.* 2020, 72(1):602. Impact Factor: 1.940, RIS: 0.702.

2) Cristian RE, Mohammad IJ, Mernea M, Sbarcea BG, Trica B, Stan MS, Dinischiotu A. Analyzing the interaction between two different types of nanoparticles and serum albumin. *Materials* 2019, 12(19), 3183. Impact Factor: 2.972, RIS: 1.405.

3) Stan MS, Nica IC, Popa M, Chifiriuc MC, Iordache O, Dumitrescu I, Diamandescu L, Dinischiotu A. Reduced graphene oxide/TiO2 nanocomposites coating of cotton fabrics with antibacterial and self-cleaning properties. *J. Ind. Text.* 2019, 49(3):277-293. Impact Factor: 1.884, RIS: 2.373.

4) Stan MS, Chirila L, Popescu A, Radulescu DM, Radulescu DE, Dinischiotu A. Essential oil microcapsules immobilized on textiles and certain induced effects. *Materials* 2019, 12(12), 2029. Impact Factor: 2.972, RIS: 1.405.

5) Voicu SN, Balas M, Stan MS, Trică B, Serban AI, Stanca L, Hermenean A, Dinischiotu A. Amorphous silica nanoparticles obtained by laser ablation induce inflammatory response in human lung fibroblasts. *Materials* 2019, 12, 1026. Impact Factor: 2.972, RIS: 1.405.

6) Strugari AFG, Stan MS, Gharbia S, Hermenean A, Dinischiotu A. Characterization of nanoparticle intestinal transport using an in vitro co-culture model. *Nanomaterials* 2019, 9(1), 5. Impact Factor: 4.034, RIS: 1.630.

7) Stan MS, Badea MA, Pircalabioru GG, Chifiriuc MC, Diamandescu L, Dumitrescu I, Trica B, Lambert C, Dinischiotu A. Designing cotton fibers impregnated with photocatalytic graphene

oxide/Fe, N-doped TiO2 particles as prospective industrial self-cleaning and biocompatible textiles. *Mat. Sci. Eng. C* 2019, 94:318-332. Impact Factor: 4.959, RIS: 1.231.

- book chapter

• Stan MS, Strugari AFG, Balas M, Nica IC. Biomedical applications of carbon nanotubes with improved properties. In: Fullerenes, Graphenes and Nanotubes: A Pharmaceutical Approach (ed. Grumezescu AM), Elsevier, Oxford, UK, 2018, p. 31-65. Print ISBN: 978-0-12-813691-1.

- review paper

• Nica IC., Stan M.S., Dinischiotu A. Current photocatalytic applications of nano-scaled titanium dioxide in the new era of "smart" technologies. Rev. Biol. Biomed. Sci. 2018; 1(2): 43-53.

• Stan MS., Badea MA., Strugari AFG. Carbon nanotubes: properties, applications and toxicity. *Rev. Biol. Biomed. Sci.* 2019; 2(1): 12-18.