Registration number,
"Petru Poni" Institute
of Macromolecular Chemistry

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SCIENTIFIC STAGE REPORT

PN-III-P4-ID-PCCF-2016-0050 Project. Contract no. 4/2018.

-- 2021 Stage --

Mimicking Living Matter Mechanisms by Five-dimensional Chemistry Approaches

Mimarea mecanismelor viului prin abordări ale chimiei supramoleculare, în cinci dimensiuni

Acronym: 5D-nanoP

Project WEB site: https://www.intelcentru.ro/5D-nanoP/ro/ and https://www.intelcentru.ro/5D-nanoP/

Objective of 2021 stage: ***Extended study of the reproducibility of nanoplatforms characteristics and functionality***

Synopsis of the stage results:

Based on the conceptual, experimental and investigative achievements accumulated until the current stage, during the 2021 refinements were done regarding all the applicative aspects of supramolecular nanoplatforms obtaining. Major advances resulted by (**i**) *ex vivo* proofing of the nanoplatforms functionality, (**ii**) *in vitro* and *ex vivo* testing of the biologic support offered by the extracellular matrices developed until now, and (**iii**) *in silico* demonstration and confirmation of the validity of the mechanisms that we have studied in laboratory regarding the nanoplatforms use in the biomedical field.

In terms of scientific production, the 2021 stage was materialized in 23 papers published in ISI ranked journals, 2 proceedings, three book chapters, 16 oral communications, two key note conferences, four posters presentation, one work-shop organized in Iasi, and one PhD thesis defended on topics related to 5D-nanoP project.

*This report summarizes work performed on the **5D-nanoP** project during the period January - December 2021.*

Stage 2021 Work Plan

Code	Activities carried out during the 2021 stage of 5D-nanoP project
4.1.	**Study of the reproducibility of synthesis pathways**
4.1.1.	- *In silico* molecular modeling and simulation studies.
4.1.2.	- 5D-chemistry approaches. Selection and synthesis of unimers. Unimers libraries building-up. Design and preliminary investigation of nanoplatforms applications.
4.1.3.	- Design, synthesis, and characterization of binding-segments between macromolecular components and substrata.
4.1.4.	- Obtaining and characterization of surrogate-substrata of biomedical relevance (tissues and/or tumors).
4.1.5.	- Design, synthesis, and characterization of functional ligands (bio-degradable and bio-active binding-segments).
4.1.6.	- Compositional and structural studies on the conformational peculiarities of the nanoplatforms.
4.2.	**Study of the reproducibility of supramolecular aggregation of unimers**
4.2.1.	- *In silico* molecular modeling and simulation studies.
4.2.2.	- 5D-chemistry approaches. Refinement of synthesis protocols. Peculiar syntheses.
4.2.3.	- Advanced compositional and structural studies on the conformational details of the nanoplatforms.
4.3.	**Study of the reproducibility of functionality and release-ability of supramolecular aggregates**
4.3.1.	- *In silico* molecular modeling and simulation studies.
4.3.2.	- 5D-chemistry approaches. Design and preliminary investigation of supramolecular constructs.
4.3.3.	- Design, synthesis, and characterization of functional ligands (chemically activatable segments).
4.3.4.	- Obtaining and characterization of surrogate-substrata applicable as bio-active scaffolds.
4.3.5.	- Design, synthesis, and characterization of functional ligands (conditionally-stable segments).
4.3.6.	- Evaluation of the functionality of nanoplatforms in cultured cells and cell-populated tissue surrogates.
4.3.7.	- Advanced compositional and structural studies on the supramolecular constructs.
4.4.	**Study on the reproducibility of the characteristics of (bio)macromolecular substrata developed for the *ex vivo* testing of nanoplatforms**
4.4.1.	- 5D-chemistry approaches. Refinement of the investigation protocols of supramolecular constructs.
4.4.2.	- Obtaining and characterization of surrogate-substrata applicable as bio-active matrices.
4.4.3.	- Evaluation of the functionality of nanoplatforms on cells cultured into bio-active matrices.
4.5.	**Study on the reproducibility of the *ex vivo* and *in vivo* applications of the developed nanoplatforms.**
4.5.1.	- 5D-chemistry approaches. Refinement of the unimer members of the building-blocks library.
4.5.2.	- Evaluation of the functionality of nanoplatforms in cultured cells and cell-populated scaffolds.
4.6.	**Study on the fate of the disassembled / degraded components of nanoplatforms in simulated biological milieus**
4.6.1.	- 5D-chemistry approaches. Processed of disassembling / degradation of supramolecular constructs.
4.6.2.	- Evaluation of the functionality of altered / modified / affected nanoplatforms.

The research results of 2021 stage
A detailed report can be found at https://www.intelcentru.ro/5D-nanoP/

4.1.1.; 4.2.1.; 4.3.1. *In silico* molecular modeling and simulation studies

In silico studies performed by applying the methods of computational chemistry to obtain details on the molecular level of structures and processes among nano complexes and assemblies interacting with biomolecules, including proteins, nucleic acids, lipid membranes, and carbohydrates. New theoretical models and computational methods and tests were developed for the novel systems, with a focus on pharmacology and drug and gene delivery. The very purpose is to intimately combine modeling and experiments. A complete reference list of publications with 5DnanoP acknowledgements can be found in the reference list of *Book of Abstracts: 5D-nanoP Workshop* on October 11th, at "Petru Poni" Institute (https://www.intelcentru.ro/5D-nanoP/). Some of this work is summarized in three separate book chapters which appeared in 2021 in two monographs published by Springer Nature: "*Soft Matter Systems for Biomolecular Applications*" (Edited by *Bulavin* et al.) and in *"New Trends in Macromolecular and Supramolecular Chemistry for Biological Applications"* (Edited by *Pinteala* et al.). In the chapters are discussed "Molecular Perspective on Solutions and Liquid Mixtures from Modelling & Experiment" focusing on their non-ideal behavior and properties as liquid medium in nano- and macro-molecular

assembly. Also new so-called "neoteric liquids", *i.e.*, ionic liquids (IL) and deep eutectic solvent (DES) mixtures are highlighted, which will find more and more applications. DNA-polyanion interactions are discussed from *in-silico* point of view in "DNA-polyamine interactions: Insight from Molecular Dynamics simulations on the sequence-specific binding of spermidine", focusing on complex-forming between charged polymers and gene material. In "Inverse Problems and Hierarchical Multiscale Modelling of Biological Matter" we review our own hierarchical multi-scale modelling methodology from the point of solving inverse problems in Physics. This method is the most accurate computational scheme to connect length and time scales in biological modelling. We show many applications and at the end we discuss how to apply it in modeling the chromosome by means of super-coarse graining (Figure 1).

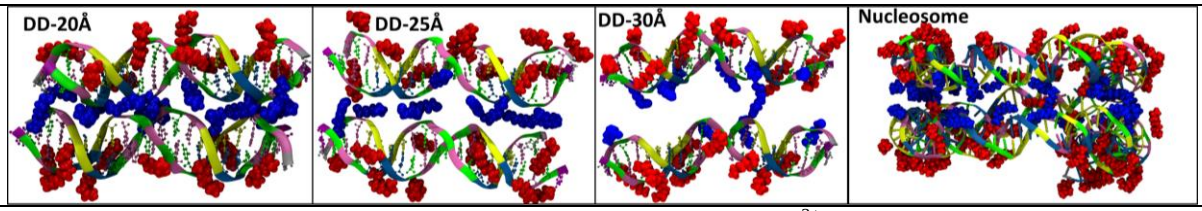


Figure 1. *Representative snapshot of the distribution of spermidine^{3+} around the two DNA molecules of each system and around the nucleosome. Water and ions are omitted for clarity. The spermidine^{3+} is colored in red and blue (Blue for the spermidine^{3+} that is situated in the space between the two DNA molecules (caged), and Red for the others (uncaged).)*

As our flagship publication in 2021 in RSC Biomaterials Science journal is the work of Vasiliu et al. "*In-silico* study of PEI-PEG-squalene-dsDNA polyplex formation: the delicate role of the PEG length in the binding of PEI to DNA" [**23**], where we study a polyplex formation mimicking very closely the experiments using a two-step protocol and large-scale high-performance MD simulations by systematically varying the cationic and anionic polymer materials to cover DNA under gene transport. It is an excellent example of unifying the competence from laboratory with computational chemistry techniques. We will continue this work in 2022 to investigate the translocation of the polyplex vector through cell membrane. To fine-tune a series of advanced experimental techniques we have systematically studied adsorption and cohesive interactions of single proteins on biocompatible surfaces such TiO_2 by varying the environmental parameters. We have used (colloid probe) atomic force microscopy (CP-AFM), surface enhanced Raman Spectroscopy (SERS) and surface force apparatus (SFA). We developed a novel coarse-grained force-field between proteins and a surface of varying characteristic (from hydrophobic to hydrophilic). Nothing like this did not exist before. These studies have led us to investigate a new category of tunable complex liquid systems, ionic liquids, which have numerous beneficial properties and are called the solvents of the new millennium. They contain all possible types of molecular interactions which can be optimized to design very specific applications, most recently in biopharma where amino acid ionic liquids are used as active pharmaceutical ingredients (API). Ionic liquid materials, including their polymers and liquid crystals with their properties and their applications were studied. Two comprehensive reviews have been published on ionic liquids and deep eutectic solvents. We have studied enzymatic catalysis by investigating if artificial co-factors work more efficiently than natural. We have chosen a NAD dependent formate dehydrogenase which catalyzes the oxidation of CO2 in yeast and bacteria to formic acid as a prototype system. As artificial cofactors we use bipyridium-based salts and reach an efficiency of almost two orders of magnitude compared to NADH . There are many applications of using this system, for example in combined electro-enzymatic conversion. Electrochemical reductions are discussed by us in a review article.

4.1.2.; 4.1.3. 4.1.5. 4.1.6. 4.2.2.; 4.2.3.; 4.3.2.; 4.3.3; 4.4.1.; 4.5.1.; 4.6.1. 5D chemistry approaches. Selection and synthesis of unimers. Building the library of unimers. Design and investigation of potential applications of developed nanoplatforms and constructs.

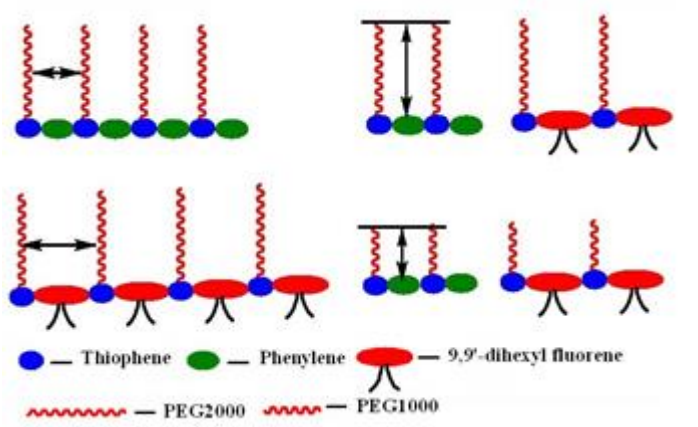
a. Enzyme-activatable nanoplatforms based on conjugated conducting amphiphilic polymers designed for combinatorial fluorescence imaging-guided diagnosis and dual-mode photodynamic and chemotherapy

Our main objective was to obtain a water-self dispersible material that spontaneously forms micelles in aqueous media having the *optimal properties for the construction of new nanoplatforms* useful

in cancer diseases treatment. *We expect that these new platforms, with an enhanced stability due to the branched architecture, can efficiently and preferentially accumulate at the tumor site via EPR effect, to release an encapsulated drug in a controlled manner, under the action of an endogenous stimulus which will allow for micellar carrier disintegration*. Moreover, due to the properties of the main chain (fluorescence, redox properties) these formulations were *designed to simultaneously allow* ***(i)*** *diagnosis of the cancer cells by fluorescence imaging, and* ***(ii)*** *tumor ablation by photodynamic therapy using light irradiation.*

As can be seen in Figure 1, we proposed a "bottom-up" manipulation of the copolymers structure to concomitantly *verify the reproducibility* of the chosen synthesis pathways and to *tune the functionality of the obtained materials* (like photophysical properties, the size of the self-assembled micellar structures, the supramolecular morphology). Both were succeeded through a combination of two ways:

- by modifying the distance between the PEG side chains by using comonomers with different size (phenylen and fluorene) (A in Figure 2);
- by changing the length of the attached PEG side chains varying their molecular weight (B in Figure 2).



(A) *(B)*

Figure 2*. Schematic representation of the adopted design criteria for synthesized fluorescent, water-self dispersible conjugated copolymers for the modulation of the micellar nanoplatforms' properties.*

A further aim of our work was to obtain poly(thienyl arylenes) based copolymers with PEG lateral chains keeping the "hairy-rod" (HR) branched architecture as we reported before. Among the HR-CPs synthesis methods, the *"macromonomer technique"* was employed due to its advantages related to well-defined grafting density and side-chain length, defect-free polymer structures and easy access to copolymer synthesis. This method enables the elaborate design, preparation, and complete characterization of the side chains prior to the ultimate step-growth polymerization. For this purpose, the followed strategy was to obtain in the first step PEG based macromonomers functionalized with 2,5-dibromothiophene moieties using post-polymerization chain-ends modification.

It can be concluded that *the PEG side chains length as well as the distance between the branching points of these side chains on the main conjugated chain are crucial influence parameters on the molecular weight of the obtained copolymers*. Thus, the increase of the PEG side chains length and the distance between the branching points is a good option for the increase of the copolymers molecular weight. Moreover, also the choice of low molecular weight comonomer size has a direct influence on the course of the Suzuki polycondensation reaction; thus, the bigger size fluorene ring most probable decreased the hindrance between two consecutive side chains allowing in this way an easier enchainment of a new thiophene macromonomer molecule at the reactive conjugated chain end. This fact could have as the consequence and could explain the obtainment of an alternating copolymer with a higher molecular weight. *The copolymers' molecular weight it is the most important aspect to be considered because it influences the copolymers behavior and functionalities in solution, in bulk and in thin film.*

In conclusion, during 2021 stage, the following findings are to be noted.

- Four amphiphilic conjugated graft copolymers were synthesized, having PEG side chains of two different lengths placed at two different distances between the adjacent ones, by combination of the "macromonomer technique" with Suzuki polycondensation, and were structurally characterized.
- It was demonstrated that the synthesis pathway is reproducible, resulting in high molecular weight materials characterized by the same architecture, regardless of the comonomer chosen.
- The chosen synthesis strategy allowed for the bottom-up structural manipulation in such a way as to give the possibility to obtain an optimal variant of material for the targeted application.

- It was demonstrated that the PEG side chains length as well as the distance between the branching are crucial influence parameters on the molecular weight of the obtained copolymers, which in its turn will determine the copolymers functionality.
- The optimal copolymeric variant having the smallest size and the highest fluorescence intensity was further used as carrier for doxorubicin drug, and its encapsulation was successfully performed.
- The capability of the obtained water-dispersible nanoplatform to deliver the cargo by enzyme-activatable disintegration was assessed by a study of the hydrolytic degradation.

All the above experimental findings are favorable to establish the foundation to further design proper structure of amphiphilic copolymers as nanocarrier systems for efficient anticancer therapy.

b. Synthetic pathways to obtain the functional ligands based on 3-amonopropylsilatrane

At this stage, the conditions, and the synthetic pathways to achieve and characterize the designed nanoplatforms and their functional ligands previously confirmed from a structural point of view by analytical, spectral, and single crystal X-ray diffraction methods, were optimized and validated (Figure 3).

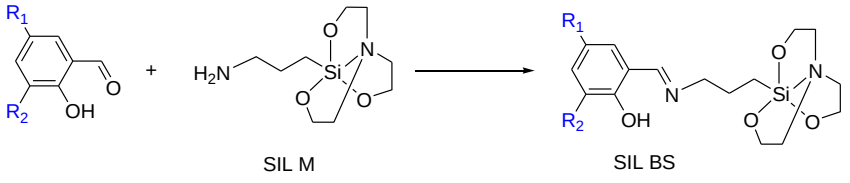


Figure 3. *The synthetic pathways to obtain the functional ligands based on 3-amonopropylsilatrane.*

The introduction of the ligand functionality was realized through aldehyde precursors with different groups: nitro, chlorine, methoxy, *tert*-butyl. The synthesis routes used in their preparation were the conventional ones (Figure 4) made in 3 stages: (I) mixing the reactants; (II) heating / magnetic stirring at room temperature of the mixture of functional precursors (with amino and carbonyl groups) leading to the formation of the Schiff base functional ligands with multiple functionalities (imine, hydroxyl, nitro / methoxy / chlorine / tert-butyl); (III) filtration and crystallization of the functional ligands, followed by their structural confirmation by single crystal X-ray diffraction.

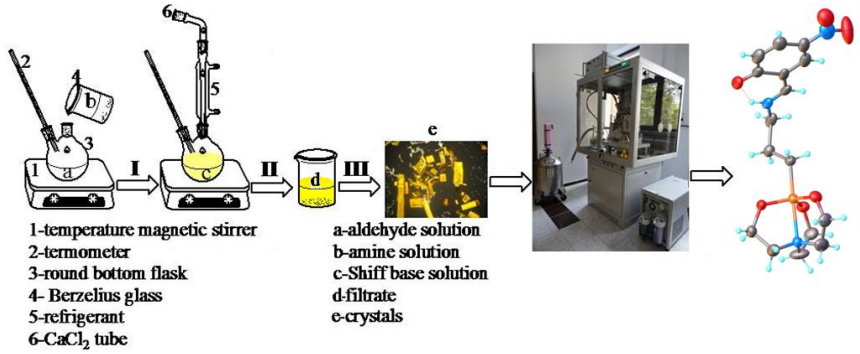


Figure 4. *The reproducible synthesis pathways in the preparation of the functional ligands.*

A major importance has been given to the selectivity of the functional ligands based on in vitro pre-experiments evaluation of their biocompatibility / cytotoxicity on normal and cancer cell lines. Based on these results, the most promising compound was the one with "nitro" groups.

The "nitro" functional group has a special importance in medicinal chemistry, the compounds having this function being applied either as drugs in the treatment of cardiovascular, ocular or tumor diseases, or as proligands with in situ bio-reduction mechanism.

c. The design and the development of "intelligent" micro-vehicles for the transport and delivery of drugs to different cell compartments

Poly(N-isopropylacrylamide-co-4-vinylpyridine) (poly(NIPAAm-co-4-VP)) was synthesized with different co-monomer composition as a dual pH/thermosensitive copolymer. The solubility characteristics (LCST) were determined at different pH corresponding to those of cell compartments and at the human body temperature. The copolymer with optimal composition was transformed in solid microparticles loaded with the model antitumoral drug, dexamethasone (Dex). Under normal physiological conditions

(PB at pH = 7.4 and T = 36 °C), the microparticles are completely insoluble and preserve the drug while at acidic conditions, simulating the pH of the cell compartments, the drug is almost completely released (Figure 5.).

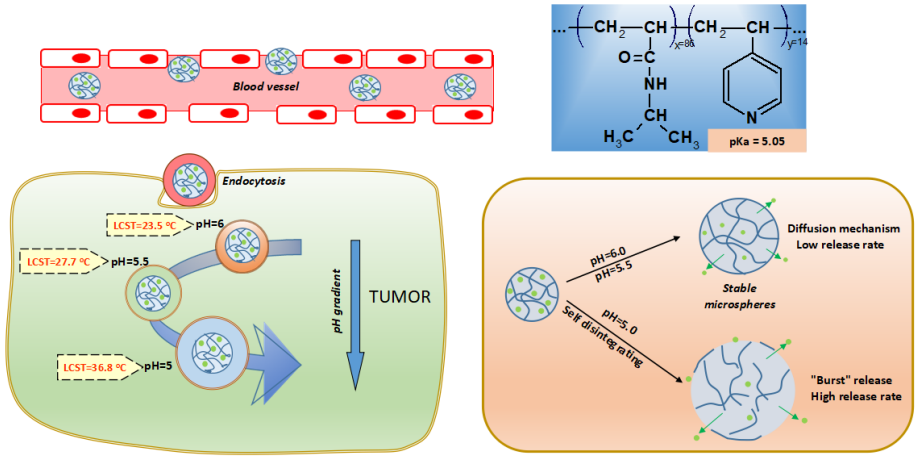


Figure 5. *Schematic representation of intelligent micro-vehicles action after i.v. administration.*

d. Carbonic anhydrases metalloenzymes are widely spread in all the biological kingdoms and catalyse the simple reaction of carbon dioxide hydratation in carbonic acid: $CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$. This reaction is involved in a wide range of physiologic reactions including respiration, photosynthesis, pH regulation, electrolyte secretion, and many biosynthetic reactions. For this reason, in 2021 stage of the project, much effort has been made to study the applications of new CA inhibitors as novel therapeutics agents.

I- Small molecules containing chalcogenide atom acting as CA inhibitors (CAIs) with antitumor activity. In the last years, tellurium compounds have fuelled a renewed interest due to its unique properties associated in diagnostics and drugs development for tumour growth inhibition. In this context, we synthetized and evaluated *in vitro* (Figure 6.A) a series of telluride bearing benzenesulfonamide moiety as effective inhibitors of the tumor relevant Carbonic Anhydrase (CA; EC 4.2.1.1) IX. The potent effects against this isoform (K_I 2.2 to 2.9 nM, Figure 6.B) gives an opportunity to explore them as possible antitumor agents. Among the series, two compounds (**3a** and **3g**) are chosen and evaluate their lethal effect *in vitro* against a breast cancer cell line (MDA-MB-231) where the expression of hCA IX is higher than other tumour cell lines and increased with the degree of hypoxia (Figure 6.C).

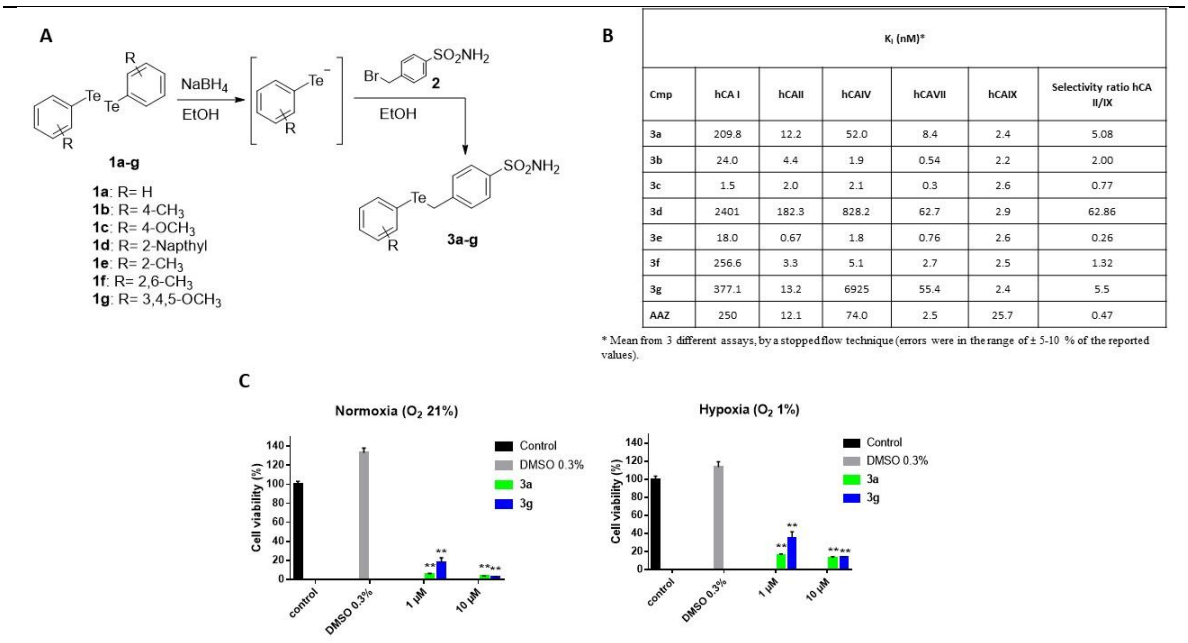


Figure 6. ***A****) Synthesis of tellurides bearing benzenesulfonamide;* ***B****) Inhibition data of human CA isoforms I, II, IV, VII and IX;* ***C****) Effects of telluride derivatives* ***3a*** *and* ***3g*** *on cell line MDA-MB231 in normoxic (21% O_2,) and hypoxic (1% O_2,) conditions.*

II- Small molecules containing chalcogenide atom acting as CA inhibitors (CAIs) with pain relief and antitumor activity. The antiproliferative properties of platinum drugs were widely used and their side effects have been attentively evaluated and the most common one is peripheral neuropathy with no existing effective treatments against it, which provokes serious difficulties to the patients for completing a full treatment schedule. The combined antiproliferative and lowering neuropathic pain properties of our organochalcogenides give an innovative approach for the counteraction and management of side effects associated with clinically platinum drugs as antitumor agents (Figure 7).

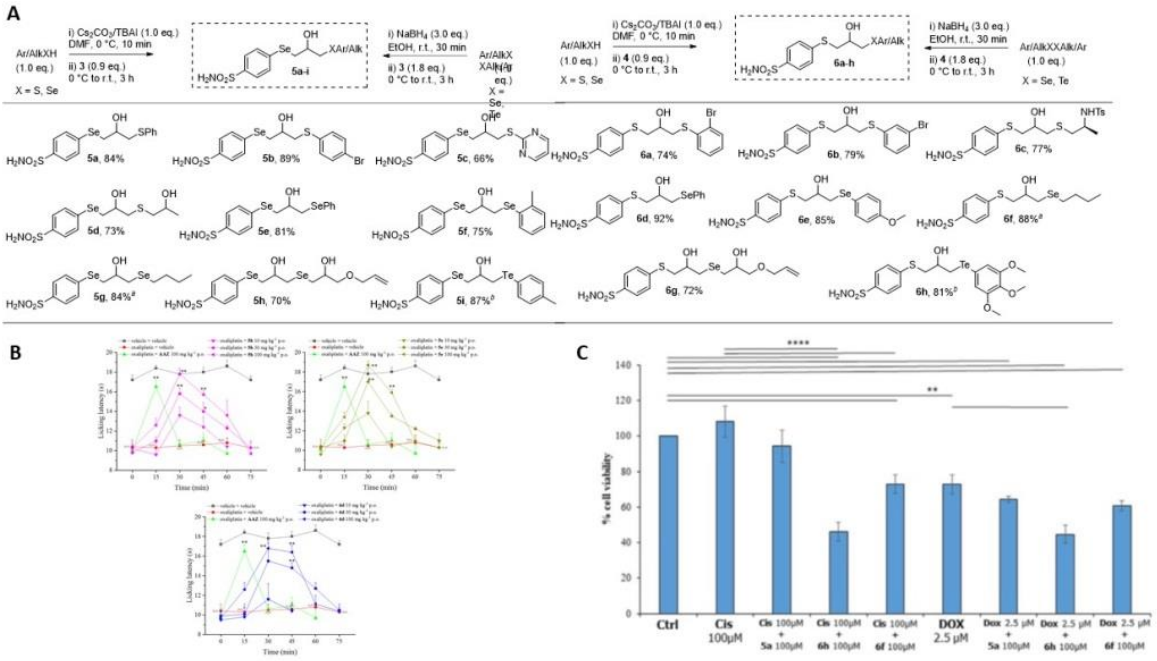


Figure 7. A*) Synthesis of organochalcogen compounds;* ***B****) Effect of acute administration of compounds on oxaliplatin-induced neuropathic pain in the mouse;* ***C****) MTT cell viability assay on MCF7 cell line.*

III. Structural insights into *Schistosoma mansoni* carbonic anhydrase (SmCA) by selenoureido-substituted benzenesulfonamides. The WHO considers schistosomiasis among the principal neglected tropical diseases afflicting subtropical and tropical regions. Tegumental carbonic anhydrase from the worm *Schistosoma mansoni* (SmCA) is considered a new anti-parasitic target because suppressing its expression interferes with schistosome metabolism and virulence. In this context, we used organochalcogen compounds, and more exactly selenoureido derivatives, to study deeply the unique features of parasitic CA by means X-ray technique solving the crystal structures of SmCA in adduct with these compounds. The key molecular features of such compounds in adduct with SmCA were obtained and compared to the human isoform hCA II, in order to understand the main structural factors responsible for enzymatic affinity and selectivity. Information from these studies provides a foundation for understanding SARs between selenoureido and its isostere derivatives and SmCA showing different favorable/unfavorable contacts between the inhibitor tail moiety and the enzyme active site, leading to different inhibition profiles for this class of sulfonamides.

e. Investigation of the antitumor properties of PEGylated derivatives synthesized in 2020 (Figure 8).

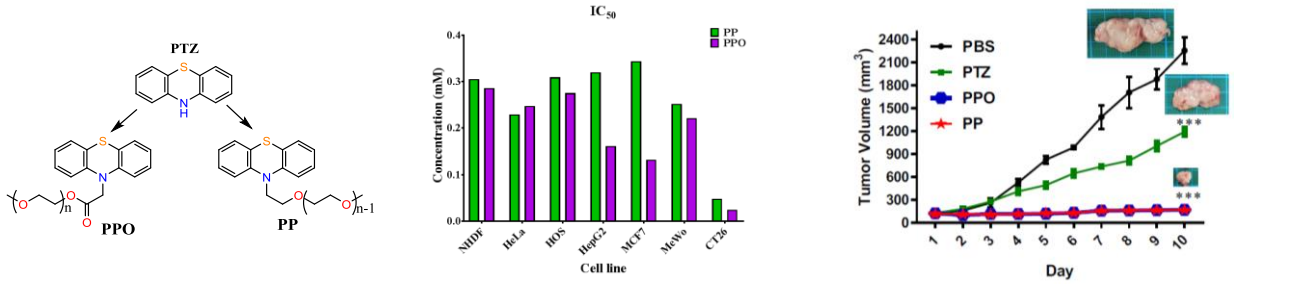


Figure 8. *The structure of the studied compounds.*

Figure 9*. Cell viability of cancerous and normal cells.*

Figure 10. *Evolution of tumor volume over the treatment period and representative images of tumors at the end of the 10-day monitoring period.*

Antitumor activity has been investigated in five human tumor lines (Figure 9): cervical cancer (HeLa); melanoma (MeWo); osteosarcoma (HOS), breast cancer (MCF7), and liver cancer (HepG2), versus normal cell line (NHDF). To explain the antitumor mechanism of action of these compounds, self-assembly tests were performed in the vicinity of cancer cells and inhibition of overexpressed enzymes in cancer cells was measured, in parallel with the observation of tumor morphology and dimensions (Figure 10).

f. The importance of exogenous natural antioxidants and how they are metabolized.

Given that knee osteoarthritis is a chronic pathology with an incompletely known pathophysiology, the concise presentation of current research state in this topic may provide a new perspective on understanding the physiological processes governed by ROS (Figure 11).

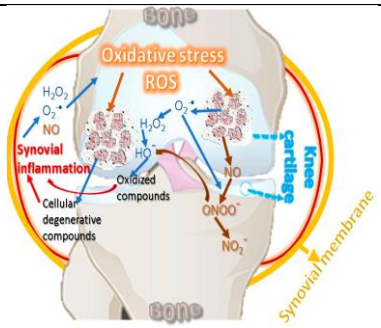


Figure 11. *Oxidative stress and inflammation reciprocal cause-and-effect cycle in KOA.*

4.1.4.; 4.3.4.; 4.3.5.; 4.3.6.; 4.3.7.; 4.4.2. Production and characterization of surrogates for bio-medical testing (tissues and / or tumors).

a. Surrogate based on collagen / hyaluronic acid / poly(ε-caprolactone)

Collagen-based 3D materials are extensively used in biomedical applications (scaffolds in tissue engineering, lab models for drug screening, modern wound dressings, and drug delivery devices) due to their intrinsic characteristics. The most envisaged are: biocompatibility and inherent bio-functionality enabling cells' proliferation and differentiation, coupled with hemostatic properties, low immunogenicity, a rich chemistry, and high water-holding capacity. A porous architecture facilitates cell adhesion and improves flexibility and substance permeability, finally promoting 3D structure biomimicry. However, the application of this protein for biomedical devices, as a unique raw source, is limited by some disadvantages, such as the poor mechanical and antibacterial properties, fast degradation, modification and processing difficulties, some batch-to-batch variations, and risk of human transgenic disease transmission. Cross-linking (by physical, chemical, or combined techniques) and combination with other polymers (natural or synthetic) or materials (i.e., ceramics) may be used as typical, convenient routes to avoid the biopolymer drawbacks and to improve/control the physical-chemical and biological properties of collagen-based materials, to meet specific needs.

In this context, aiming to develop new modern tissue surrogates, 2021 stage study was dedicated to a comparative evaluation of hybrid 3D structures, containing natural and synthetic polymers (i.e., atelocollagen, hyaluronic acid and poly(ε-caprolactone) derivatives), with different architectures. The hybrid matrices were obtained according to previous published procedures, i.e., by: (**i**) vitrigel preparation strategy (implying a vitrification step), (**ii**) simple lyophilization, or (**iii**) cryogelation followed by lyophilization (Figure 12). The characterization is presented in Figures 13 and 14.

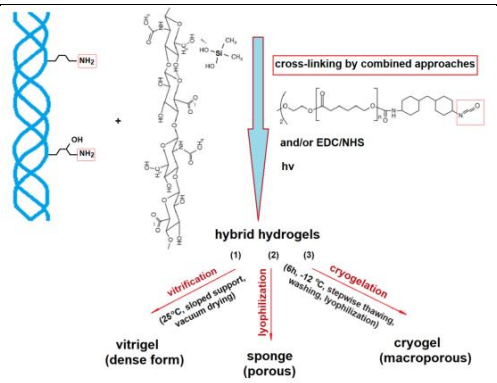


***Figure 12**. Schematic representation of the preparative protocol for the investigated hybrid 3D constructs.*

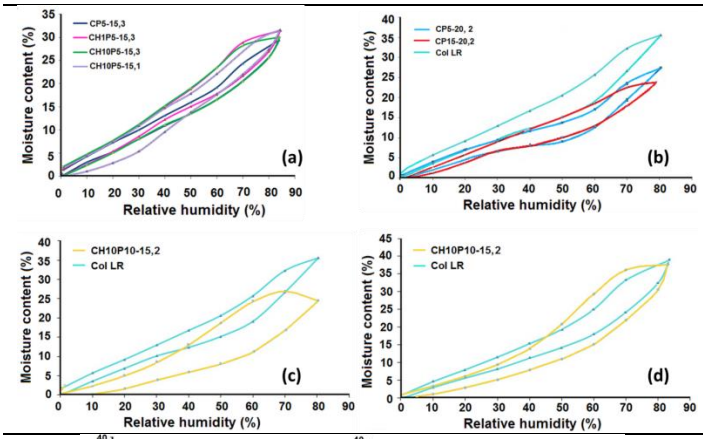


***Figure 13.** Typical water vapor sorption–desorption isotherms - effect of composition. Registration conditions: (a), (b) and (c) equilibrium time 10/20 min; (d) equilibrium time 40/60 min.*

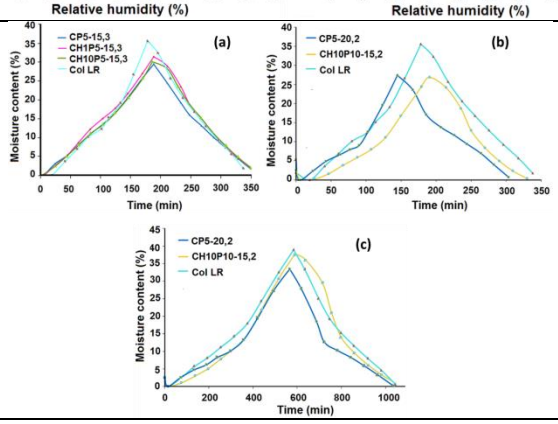


***Figure 14.** Kinetics of water sorption/desorption for different experimental conditions (equilibrium time): (a) and (b) equilibrium time 10/20 min; (c) equilibrium time 40/60 min.*

Hybrid collagen-based 3D structures comprising PCL and a hyaluronic acid derivative (in dense, porous, or macroporous form) were investigated in comparison with a commercially available collagen sponge, for intended applications in biomedical area (for tumor or tissue surrogates)). According our results, all samples favour water absorption and moisture vapour penetration, but only the porous/macroporous samples exhibited a comparable behaviour with the commercial product (reference sample), pointing on the important effect of porosity, followed by the crosslinking degree and formulation.

b. Tissue surrogates based on thermosensitive and injectable poloxamer-*graft*-carboxymethyl pullulan hydrogel.

The synthesis of an injectable carboxymethyl pullulan-graft-poloxamer copolymer (Plx-g-CMP) is presented in Figures 15 and 16. The effect of polymer concentration on the gelation behavior was analyzed by different methods. The influence of the rest temperature before tests on the gelation starting time was also analyzed. Finally, various physico-chemical properties of the new copolymer related to its potential application as a drug delivery system were investigated.

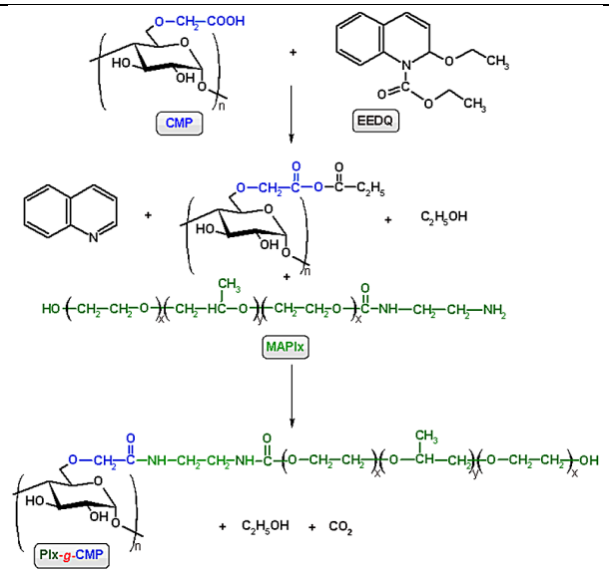


***Figure 15**. Synthesis route of poloxamer-graft-carboxymethyl pullulan copolymer*

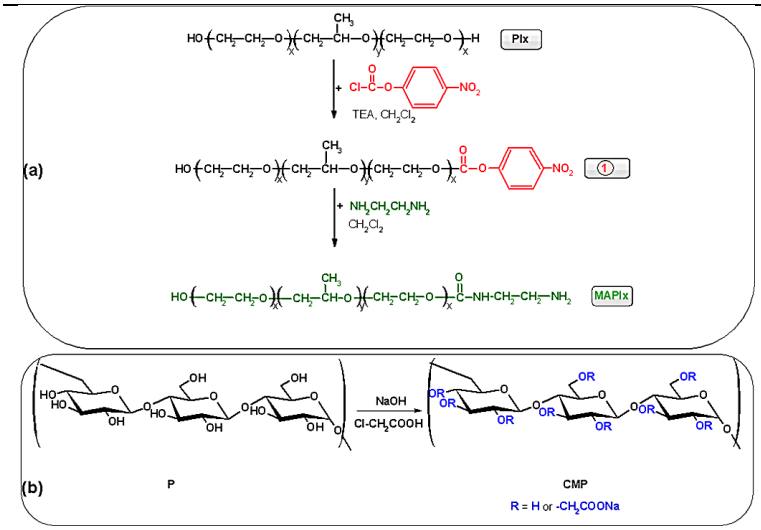


***Figure 16**. Synthesis route of monoamine derivative of poloxamer (MAPlx) (a) and carboxymethyl pullulan (b).*

In conclusion, poloxamer-*graft*-carboxymethyl pullulan (Plx-*g*-CMP) copolymer was succesufully synthesized by coupling reaction between carboxymethyl groups of pullulan and amine groups introduced on poloxamer. The grafted copolymer has the ability to form gels under simulated physiological conditions (phosphate buffer at pH=7.4, and 37 °C). The sol-gel transition temperature of the copolymer is lower (20 °C) than that of Plx (25 °C) at the same concentration (18%, w/v). Moreover, the sol-gel transition of grafted copolymer occurs down to a concentration of 11% (w/v), while the gelation of Poloxamer 407 does not occur at concentrations lower than 18% (w/v).

c. Silatranes and their derivatives: Design, synthesis, and characterization of functional ligands (including bio-degradable and bio-active segments).

Based on the studies of the reproducibility of the functionality of the ligands in environments with different pH, done by UV-VIS and NMR spectral methods, the stability conditions of the functional ligands were established by evaluating: the interaction with the biological environment (study of biocompatibility / cytotoxicity, bio- / mucoadhesivity); the mechanism of interaction with proteins / enzymes considered "target" in antitumor / antiviral therapy (cancer, SARS-CoV); The interaction with the biological environment was studied *in vitro* on normal cells (fibroblasts) and tumor cells (MCF-7 and HEPG2) by CellTiter-Glo technique, a procedure that involves the addition of a single reagent (CellTiter-Glo® Reagent) directly to the cells grown in the medium. The addition of the CellTiter-Glo reagent results in cell lysis and the generation of a luminescent signal proportional to the amount of ATP in cells, this one being directly proportional to the number of cells in the culture. The results of silatrane (SIL M) and SIL BS ligand tests indicated a very good compatibility with the normal cells, the cell viability of NDHF (fibroblasts) being over 90% at concentrations of 0.8 μg / ml for SIL M and 0.05 μg / ml for SIL BS, respectively (Figure 17).

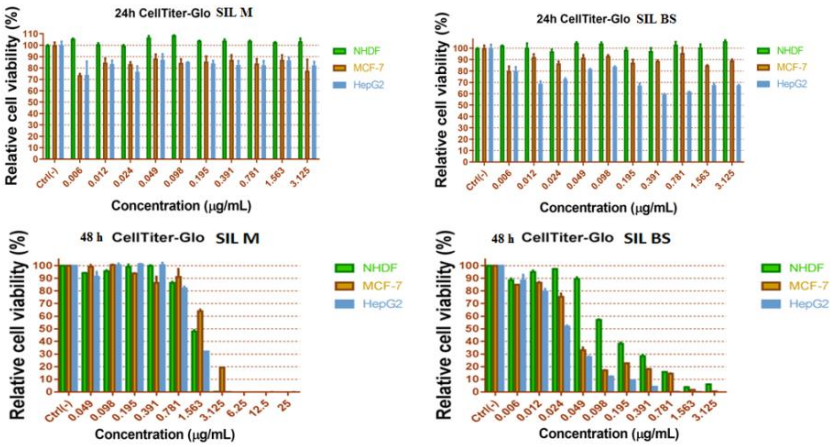


Figure 17. *The cell viability on normal cells (NHDF) and cancer cells (MCF-7 and HEPG2) against the studied silatrane compounds.*

	NHDF	MCF-7	HepG2	
IC50	0.1623	0.03346	0.02586	ug/mL

	NHDF	MCF-7	HepG2	
IC50	1.531	1.968	1.259	ug/mL

Bio / mucoadhesivity tests (Figure 18) were performed on cellulose dialysis membranes and pig intestine at pH 7.4 and 37 °C, respectively. The tests were performed with a TA.XT Plus analyzer that allowed the evaluation of the maximum adhesion force and mechanical adhesion work after applying a contact force of 1 gF, for 30 s.

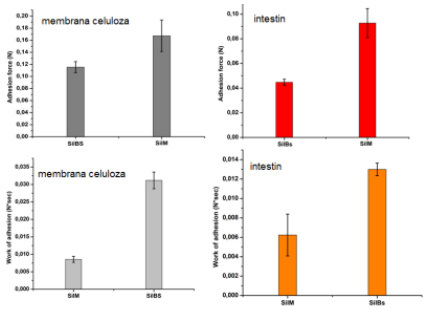


Figure 18. *The results of the bio/mucoadhesion tests of the studied silatrane compounds.*

The ability of the ligands to bind proteins in human / bovine serum and to inhibit certain enzymes considered as "target" for certain diseases (cancer, SARS-CoV) have been studied by UV-vis, fluorescence, circular dichroism, and molecular docking (Table 1, Table 2, Figure 19).

Table 1. *Contacting receptor residues in docked complexes (by studied ligands SIL-M and SIL-BS), and calculated affinity estimators (relative binding energy and dissociation constant).*

Docked complex (Receptor@Ligand)	No. of contacting residues	Contacting residues in the receptor (HSA)	E_b (kcal/mol)	K_d (µM)
HSA@**SIL-M**	14	LEU^{22}, VAL^{23}, ALA^{26}, PHE^{27}, TYR^{30}, VAL^{46}, LEU^{66}, HIS^{67}, PHE^{70}, ASN^{99}, ASP^{249}, LEU^{250}, LEU^{251}, GLU^{252}.	-7.470	3.345
HSA@**SIL-BS**	12	ARG^{114}, LEU^{115}, LEU^{135}, TYR^{138}, LEU^{139}, ILE^{142}, ARG^{145}, HIS^{146}, ALA^{158}, TYR^{161}, ARG^{186}, LYS^{190}	-9.315	0.148

Table 2. *Contacting receptor residues by ligand in docked complex M^{PRO}@SIL-M, and calculated affinity estimators (relative binding energy and dissociation constant).*

Docked complex (Receptor@Ligand)	No. of contacting residues	Contacting residues in the receptor (HSA)	E_b (kcal/mol)	K_d (µM)
M^{PRO}@**SIL-M**	14	PHE^{8}, LYS^{102}, GLN^{110}, THR^{111}, PHE^{112}, GLN^{127}, ASN^{151}, ILE^{152}, ASP^{153}, SER^{158},	-5.794	56.61

		THR[292], PHE[294], ASP[295], ARG[298]		

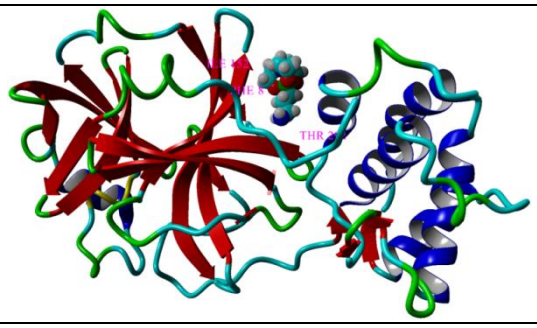


Figure 19. *Molecular rendering of the best docked pose showing the interaction between M^{PRO} receptor (COVID-19 main protease) and silatrane SIL-M.*

4.4.3. Study of the reproducibility of the characteristics of (bio)macromolecular matrices intended for *ex vivo* testing of nanoplatforms

A novel 3D cell model of human hepatocellular carcinoma (HepG2 cells) for drug testing was developed. The 3D platform was tested comparatively versus 2D by following the cytotoxicity and the apoptotic response to an anti-tumor agent, cisplatin. The synthesis of the new 3D scaffold was described in the previous 5D-NanoP report (2020). The scaffold was developed by the group of dr. Fundueanu and consists of a hyaluronic-based-hydrogel, namely HA^3P50, where "3" is the concentration of both polymers (%, w/v) in the initial mixture, and "50", the P(MVE-*alt*-MA) content (%, w/w) (Figure 20).

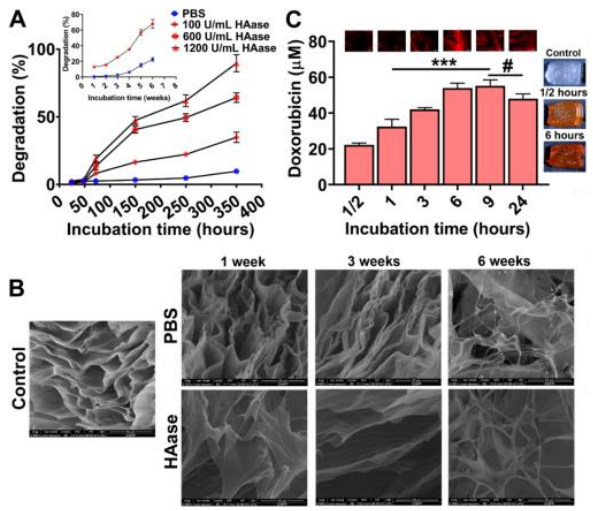


Figure 20. *In vitro degradation and drug-diffusion assessment for HA^3P50 scaffold. (A) Hydrolytic (PBS) and enzymatic (HAase, hyaluronidase) time and dose-depended degradation of the scaffold. (B) Scanning electron micrographs depicting the HA3 P50 scaffold prior (control) and after incubation in PBS and 100 U/mL HAase for 1, 3, and 6 weeks (scale bar: 50 μm). (C) Time-course of doxorubicin (MW: 543.52 g/mol) diffusion in the scaffolds. Above the graph, the representative fluorescence images are inserted (scale bar: 200 μm). Stereomicroscopic images of control scaffold and doxorubicin-loaded scaffolds after ½ and 6 hours of incubation are shown on the right. Results are shown as mean ± standard deviation (SD) from two experiments performed in duplicate and analyzed using unpaired two-tailed Student's t-test. ***$p < 0.001$ versus ½ hours and #$p < 0.05$ versus 24 hours.*

The resulting images indicated the spread of the fluorescent drug (doxorubicin) throughout the porous scaffold in close connection with its fibers. These observations were also supported by the stereomicroscopic images of the control and scaffolds incubated with doxorubicin for ½ hours and 6 hours (Figure 21.C, the right side of the graph). The following conclusions were drawn.

1. HepG2 cells grown in HA^3P50 scaffold exhibit liver-like functionality (Figure 21).

2. Culturing HepG2 cells in the HA^3P50 scaffold increases cell metabolism (Figure 22).

3. Culturing HepG2 cells in the HA3P50 scaffold decreases the secretion of transaminases (Figure 23).

4. Culturing HepG2 cells in the HA3P50 scaffold enhances cell chemosensitivity to cisplatin (Figure 24).

5. Cisplatin treatment of HepG2 cells induces DNA fragmentation and rearrangements of the cytoskeleton (Figure 25).

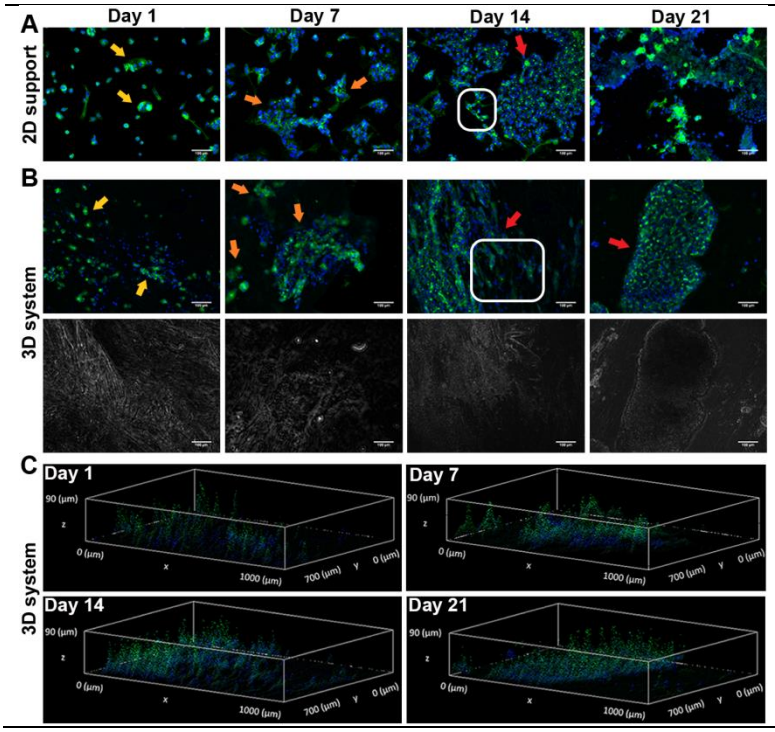


Figure 21. *Assessment of the distribution of F-actin in HepG2 cells cultured on 2D support and in HA[3]P50 scaffold (3D). HepG2 cells were grown on 2D supports and in 3D systems for 1, 7, 14 and 21 days (A) Representative phalloidin-FITC/DAPI merged images for 2D cell culture. (B) Representative phalloidin-FITC/DAPI merged images (up) and phase-contrast images (down) for 3D cell system (green, actin and blue, cell nuclei). The yellow arrows point to the closely grouped cells attached to the scaffold's fibers or plastic support, the orange arrows show cell clusters, the red arrows indicate the cellular aggregates, and white squares indicate the extension of cluster cells. Scale bar: 100 µm. (C) 3D reconstruction images of Z-stacking sections for phalloidin-FITC and DAPI staining of cellularized 3D systems.*

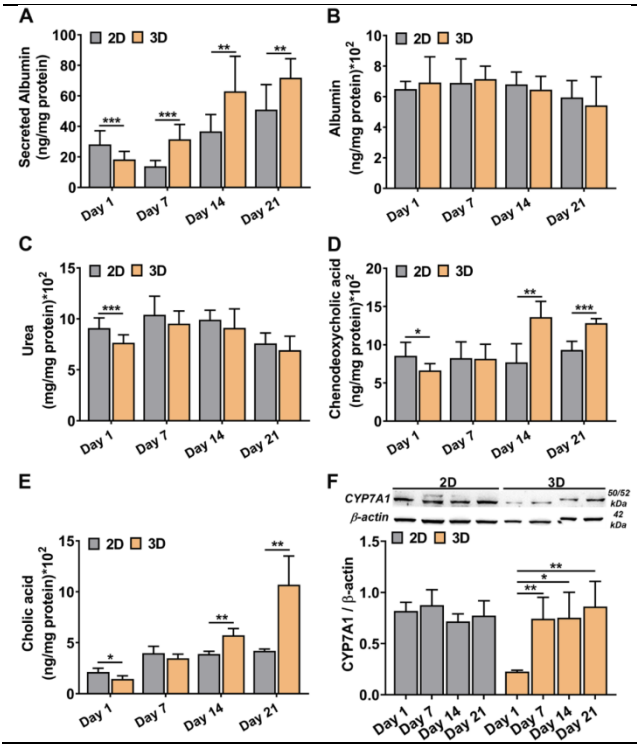


Figure 22. *The metabolic status of HepG2 cells cultured on 2D support and in HA[3]P50 scaffold (3D). HepG2 cells were grown on 2D supports and in 3D systems for 1, 7, 14, and 21 days. The secreted (A) and intracellular (B) concentrations of albumin; the levels of urea (C), chenodeoxycholic acid (D), and cholic acid (E) in the cell medium, and the CYP7A1 protein expression (F). The results are shown as mean ± SD and analyzed using unpaired two-tailed Student's t-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.*

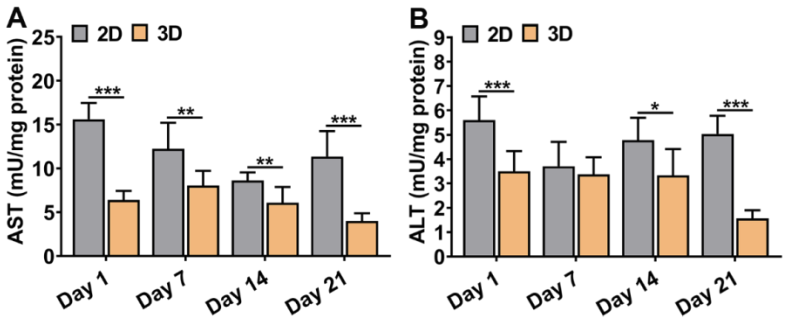


Figure 23. *The assessment of liver-injury biomarkers released by HepG2 cells cultured on 2D support and in HA³P50 scaffold (3D). HepG2 cells were grown on 2D supports and in 3D systems for 1, 7, 14, and 21 days. The enzymatic activities of aspartate aminotransferase (AST) (A) and alanine aminotransferase (ALT) (B). The results are presented as mean ± SD and analyzed using unpaired two-tailed Student's t-test: * p < 0.05, **p < 0.01, ***p < 0.001.*

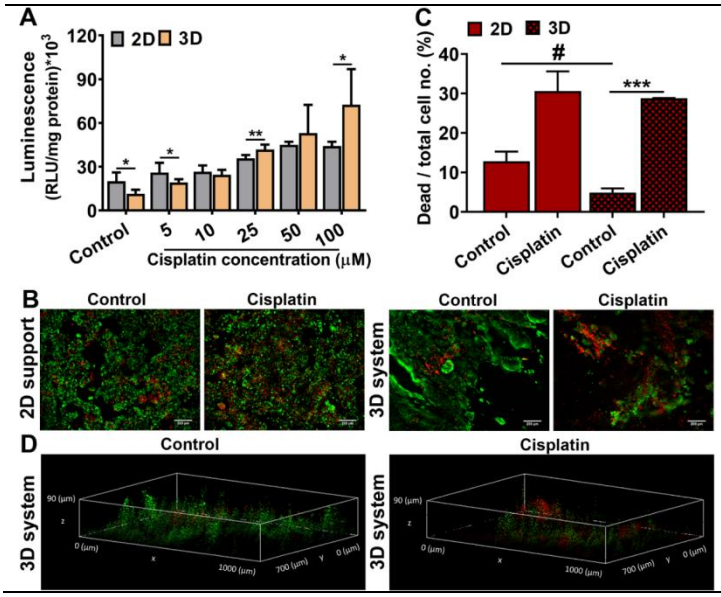


Figure 24. *Cisplatin-induced cytotoxicity in HepG2 cells cultured on 2D support and in HA³P50 scaffold (3D). HepG2 cells grown on 2D and in 3D systems for 7 days were exposed for 24 hours to 5-100 μM cisplatin or 0.1% DMSO (control cells). (A) The cisplatin cytotoxicity in HepG2 cells was investigated by the release of the adenylate kinase in the culture medium. (B) Live (green)/dead (red) assay of control cells and 25 μM cisplatin-treated HepG2 cells. Scale bar: 200 μm. (C) The percentage of dead/total cell number obtained by live/dead cell assay. (D) 3D reconstruction images of the Z-stacking sections for live/dead assay of control and cisplatin-treated HepG2 cells grown in HA3 P50 scaffold. The results were shown as mean ± SD and analyzed using unpaired two-tailed Student's t-test: * p < 0.05, **p < 0.01, ***p < 0.001 and # p < 0.05.*

The 3D reconstruction images for cells cultured in the HA-based 3D system (control and cisplatin-treated cells) support the above-mentioned results showing that the cells spread in the depth of the scaffold, and the exposure to cisplatin induces an increase in red fluorescence, which is indicative of a high number of dead cells versus control cells grown in the 3D system (Figure 24.D).

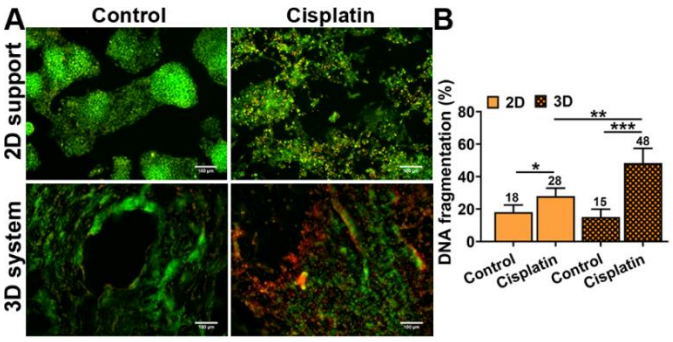


Figure 25. *The effect of cisplatin treatment on DNA fragmentation in HepG2 cells cultured on 2D support and in HA³P50 scaffold (3D). HepG2 cells grown on 2D supports and in 3D systems for 7 days were exposed for 24 hours to 25 μM cisplatin or 0.1% DMSO (control cells). (A) Representative fluorescence microscopy images of acridine orange (AO) staining of dsDNA (green) and ssDNA (red) in HepG2 cells. Scale bar: 100 μm. (B) DNA fragmentation expressed as a percentage of red to (red + green) fluorescence obtained from the AO staining. The results are the mean ± S.D. of three experiments. Statistically significant differences were determined by unpaired two-tailed Student's t-test: * p < 0.05, **p < 0.01, ***p < 0.001.*

The 3D projections of fluorescence images highlighted the homogeneous arrangement of the cytoskeleton in control HepG2 cells cultured in the HA³P50 scaffold, compared to cells grown on 2D support that become overcrowded since they are forced to keep their monolayer alignment. Also, differences in the spatial arrangement of the actin filaments and microtubules can be observed for cisplatin-treated HepG2 cells cultured in both culture systems compared to their corresponding control cells. The levels of β-tubulin in HepG2 cells treated with cisplatin were reduced by ~ 40% (for a

concentration of 25 μM and 50 μM cisplatin, $p < 0.05$) in the HA-based 3D cell model compared to the control HepG2 cells cultured in the 3D system. Moreover, the protein levels of β-tubulin were progressively decreased by the cisplatin treatment of 2D cell culture. Also, the levels of β-tubulin were lower for control (~ 45%, $p < 0.01$) and cisplatin-treated cells cultured in the HA^3P50 scaffold (~34% for 5 μM and ~50% for 25 μM) compared to the corresponding conventional 2D culture.

Cisplatin treatment of HepG2 cells induces the phosphorylation of ERK and p38-MAPK Cisplatin treatment of HepG2 cells induces the dysregulation of the NF-kB/STAT3/Bcl-2 pathway were investigated also. The results showed that: (**i**) the HA^3P50 scaffold is hydrolyzed by the enzymatic action of hyaluronidase and is highly absorptive for doxorubicin; (**ii**) HepG2 cells cultured for 21 days in the HA^3P50 scaffold proliferate to large cellular aggregates and gain liver-like functions such as the improved release of albumin, urea, bile acids, and transaminases, and the enhanced synthesis of CYP7A1; (**iii**) after 7 days, HepG2 cells cultured in HA^3P50 scaffold exhibit enhanced chemosensitivity to cisplatin (increased cytotoxicity and DNA fragmentation and rearrangement of the cytoskeleton); and (**iv**) the cisplatin-induced signaling response of HepG2 cells grown in HA^3P50 scaffold involves increased phosphorylation of ERK and p38α-MAPK and decreased synthesis of NF-kB, STAT3, and Bcl-2. The data suggest that the HA^3P50 scaffold can be used as an HA-based 3D cell platform for testing the effect of chemotherapeutic drugs on hepatocellular carcinoma. Also, the newly developed HA-based 3D model can be adapted and employed as an experimental platform for drug testing in other pathologies.

4.5.2. Study of the reproducibility of the effects of using nanoplatforms *ex vivo* and *in vivo*

To accomplish this activity, we used the newly developed microspheres loaded with doxorubicin (MS-DXR), which comprise a pH-sensitive poly(N-isopropylacrylamide-co-vinylimidazole) copolymer synthesized by the group of dr. Fundueanu to study the microspheres capacity to deliver the encapsulated DXR to cancer tissues. To accomplish this purpose, we used two cancer cell lines, hepatic carcinoma HepG2 cells and lung adenocarcinoma A549 cells. Also, we studied the *ex vivo* hemolysis and erythrocytes aggregation for MS-DXR particles compared to plain MS. In addition, in order to study the *in vivo* MS-DXR distribution and pharmacokinetic, we administered *the* MS-DXR to C57BL/6 mice and compared the results with free DXR and control untreated mice.

In vitro/in vivo tests (Figure 26) revealed the fact that the microspheres encapsulating DXR (MS-DXR) are stable in conditions similar to that found in the bloodstream (pH = 7.4, T = 36 ºC) protecting the drug, but solubilize after internalization (pH = 6.0-5.0) releasing the payload. The plain particles are cytocompatible and deliver the loaded DXR in a dose and time-dependent manner in both HepG2 and A549 cells.

The accumulation of MS-DXR particles and free DXR in HepG2 and A549 cells was investigated using fluorescence microscopy. The fluorescence images revealed that both MS-DXR and free DXR were internalized by the cells in a dose- and time-dependent manner. The DXR delivered by MS-DXR is mainly localized in the nuclei of HepG2 and A549 cells, similar to free DXR. The MS-DXR are internalized at a greater extent by HepG2 cells compared to A549 cells, and this correlates with increased cytotoxicity induced by MS-DXR in HepG2 cells as compared with A549 cells.

The stability of DXR-loaded MS in biological media was evaluated by measuring the in vitro release of the DXR in mouse plasma and fetal bovine serum (FBS). We found that DXR was progressively released from MS-DXR in the first 180 minutes, reaching a similar value of about 50% DXR release for both incubation conditions, plasma, and serum.

Before the preclinical investigations of MS-DXR in animal models, we assessed the hemolytic behavior of MS, MS-DXR, and free DXR. We evaluated their effect on red blood cells membrane integrity by quantifying the hemoglobin released from erythrocytes, isolated from C57BL6 mice, after incubation with MS, MS-DXR, and free DXR. The results showed that MS-DXR particles do not cause hemolysis and erythrocytes aggregation and moreover the MS-DXR and free DXR localize in the liver and kidneys of mice, and the loading of DXR into MS resulted in the reduced renal clearance of DXR.

Then, the localization of MS-DXR compared to free DXR in different organs, after retro-orbital injection in C57BL6 mice, was investigated by fluorescent optical imaging, based on DXR's fluorescent properties. Thus, one hour after MS-DXR, free DXR or PBS administration, the blood was drawn by cardiac puncture and, after thoroughly washing of the vasculature with PBS through the left ventricle, the organs (brain, lungs, heart, liver, spleen, kidneys) were harvested. The fluorescence emission data show, at 1 hour after administration, an accumulation of DXR, either free or encapsulated into MS, in the liver and kidneys.

Altogether, the data suggest that the newly developed microspheres are biocompatible and may be introduced as delivery carriers for the antitumoral drug, doxorubicin. The MS-DXR have the potential to deliver DXR to hepatic tumors and besides, the microparticulate system can be adapted and endowed with targeting properties by conjugating specific ligands to direct them more efficiently to a certain tumor.

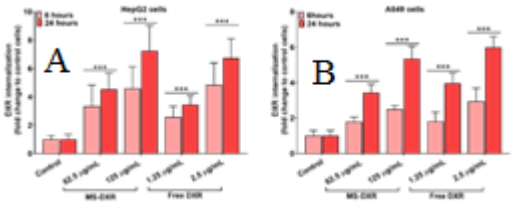


Figure 26. *Quantification of MS-DXR and DXR uptake by HepG2 cells (A) and A549 cells (B) expressed as the ratio of red fluorescence to nuclei number (DAPI staining). Each point represents a mean of almost 27 fields. The bar graph shows results expressed as means ± standard deviation (S.D.) of three experiments performed in triplicate.*

4.6.2. Study of the effects of disassembly/degradation components of nanoplatforms in simulated biological environments

a. Biological evaluation of magnetic nanoparticles developed for doxorubicin delivery, synthesized and provided by the team P1 (Figure 27).

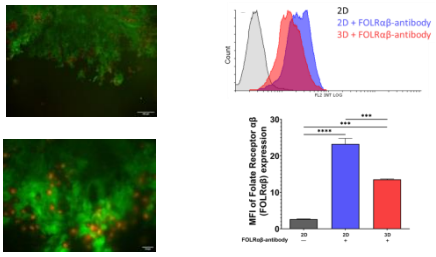


Figure 27. *Live/dead cell assay of MCF-7 cells grown in HA^{3}P50 scaffold for 7 days. Representative images with scale bar: 200 µm (A) and scale bar: 20 µm (B). Green fluorescence depicts the live cells marked with Calcein-AM and the red fluorescence evidences dead cells marked by propidium iodide. (C) The Expression of α and β isoforms of the folic acid receptor (FOLR-αβ) in MCF-7 cells at 7 days after seeding in 2D and 3D culture system. ***p < 0.001, ****p < 0.0001.*

The biological investigations indicate that the hyaluronic acid-based HA^{3}P50 scaffold is non-cytotoxic and support the attachment, proliferation, and viability of breast cancer (MCF-7) cells, being thus suitable for use as tissue/tumor surrogate of breast cancer. The scaffold can be used as 3D tumor cell model to investigate the interaction of nanoparticles/nanoplatforms, for example to study the anti-tumoral effect of Fe_3O_4-DXR-FA nanoparticles.

b. Pentacoordinate silicon complexes with Schiff base - silatrane derivatives

The Schiff base derived from 3-aminopropylsilatrane and 5-nitrosalicylaldehyde (Sil SB) and its precursor 3-aminopropylsilatrane (Sil M) were synthesized by the team lead by Dr. Maria Cazacu (P4). These two compounds were analyzed for cytotoxicity against HepG2 cells by XTT and ToxiLight assays (Figure 28).

The condensation of 5-nitrosalicylaldehyde to 3-aminopropylsilatrane (Sil SB) increases the cytotoxicity of the resulting silatrane derivative compared to the precursor 3-aminopropylsilatran (Sil M). While the Sil M has no cytotoxic effect on HepG2 cells, the Sil SB expresses an anti-tumor effect by inducing the death of 50% cells at approximately 200 µg/mL.

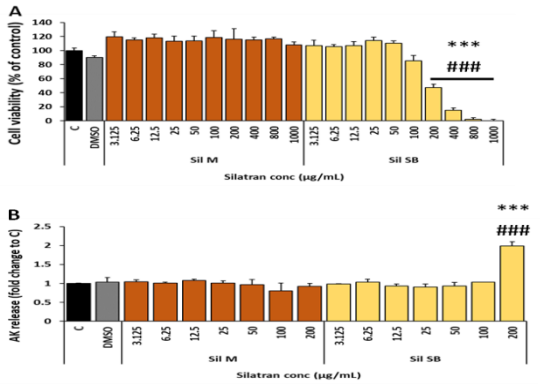


Figure 28. *(A) The viability of HepG2 cells treated with (3.125 – 1000 µg/mL) Sil M and Sil SB for 24 hours. (B) Cytotoxicity induced by Sil M and Sil SB against HepG2 cells.* ***$p < 0.001$ *vs. control cell and* $^{###}p < 0.001$ *vs. DMSO treated cells.*

DISSEMINATION of the results obtained during the stage 2021 of 5D-nanoP project

***In parvo*: 23 papers in ISI journals; 2 proceedings, 3 book chapters; 16 oral communications; 2 key note conferences; 4 posters presentation; 1 work-shop organized; 1 PhD thesis defended.**

Articles published in ISI journals

1. Turtoi M, Anghelache M, Bucatariu SM, Deleanu M, Voicu G, Safciuc F, Manduteanu I, Fundueanu G, Simionescu M, Calin M. *A novel platform for drug testing: Biomimetic three-dimensional hyaluronic acid-based scaffold seeded with human hepatocarcinoma cells*. Int J Biol Macromol. **2021** Aug 31;185:604-619. doi: 10.1016/j.ijbiomac.2021.06.174. Epub 2021 Jul 1. PMID: 34216662.

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1. Leon Engelbrecht, Francesca Mocci, Yonglei Wang, Sergiy Perepelytsya, Tudor Vasiliu and Aatto Laaksonen. Molecular Perspective on Solutions and Liquid Mixtures from Modelling & Experiment. Chapter 3 in "*Soft Matter Systems for Biomedical Applications"***,** Ed**itors: Bulavin** Leonid, **Lebovka** Nikolai, Springer Nature Switzerland AG, 2021**.** DOI: 10.1007/978-3-030-80924-9.
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Participation at national and international conferences

Oral presentations

1. Mihaela Turtoi, *Hyaluronic acid-based 3D cell model of human hepatocarcinoma for chemotherapeutic drug testing*, 5DnanoP Workshop2021 at Petru Poni Institute, 11 October **2021**, online session.

2 Manuela Calin, *P-selectin directed RAGE-shRNA nanocarriers reduce atherosclerosis-associated inflammation in ApoE-deficient mice*, The 42nd Anniversary Symposium of the Institute of Cellular Biology and Pathology "Nicolae Simionescu" and the 38th Annual Scientific Session of the Romanian Society for Cell Biology, 4-6 November **2021**, online session.

3. C. Deleanu, *NMR metabolomics and lipidomics in clinical diagnosis*, Adriatic NMR Conference, 13-15.09.**2021**, Primosten, Croatia. Book of Abstracts, pp. 19, ISSN 2806-6227. (Conferinţă Plenară, cu prezenţă fizică)

4. A. Arvinte, *Bimetallic Based Nanostructures for Electrochemical Sensing Applications*; 6th International Congress on Biomaterials and Biosensors (BIOMATSEN), Oludeniz, Turkey, 17-23 October **2021**.

5. B.F. Craciun, L. Clima, A. Angeli, A. Petreni, S.A. Ibanescu, M. Pinteala, C.T. Supuran, *Squalene functionalized with coumarines or benzenesulfonamides as hybrid inhibitors for Carbonic Anhydrase*, Progress in Organic and Macromolecular Compounds 28th edition "MACROIasi 2021", Iasi, Romania, October 7-9 **2021** (Poster Presentation).

6. C. Racleș, M.-F. Zaltariov, D. Peptanariu, T. Vasiliu, M. Cazacu, *Functionalized silica as doxorubicin carriers*; 5DnanoP Workshop2021, 11 Oct. (**2021**) (oral presentation).

7. M.-F. Zaltariov, M. Cazacu, D. Peptănariu, C. Cojocaru, L. Clima, B.-I. Ciubotaru, A. Bargan, *New silatranes possesing biodegradable and bioactive functionalities*, 5DnanoP Workshop2021 at Petru Poni Institute Monday October 11th (**2021**) (oral presentation).

8. G. Fundueanu, *Stimuli-sensitive polymers for self-regulated drug delivery systems*, United Conference of Pharma B2B, Novel Trends and Approaches in Pharmaceutical Industry, July 15, **2021**, New Jersey, USA (keynote Conference).

9. I. Popescu, M. Turtoi, R.N. Darie-Nita, M. Calin, *Degradable Pluronic/alginate hydrogels for skin wound healing-in vitro studies*, International Congress of the „Apollonia" University from Iași „By promoting excellence, we prepare the future" XXXIth Edition, 01-03.03. **2021**, Iași, Romania.

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11. Aatto Laaksonen (Plenary speaker): *Multiscale modelling of ionic liquids and biomass*. Young Scientists Workshop on Conversion and Utilization of Biomass, September 28-29, **2021**, Beijing China.

12. A.-D. Bendrea, L. Cianga, I. Cianga, *Thiophene end-group functionalized oligo(2-methyl-2-oxazoline) as an amphilphilic reactive macromonomer and as a non-conventional intrinsic luminescent material*, Conference "Progress in Organic and Macromoleculecular Compounds", 7-9 Otober **2021**, Iasi, Romania.

13. P. Tîrnovan, F. Mocci, T. Vasiliu, L. Cianga, I. Cianga, M. Pinteală, A. Laaksonen, *In silico studies of a novel amphiphilic graft conjugated polymer for biomedical applications*, 5DnanoP Workshop2021 at Petru Poni Institute Monday October 11th (**2021**) (oral presentation).

14. A.-D. Bendrea, L. Cianga, I. Cianga, *Regarding some of the potential application uses of the unexpected scientific findings during the research of 5DnanoP*, 5DnanoP Workshop2021 at Petru Poni Institute Monday October 11th (**2021**) (oral presentation).

15. I. Cianga, A.-D. Bendrea, L. Cianga, G.-L. Ailiesiei, *Conducting conjugated polymers as biomaterials in the frame of 5DnanoP project*, 5DnanoP Workshop2021 at Petru Poni Institute Monday October 11th (**2021**) (oral presentation).

16. A.-D. Bendrea, L. Cianga, I. Cianga, *Conjugated polymers: a step forward to smarter and more advanced synthetic polymers biomaterials*, The International Congress of Apolonia University of Iasi, 1-3 March **2021**, Iasi, Romania (oral presentation).

Posters

1. Geanina Voicu, Mihaela Turtoi, Maria Anghelache, Sanda-Maria Bucatariu, Mariana Deleanu, Florentina Safciuc, Ileana Manduteanu, Gheorghe Fundueanu, Maya Simionescu, Manuela Calin. *A*

three-dimensional hyaluronic acid-based scaffold seeded with human cancer cells functions as a suitable platform for antitumoral drug screening. The 18th International Conference on Nanosciences & Nanotechnologies (NN21), 6-9 July **2021**, Thessaloniki, Greece.
2. Mihaela Turtoi, Maria Anghelache, Andrei-Alunel Patrascu, Catalin Maxim, Delia-Laura Popescu, Ileana Manduteanu, Manuela Calin. *Designing of new biocompatible coordinating compounds of oxidovanadium(V) as insulin mimetics*. The 18th International Conference on Nanosciences & Nanotechnologies (NN21), 6-9 July **2021**, Thessaloniki, Greece.
3. Mihaela Turtoi, Maria Anghelach, Delia-Laura Popescu, Ileana Manduteanu, Manuela Călin. *Design of new oxidovanadium(V) compounds as insulin-mimetics*. The 42nd Anniversary Symposium of the Institute of Cellular Biology and Pathology "Nicolae Simionescu" and the 38th Annual Scientific Session of the Romanian Society for Cell Biology, 4-6 November **2021**, online session.
4. F. Zaltariov, C. D. Varganici, D. Filip, D. Macocinschi, *Stability of the HPC/PU Polymeric Blends in Accelerated Weathering and Biological Environments*, M. 1st Corrosion and Materials Degradation Web Conference, 17-19 May **2021**, sciforum ID-045074 (poster).

5D-nanoP Workshop, Ocober 11, 2021, Petru Poni Institute, Iasi, Romania
(Book abstract on https://www.intelcentru.ro/5D-nanoP/)

PhD thesis defended
Thesis title: „Noi rețele polimerice pe bază de polizaharide modificate, cu potențiale aplicații medicale" („New polymer networks based on modified polysaccharides, with potential medical applications")
Autor: Bioeng. Ioana A. Tanasa (Duceac)
Supervisor: Dr. Sergiu Coseri
Date of defense: October 21, **2021**.

All activities were discussed.

Project director,

Prof. Aatto Laaksonen