SCIENTIFIC REPORT
Stage 2 - Year 2023
Nanovehicle fine-tuning for improved anticancer drug delivery (NanoCanTune)

Project code: PN-III-P1-1.1-PD-2021-0786
Contract no.: PD66/2022
Phase 2/2023: Nanovehicle stability and behavior in a biological environment (WP2 part I)
Stage 2 Budget: 149.456,00 lei

Stage 2 summary:

In this stage, which corresponds to months 10-21 of the project, 8 activities were undertaken for the purpose of characterizing the behavior of liposomal nanovehicles encapsulating the antitumor drug 5-fluorouracil (5-FU), both in an *in vitro* and an *in silico* environment, according to Annex II of the funding contract. The main objective of this stage, namely: **Investigation of the stability of liposomal formulations and drug distribution in the nanovehicle by molecular dynamics simulations (O3)**, was achieved. In addition, the first part of the last objective of a total of the four objectives proposed in this project was completed, namely: **Investigation of the behavior of liposomal formulations in rat plasma from the point of view of protein corona formation (O4 part I)**. Two of the eight activities undertaken had the role of complementing the information obtained in stage 1 of the project regarding the physico-chemical characterization of new liposomal formulations with 5-FU, as well as the *in vitro* investigation of the controlled release properties of 5-FU from liposomes, also in accordance with Annex II of the financing contract.

According to the work plan, *20 optimized liposomal formulations* with different lipid compositions were generated. The main used lipid was 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), and liposomes varied in cholesterol (CHOL) or the positively charged lipid 1,2-dioleyl-3-trimethylammonium-propane (DOTAP) content, with or without the antitumor drug 5-FU. The liposomal suspensions were obtained by thin film hydration, followed by sonication and extrusion, based on the protocol optimized in stage 1 of the project. DPPC was chosen as the main lipid because it was previously observed that formulations with DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) aggregated over time, and the formulation containing DPPC:CHOL in a molar ratio of 60:40 allowed for the best encapsulation of the antitumor drug 5-FU in terms of estimated intravesicular concentration. Egg phosphatidylcholine was excluded due to its compositional complexity, which makes accurate *in silico* modeling impossible. Computational models were generated for all 10 liposomal formulations, in the form of a stacked double lipid bilayer (to mimic the intraliposomal compartment). Free of drug systems were generated for each composition, as well as 3 systems with various degrees of 5-FU loading for each composition (21, 42, and 84 5-FU molecules, respectively), with a total of 40 investigated systems in the form of a stacked double lipid bilayers. Moreover, 9 vesicular systems were also generated. The behavior of all 5-FU formulations was studied in rat plasma by proteomic techniques, and some adsorbed proteins on the liposomal surface could be comparatively identified.

The degree of objective completion for Stage 2 is 100%.