SCIENTIFIC REPORT

PN-III-P4-ID-PCE-2020-1523, Contract No. PCE 161 / 2021

Versatile molecular vectors with tailored carrying and actuating abilities, dedicated to gene and drug delivery in fight against cancer (TM-Vector)

Acronym: **TM-Vector**

Web page: https://intelcentru.ro/tm-vector/

The TM-Vector project aimed to design, develop and evaluate a new type of nanoparticulate (macro)molecular gold-core core-shell tool, hereafter referred to as a nonviral or conjugate vector, which combines the transport abilities, release, and pharmacodynamic action to provide a versatile delivery system for nucleic acids, drugs, or radionuclides, with improved efficacy, selectivity, and capacity for target release or labeling.

Activities for the design, synthesis and physico-chemical and biological characterization of two nonviral vector libraries (I, II) were mainly carried out within this project, including their testing for applications in gene therapy, targeted drug delivery and medical imaging. Support activities (Activities for the design, synthesis and physico-chemical and biological characterization of two nonviral vector libraries (I, II) were mainly carried out within this project, including their testing for applications in gene therapy, targeted drug delivery and medical imaging. Support activities (**III**) were also considered, for the development of appropriate experimental plans.

I. Gold-core nonviral vectors functionalized with polyethylene glycol (PEG) of various molecular weights and then post-functionalized with polyethyleneimine (PEI), the latter functionalized with glycosamine (GA) groups. The vectors were (**a**) post-functionalized with drugs for their transport and delivery to the target; (**b**) complexed with a plasmid to prove their ability to function as vectors for gene therapy; (**c**) radiolabeled to prove their applicability in medical imaging.

II. Gold-core nonviral vectors functionalized with cyclodextrin-pre-functionalized PEI for binding to specific targeting peptides in the breast cancer model MCF7 cell line. After polyplex formation with a specific plasmid, the system was tested *in vitro* and *in vivo* to prove its efficacy in gene therapy applications.

III. Support activities for the proper design of experiments with nonviral gene vectors.

The results obtained ensured the fulfillment of the result indicators, presented in the table below.

Proposed indicators	Realized indicators
2021: A scientific paper. Attending at least one conference. TM-vectors WEB site. Research report. Synthesis and characterization protocols	2021: Two ISI papers. Two key note speaker conferences, and three oral communications. Web page. Research report including working protocols.
2022: Two scientific papers. Participation in conferences and symposia. Updating the WEB page. Research report. Synthesis and characterization protocols.	2022: Three ISI papers. Two keynote speaker conferences, six oral communications and posters. Web page. Research report including work protocols.
2023: Three scientific papers. Participation in conferences and symposia. Updating the WEB page. Research report. Synthesis and characterization protocols.	2023: Two ISI papers published. 4 ISI papers in evaluation stage (1/RSC, 1/CellPress, 1/Taylor & Francis, 1/Elsevier). A patent application. Five communications and posters. Web page. Research report including work protocols.

The scientific report, presented below, briefly includes the activities carried out according to the proposed implementation plan of the project:

TM-vector implementation plan. Stage 2021.

Stage I	Activities
Preliminary, documentary and experimental studies on the design and synthesis of generation I gold core nonviral vectors.	**A.1.1. Design and preparation of nonviral vectors** A.1.1.1. Synthesis of gold nanoparticles (AuNPs) with controlled size and increased stability in aqueous media. A.1.1.2. Conjugation of AuNPs with a cationic polymer (CP) functionalized with cyclodextrin (CD). A.1.1.3. Synthesis of complex combinations as potential fluorophores for obtaining inclusion complexes with β-cyclodextrin.
	A.1.2. Physicochemical characterization of reaction intermediates and AuNPs
	A.1.3. Preliminary *in vitro* evaluation of nonviral vectors A.1.3.1. *In vitro* evaluation of nonviral vectors cytotoxicity. A.1.3.2. Evaluation of transfection capacity of Au-CP-CD-Plasmid/Peptide polyplexes.
	A.1.4. Evaluation of vector functionality on animal blood samples
	A.1.5. Project management and results dissemination

TM-vector implementation plan. Stage 2022.

Stage II	Activities
Synthesis and physicochemical characterization of a second-generation nonviral vector library for *in vitro/in vivo* testing.	**A.2.1. Design and preparation of generation II nonviral vectors** *A.2.1.1.* Conjugation of gold NPs with appropriate functionalized PEG of different molecular masses (Au-S-PEGi-X, eg. X: -COOH); *A.2.1.2.* Conjugation of Au-S-PEGi-X with cationic polymer (CP) functionalized with glucoxamine (GA)(Au-S-PEGi-CP-GP); *A.2.1.3.* Loading of Au-S-PEGi-CP-GP conjugates with a plasmid or other biologically active molecules (Au-PEGi-CP-GP-Plasmid/GP polyplexes).
	A.2.2. Physico-chemical characterization of II generation gold nanoparticles *A.2.2.1.* Physico-chemical characterization of precursors and reaction intermediates by advanced methods: NMR, ATR-FTIR, Raman, ESI-MS, MALDI-Tof/MS. *A.2.2.2.* Characterization of nonviral vectors in dry state, by ATR-FTIR, NIR, Raman, XPS, XRD, SEM/EDX, micro-ATR-FTIR, TG/DSC, SEM, TEM, AFM, fluorescence spectroscopy. *A.2.2.3.* Characterization of nonviral vectors in wet state: UV-Vis, DLS, fluorimetry, micro-ATR-FTIR, Cryo-TEM, Raman-AFM. *A.2.2.4.* Nanoscale investigation of nonviral vectors, *in vitro*, in simulated biological fluids. *A.2.2.5*. Investigation of the ability of tellurium-based unimeric compounds (Te-AC) to

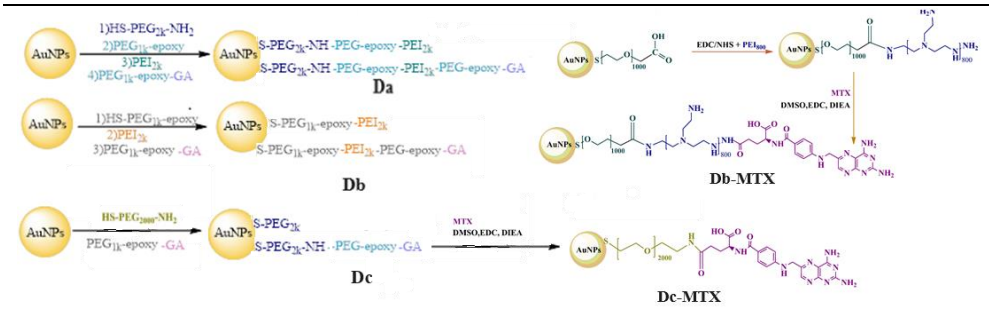
	generate free radicals, *in vitro*.
	A.2.3. Preliminary *in vitro* evaluation of nonviral vectors *A.2.3.1.* Evaluation of transfection capacity of Au-PEGi-CP-Plasmid / targeting molecule polyplexes. *A.2.3.2.* Designing a cancer therapy with prodrug-loaded in the synthesized nonviral vector.
	A.2.4. Confirmation of functionality of synthesized nonviral vectors by *in vivo* assays *A.2.4.1. In vivo* testing of the functionality of complex systems that have shown promising properties after their in vitro testing.
	A.2.5. Administrative management of the project and results dissemination

TM-vector implementation plan. Stage 2023.

Stage III	Activities
Synthesis and physicochemical characterization of a library of nonviral vectors. Testing the functionality of generation I nonviral vectors by *in vivo* assays.	**A.3.1. Design and preparation of nonviral vectors** A.3.1.1. Loading of AuPEI-CD conjugates with a plasmid or with biologically-active molecules and functional peptides. AuPEI-CD-peptide/Plasmid polyplexes for gene therapy. A.3.1.2. Plasmid loading of Au-PEG-PEI-GA conjugates for transfection of breast tumor cells. A.3.1.3. Loading of Au-PEG-PEI-GA conjugates with methotrexate with antitumor properties. A.3.1.4. Radiolabeling of Au-PEG-PEI-GA conjugates for diagnosis. A.3.1.5. Cyclodextrin/drug inclusion complex formation studies. A.3.1.6. Electrochemical studies of Au nanoparticles electrodeposited on different 2D materials
	A.3.2. Physico-chemical characterization of gold nanoparticles, Au-S-PEGi-CP-CD conjugates and Au-PEGi-CP-CD-Plasmid/Peptide polyplexes A.3.2.1. Nanoscale investigation of nonviral vectors *in vitro*, in simulated biological fluids.
	A.3.3. Preliminary *in vitro* evaluation of nonviral vectors A.3.3.1. Designing a cancer therapy with prodrug-loaded in the synthesized nonviral vector.
	A.3.4. Confirmation of the functionality of the synthesized nonviral vectors, by *in vivo* tests A.3.4.1. *In vivo* testing of the functionality of complex systems that have shown promising properties after *in vitro* testing.
	A.3.5. Administrative management of the project and results dissemination

Presentation of project results

I. Gold core nonviral vectors functionalized with polyethylene glycol (PEG) of different molecular weights and then post-functionalized with polyethyleneimine (PEI, cationic polymer) pre-functionalized with glucosamine (GA) groups (vectors **Da** and **Db**, according to Scheme 1). The establishment of the work protocol was carried out during Stage II/2022, through the synthesis of some libraries of compounds to develop the synthesis method that ensures the reproducibility of the reaction products. The chemical structure was proved by FTIR spectroscopy, which highlighted the characteristic peaks of each studied vector. The UV-Vis spectra preserved the surface plasmon resonance between 520 and 528 nm (specific range of gold nanoparticles) in all reaction steps. The successive addition of polymer layers induces a bathochromic shift. The ruby-red color was retained in all reaction steps. DLS histograms revealed uniform assemblies with a clear trend of increasing hydrodynamic diameter with increasing length of the side chain attached to the gold nanoparticles. In the case of the **Da** vector, the hydrodynamic diameter measured by the DLS technique was 324 nm, with a zeta potential of 19.2 mV. For the **Db** vector (with shorter PEG chain compared to the PEG chain in **Da**) the hydrodynamic diameter is about 242 nm and the zeta potential is 21.5 mV. The values of the zeta potential attest the fact that the systems exhibit colloidal stability.



***Scheme 1**. Reaction scheme for obtaining **Da, Db, Dc, Db-MTX** and **Dc-MTX conjugates**.*

In vitro evaluation of the biocompatibility and cytotoxicity of the two systems (Da and Db) on human gingival fibroblasts (HGF) showed that the nanosystems are biocompatible, not affecting cell viability at the tested concentrations.

In vivo tests on BALB/c mice allowed the determination of acute toxicity doses (LD_{50}), which were 1.9 mg/mouse for **Da** and 6 mg/mouse for **Db**. During the monitoring period, surviving animals in both groups did not show behavioral changes or signs of toxicity. After repeated dose toxicity of the two vectors, no behavioral changes, body weight differences, or other signs of late clinical toxicity were observed. The mice maintained their interest in food and water during the 7 days of monitoring, and histopathological examination of the liver and kidney revealed that their administration induced early-stage pathological changes.

The Da, Db and Dc systems (Scheme 1) were post-functionalized appropriately depending on the desired application:

I.1. In gene therapy (*Stage II/2022 and Stage III/2023*): the AuNP-PEG-PEI-GA systems were complexed with the plasmid pCS2+MT-Luc (5991 bp), forming the corresponding polyplexes.

The ability of the vectors to complex nucleic acids was tested by electrophoresis on agarose gel, in solutions of simulated biological fluid with different concentrations. From electrophoretic studies, the two vectors form polyplexes by complexing the plasmid at different vector concentration values. The **Da** vector completely complexes the plasmid at a concentration of 8·10-9 M, while the **Db** vector ensures a total packaging of the plasmid at the concentration of 8·10-11 M (plasmid concentration: 1 µg/µL).

Cytotoxicity (CellTiter-Glo test) and transfection (Bright Glo Luciferase test) tests applied to **Da** and **Db** vectors at 48 hours on the HeLa cell line, showed that they do not show cytotoxicity for concentrations lower than 1200 µM gold and that they provide a transfection effective at concentrations lower than 800 µM gold for **Da** and respectively around 2000 µM gold for **Db**. In stage 2023, it was proved (by tests of protection against enzymatic degradation in the presence of deoxyribonuclease, which catalyzes the hydrolytic cleavage of phosphodiester bonds in the DNA composition) that the plasmid embedded in the nonviral vectors is not exposed to degradation.

In conclusion, the study carried out in during 2022-2023 concluded that the synthesis of the **Da** and **Db** systems (Scheme 1) is reproducible, the respective systems can be used in the targeted gene therapy of sugar-consuming tumor cells. The synthesis method of **Da** and **Db** vectors is original, the products ensuring high efficiency in the packaging of DNA plasmid. Their polymer coating gives good protection to the transferred genes. The systems show good biocompatibility, low *in vitro* toxicity on the

HGF cell line (non-tumor) and low *in vivo* toxicity after intravenous administration in mice. The proposed vectors may represent an alternative for the transport and delivery of genetic material in cancer cells, with minimal negative effects on healthy cells, at a low N/P ratio (nitrogen concentration in the vector/phosphorus concentration in the DNA), a fact that recommends them to further studies for clinical therapeutic purposes.

I.2. Designing a cancer therapy with prodrug-loaded in the synthesized nonviral vectors Db and Dc (presented in Scheme 1) (*Stag II/2022 and Stage III/2023*)

Methotrexate (MTX), a folic acid antagonist used in cancer therapy, was chosen as the pro-drug in this study. Methotrexate has low solubility in aqueous media and shows low skin permeability. These deficiencies can be alleviated by using appropriate drug delivery systems, including appropriately functionalized gold nanoparticles. For these reasons, **Db** conjugates that showed low cytotoxicity on non-tumor fibroblasts were functionalized by covalent binding with methotrexate (**Db-MTX**; Scheme 1). To highlight the influence of the presence/absence of polyethyleneimine (PEI) on the biological properties, PEG-only and PEG-GA functionalized gold core conjugates (product Dc; Scheme 1) were also synthesized, and were covalently functionalized with MTX (**Dc-MTX**; Scheme 1). The non-viral vectors Db and Dc expose on the surface of the nanoparticles amino groups that are capable of interacting with the carboxylic groups of MTX structure. Confirmation of the chemical structure and morphology was achieved by UV-Vis spectroscopy, FTIR spectroscopy, DLS and STEM. The STEM images revealed that the investigated systems have spherical morphology and diameters of around 16 nm. DLS analysis indicates zeta potential values of 39 mV for **Dc** and 15 mV for **Db**, confirming that the nanoparticles show good colloidal stability even when they possess amine groups on the surface.

As a result of the presence of PEI chains on their surface, the nonviral **Db-MTX** vectors (Scheme 1) can condense a plasmid, and following the attachment of MTX to their surface through ester bonds, the pharmacologically active compound can be released, restoring the initial structure of the vector, under the action of esterase enzymes. A double therapeutic effect is therefore ensured: the simultaneous delivery of genes and pharmacologically active principles to the targeted cells (through two distinct mechanisms, namely the electrostatic condensation of DNA and the covalent binding of drugs and glucosamine).

***In vitro* evaluation of cytotoxicity (MTT assay) and antioxidant action (DPPH assay) of Db (AuNP-PEG-PEI-MTX) and Dc (AuNP-PEG-MTX) conjugates** (Scheme 1) (*Stage II/2022 and Stage III/2023*)

Cytotoxicity tests (MTT) on human gingival fibroblasts (HGF), indicate that **Db** (AuNP-PEG-PEI-MTX) and **Dc** (AuNP-PEG-MTX) vectors do not affect cell viability at the tested concentrations. On the MCF7 (breast cancer) cell line, the vectors in question induce cytotoxic effects with higher efficacy than free MTX at the same concentrations of MTX. The DPPH test demonstrated that the studied systems have an increased antioxidant activity compared to free MTX, in the order: **Dc** > **Db** >> MTX.

In conclusion, through the obtained results, it was highlighted that the system that has in its composition only PEG chains functionalized with MTX and glucosamine units shows increased antitumor and antioxidant activities compared to both the system with PEI and free MTX. The overall antitumor activity of the **Db** (AuNP-PEG-PEI-MTX) conjugate is enhanced by the transfection ability of the carried

plasmids. This fact was highlighted by transfection tests on the HeLa cell line, using a plasmid that produces a fluorescent protein (pCS2+NLS+eGFP).

I.3. Design of nonviral vectors for PET and SPECT medical imaging *(Stage III/2023)*

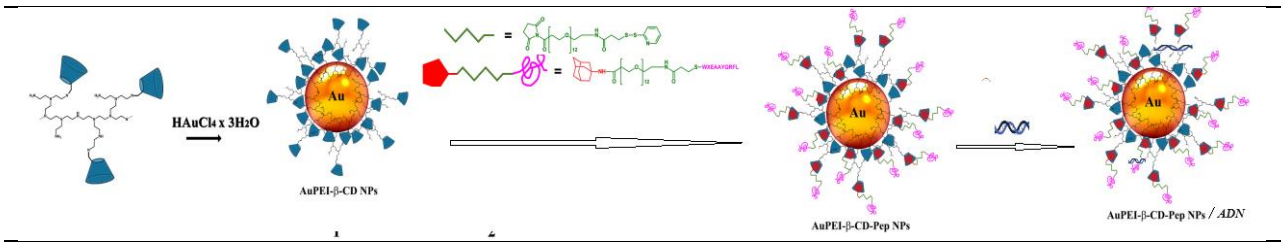
The demand for radiopharmaceuticals products that are accessible and appropriate for the particular diseases is one of the most persistent concerns of precision medicine that calls on nuclear imaging. The nonviral vectors AuPEG-PEI and AuNP-PEG-PEI-GA include in their composition versatile components that can be functionalized in a nuanced way, such as (**i**) the biocompatible gold nanoparticle core, (**ii**) the presence of PEI at the periphery of the nanoparticle (ensuring radioisotopes capture), (**iii**) the presence of glucosamine sequences (which ensures the accumulation of vectors in areas of high glucose consumption) and (**iv**) the presence of PEG in the shell (a fact which increases the biocompatibility and colloidal stability of vectors in biological fluids). Due to these compositional and functional features, we believe that the developed vectors are suitable to be tested for applications in SPECT and PET imaging.

The obtained AuPEG-PEI and AuNP-PEG-PEI-GA conjugates were radiolabeled with ^{99m}Tc (for SPECT) or ^{68}Ga (for PET). The biocompatibility of the unlabeled and labeled conjugates was evaluated on non-tumor fibroblasts, and the radiolabeling yield of the vectors was determined by the flash thin layer chromatography technique.

The newly developed multimodal materials exhibited good biocompatibility and excellent radiolabeling ability, offering outstanding prospects for use as radiotracers in SPECT and PET imaging. The promising potential of using these agents as diagnostic tools in further *in vivo* evaluations was demonstrated by *in vitro* biocompatibility on normal fibroblasts.

II. Non-viral vectors with gold core functionalized with branched polyethyleneimine, pre-functionalized with β-cyclodextrin units (AuPEI-CD; Scheme 2) (*Stage I/2021 and Stage II/2023*).

As a result of the studies carried out in the 2021 stage, in which the synthesis methods found in the specialized literature were evaluated, in the 2023 stage, in order to obtain AuPEI conjugates functionalized with β-cyclodextrin (β-CD), we decided to apply a modified synthesis method proposed by Forrest[1] and Pun[2], where the molecular mass of branched polyethyleneimine (bPEI) was 25kDA. The first step of the synthesis consisted in the functionalization of bPEI with cyclodextrin units, through the reaction between mono-6-O-(p-toluenesulfonyl)-β-cyclodextrins (previously synthesized and reported in Stage I/2021) with bPEI in DMSO. The reaction mixture was stirred under an inert atmosphere for 72 hours at 70°C and the reaction product was separated by centrifugation, washed with acetone and resolubilized in water, followed by filtration on an Amicon Ultra-15 filter, with MWCO 10,000 Da. From the ^{1}H-NMR spectra a molar ratio β-CD/bPEI=26 was determined.



Scheme 2. *Graphical summary for preparation of AuPEI-CD-Pep nanoparticles and derived polyplexes.*

The second synthesis step consisted in the preparation of AuPEI-β-CD conjugates. An aqueous solution of bPEI-β-CD was added dropwise and stirred at room temperature over an aqueous solution of chloroauric acid ($HAuCl_4 \cdot 3H_2O$) to obtain a $HAuCl_4$/bPEI-β-CD with mass ratio of 1:4 g/g. The resulting mixture was irradiated for 2.5 minutes in a microwave oven until the color turned red. The solid product was recovered by filtration on an Amicon Ultra-15 filter with MWCO 50,000 Da, yielding a viscous, red mass. The physico-chemical characterization of the nanoparticles was carried out by UV-Vis, FT-IR, TGA, SEM, TEM, and DLS. According to the same protocol, gold nanoparticles coated with unmodified bPEI (AuPEI-PEI) were also obtained.

The third step of the synthesis consisted in the functionalization of peptide WXEAAYQRFL (Chempeptide Ltd., Shanghai, China), that is specific for targeting MCF7 (breast cancer) cells, by coupling it in aqueous solution with 1-adamantylamine (Ad), in a molar ratio of 1:1, through the ligand 3-(2-pyridyldithio)propionate of polyethylene glycol-succinimidyl (PEG12 SPDP). The reaction mixture was stirred for 24 h at room temperature, then centrifuged, and the supernatant (containing the reaction product) was further used as such. The chemical structure of the reaction product was confirmed by MALDI-Tof (DHB matrix), presenting an m/z ratio of 2705, corresponding to the targeted compound.

The TEM images indicated a spherical morphology of the conjugates, with an average diameter of about 10 nm. It is also noticeable the presence of a small number of nanoparticles with larger sizes, resulting from aggregation, which leads to the increase of polydispersity for the system. However, the zeta potential value of 53 mV determined for the nanoparticles indicates their good stability in biological fluids, unaffected by the formation of aggregates.

In the last stage of the tests, the AuPEI-β-CD nanoparticles were incubated for 24 hours with the adamantyl-functionalized peptide, after which the plasmid pCS2+MT-Luc was added, ratio N (nitrogen from the conjugate) : P (from the plasmid) of 1:50 (determined from electrophoresis studies), and the resulting mixture was incubated at 25°C for 30 min (Scheme 2). After performing the protection test against enzymatic degradation with deoxyribonuclease, it was found that the plasmid pCS2+MT-Luc, embedded in the nonviral vectors, is not subject to enzymatic degradation.

To evaluate the cytotoxicity of *AuPEI-β-CD*, *AuPEI-β-CD-pep* vectors and the *AuPEI-β-CD-pep* / pCS2+MT-Luc complex (hereafter named polyplex) MTT assays on HeLa cells and MCF7 cells of interest (breast cancer) was performed. The results showed that the cytotoxicity is good and acceptable (>70%) for the polyplexes formed, while the non-plasmid-complexed vectors show higher cytotoxicity against the tested cells. The relatively high values of cytotoxicity on HeLa and MCF7 cells are contradicted by the *in vivo* tests (presented below) because their administration to mice did not produce negative effects on the investigated organs. Transfection capacity assays indicate that the *AuPEI-β-CD-pep/pCS2+MT-Luc* polyplex exhibits a much higher transfection capacity towards MCF7 cells, concluding that it provides the necessary specificity in transfecting MCF7 cells.

The ***in vivo*** **testing** was carried out by a team from the University of Life Sciences, "Ion Ionescu de la Brad" from Iași, in accordance with the EU directive 63/2010, and all experimental procedures were approved by the Ethics Committee of the University (opinion no. 404/18.07.2023). The *in vivo* tests were the subject of the Service Contract for research projects, no. 1333/3.04.2023, concluded between the Institute of Macromolecular Chemistry "Petru Poni" and the University of Life Sciences "Ion Ionescu de la Brad".

***In vivo* testing of AuPEI-CD-Pep/pCS2+NLS-eGFP polyplex**

The animals used in the study were CD1 mice - 8-week-old females, purchased from the Cantacuzino Institute Bucharest, Băneasa resort. The acclimatization of the animals was done under identical conditions of temperature (25°C) and humidity (60%), with permanent access to water (*ad libitum*) and food, ensuring a 12 hours light/dark cycle. The animals were housed in autoclavable polycarbonate cages of 1500 cm^2, approximately 300 cm^2/mouse. The animals were provided with standardized food - Cantacuzino Institute.

The pCS2+NLS-eGFP plasmid, AuPEI-CD-Pep transfection vector and MCF7 cells were used for the experiment. The plasmids used in the experiments contain the reporter genes for *green fluorescent protein*, and the concentration in the DNA and the degree of purity were determined spectrophotometrically with the help of a microvolume spectrophotometer (Denovix), by measuring the absorbance at 260 nm and by calculating the ratio of absorbance at 260 and 280 nm.

The results of testing the AuPEI-CD nonviral vector on BALB/c mice showed that it is non-toxic and does not induce changes in the evaluated parameters at the repeated dose of 500 mg/kg. Therefore, the AuPEI-CD vector did not affect the health of the animals used in this study. This result allowed us to continue the study by administering AuPEI-CD-Pep/pCS2+NLS-eGFP polyplexes (Pep: peptide specific for targeting MCF7 tumor cells; plasmid used: pCS2+NLS-eGFP capable of inducing fluorescent protein expression) to mice that had previously been given MCF7 tumor cells. Bioluminescence studies showed that the polyplexes transfected MCF7 cells, recommending the AuPEI-CD vector for gene therapy applications. Studies are currently underway, the vectors are being tested on animals with induced tumors.

III. Support activities:

III.1. Synthesis and characterization of complex combinations with rare metals, as potential fluorophores for obtaining inclusion complexes of β-cyclodextrin.

III.2. Preliminary studies on the coating of gold particles with supramolecular networks of cyclodextrins.

III.3. Characterization of tellurium-based complex combinations as potential providers of free radicals in tumor tissue.

III. 4. Synthesis and characterization of ibuprofen/β-cyclodextrin inclusion compounds. Physicochemical characterization and evaluation of the *in vivo* release profile of the drug

III.5. Obtaining supramolecular aggregates of compact gold nanoparticles, with a wide absorption spectrum in the visible range, intended for photothermic applications.

III.6. Electrodeposition of gold nanoparticles and application of modified electrodes in the electrochemical detection of nitrite in waters.

SCIENTIFIC RESULTS DISSEMINATION

The results of the TM-Vector project were published in ISI rated journals, as follows: seven published articles, four articles under evaluation (sent for publication to RSC, CellPress and Taylor & Francis, Elsevier) and a patent application. Also in Stage I/2021 there were presented two conferences and three oral communications; in Stage II/2022 two conferences and six oral/poster communications,

and in Stage III/2023 4 conferences and 1 poster at national and international scientific events. Conferences / communications / posters are presented below. In the 2023 stage, a short-term research internship was also carried out at the Ilie Murgulescu Institute, Bucharest, for training in the issue of electronic spin resonance.

List of papers published or under evaluation for 2021-2023

1. Sardaru MC, Rosca I, Morariu S, Ursu EL, Ghiarasim R, Rotaru A**.** Injectable Thixotropic β–Cyclodextrin–Functionalized Hydrogels Based on Guanosine Quartet Assembly. *Int. J. Mol. Sci.* **2021**, *22*(17), 9179; https://doi.org/10.3390/ijms22179179

2. Sardaru MC, Marangoci NL, Shova S, Bejan D. Novel Lanthanide (III) Complexes Derived from an Imidazole–Biphenyl–Carboxylate Ligand: Synthesis, Structure and Luminescence Properties. *Molecules* **2021**, *26*(22), 6942; https://doi.org/10.3390/molecules26226942

3. Petreni A, Iacobescu A, Simionescu N, Petrovici AR, Angeli A, Fifere A, Pinteala M, Supuran CT. Carbonic Anhydrase inhibitors bearing organotelluride moieties as novel agents for antitumor therapy. *Eur J Med Chem*, **2022,** 244, 114811; https://doi.org/10.1016/j.ejmech.2022.114811

4. Dimofte A, Simionescu N, Petrovici A-R, Spiridon I. Probiotic Properties of Weissella confusa PP29 on Hibiscus sabdariffa L. Media. *Fermentation* **2022**, 8(10), 553; https://doi.org/10.3390/fermentation8100553

5. Condurache M-I, Petrovici A-R, Simionescu N, Profire B-S, Confederat L-G, Bujor A, Miron A, Profire L. Simultaneous Determination of Glibenclamide and Silymarin Released from Chitosan Microparticles by HPLC-ESI-MS Technique: Method Development and Validation. *Pharmaceutics* **2022**, 14(10), 2164; https://doi.org/10.3390/pharmaceutics14102164

6. Craciun BF, Clima L, Bostiog DI, Silion M, Calin M, Peptanariu D, Pinteala M. Multilayer gold nanoparticles as nonviral vectors for targeting MCF-7 cancer cells. *Biomater Adv.* **2023**, 144, 213201; https://doi.org/10.1016/j.bioadv.2022.213201

7. Vasincu IM, Apotrosoaei M, Lupascu F, Iacob AT, Giusca SE, Caruntu ID, Marangoci NL, Petrovici AR, Stanciu GD, Tamba BI, Profire BS, Focsa AV, Pinteala M, Profire L. Complexes of Ibuprofen Thiazolidin-4-One Derivatives with β-Cyclodextrin: Characterization and In Vivo Release Profile and Biological Evaluation. *Pharmaceutics* **2023**, 15(10), 2492; https://doi.org/10.3390/pharmaceutics15102492

8. Bostiog DI, Natalia Simionescu N, Coroaba A, Marinas IC, Chifiriuc MC, Gratiela Gradisteanu Pircalabioru G, Maier SS, Pinteala M. Multi-Shell Gold Nanoparticles Functionalized with Methotrexate: A Novel Nanotherapeutic Approach for Improved Antitumoral and Antioxidant Activity and Enhanced Biocompatibility. *Drug Delivery*, *Submission ID: 236177022*

9. Uritu CM, Al-Matarneh C, Bostiog D, Coroaba A, Ghizdovat V, Filipiuc S, Simionescu N, Stefanescu C, Nastasa V, Tamba B, Maier S, Pinteala M. Surface-modified gold nanoparticles: A step toward structural-functional hybrid PET/SPECT radiopharmaceuticals. *Journal of Materials Chemistry B*, în etapa de recenzie (https://submissions.rsc.org/tracker/TB-ART-11-2023-002654?t=YqB9CQL792c%2FxdmpilgzYUtTCpIxszlfF80TCVkq8BuHVQ%3D%3D)

10. Bostiog DI, Nastasa V, Al-Matarneh MC, Peptanariu D, Mares M, Pasca S-A, Bostanaru-Iliescu A-C, Craciun BF, Maier SS, Pinteala M. Simple yet versatile conjugation and decoration strategy to produce

nanoparticulate gene vectors. In vitro and in vivo evidence of their functionality and safety in use. *Heliyon*, HELIYON-D-23-53295 (https://www2.cloud.editorialmanager.com/heliyon/default2.aspx)

11. Carp OE, Pinteala M, Arvinte A. Electrodeposition of Au nanoparticles on 2D layered materials and their applications in electrocatalysis of nitrite. *Nano Materials Science*, în etapa de evaluare, *manuscript ID: NMS-2023-0176.*

Invention Patent Application no. A/00143/2023 entitled: "Procedure for obtaining supramolecular compact aggregates of gold nanoparticles, with a wide absorption spectrum in the visible range, with applications in photothermia", Authors: Ursu Elena-Laura, Sardaru Monica-Cornelia, Ursu Cristian, Rotaru Alexandru.

Research internship:

1. Fifere Adrian - Research internship at "Ilie Murgulescu" Institute of Physical Chemistry in Bucharest, from February 5 to 11, 2023

Director proiect,

Dr. Mariana Pinteală

Bibliografie

[1] M.L. Forrest, N. Gabrielson, D.W. Pack, Cyclodextrin–polyethylenimine conjugates for targeted in vitro gene delivery, *Biotechnol. Bioeng*. 89 (4) (2005) 416–423, https://doi.org/10.1002/bit.20356.

[2] S.H. Pun, N.C. Bellocq, A. Liu, G. Jensen, T. Machemer, E. Quijano, et al., Cyclodextrin-modified polyethylenimine polymers for gene delivery, *Bioconjug. Chem*. 15 (4) (2004) 831–840, https://doi.org/10.1021/bc049891g.