Wrocław University
of Science and Technology

Doctoral dissertation

Designing and obtaining novel bilosomes as a biocompatible nanoplatform for enhancing phyto-photodynamic therapy

The current doctoral dissertation deals with the synthesis, characterization of physicochemical properties, and evaluation of the applicability of new-generation vesicular nanostructures (bilosomes) designed to co-encapsulate active compounds of different solubility and their potential application in phyto-photodynamic therapy (phyto-PDT). Precisely planning the various stages of the experiments, including the selection of appropriate building components forming the nanocarrier (NC) envelope, as well as process conditions and parameters, led to the development of an efficient and reproducible method for obtaining modern bilosomes with a well-defined structure and significant functional properties: (i) nanoscopic size (< 100 nm); (ii) spherical shape; (iii) low polydispersity index (< 0.3); (iv) long-term colloidal stability; (v) high encapsulation efficiency; (vi) multidirectional biological activity (antimicrobial and anticancer).

The designed colloidal systems, with both negative and positive surface charges, were obtained based on self-assembling processes involving amphiphilic biomolecules, including the non-toxic and highly biodegradable phospholipid 1,2-diacyl-*sn*-glycero-3-phosphocholine (phosphatidylcholine, PC), a natural component of biological membranes. In the present study, sodium cholate (SC) derived from cholic acid (the predominant human bile acid), with or without cholesterol (Chol) as an auxiliary lipid, was used to stabilize individual NCs. While the surface of the novel vesicles was coated with a biocompatible poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) triblock copolymer (PEO-PPO-PEO) with the trade name Pluronic® P123 (for a negatively charged system) or polyethylene glycol, conjugated with a cross-linked phospholipid (*N*-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine, DSPE-PEG 2000) for a positively charged one whose lipid bilayer was enriched with a cationic lipid with a well-documented safety profile (i.e., 1,2-dioleoyl-3-trimethylammoniopropane, DOTAP).

To confirm the feasibility of using the developed vesicular systems as carriers of active compounds, as well as due to the lack of literature reports on enhancing photodynamic activity

by encapsulating a conventional photosensitizer (PS) together with a naturally occurring chemopreventive compound in next-generation bilosomes, the optimized nanostructures were loaded with a hybrid cargo, i.e., a hydrophilic PS (Methylene Blue or Rose Bengal) and a hydrophobic phytochemical (curcumin or astaxanthin). Physicochemical characterization of the obtained bilosomal systems involved the determination of fundamental parameters, including size (expressed as hydrodynamic diameter, D_H) and polydispersity index (PdI) using dynamic light scattering (DLS), as well as zeta potential (ζ) based on electrophoretic light scattering technique (ELS). The morphology of the synthesized NCs was determined through transmission electron microscopy (TEM) and atomic force microscopy (AFM). The above studies showed that the encapsulation of active compounds does not affect the size, surface electric potential, and morphology of the developed bilosomes. The effectiveness of the encapsulation process was confirmed using ultraviolet-visible spectroscopy (UV-Vis) and/or attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR).

The substantial element during the dissertation was to show and highlight the versatility of the developed NCs in biological applications, so the last part of the research was devoted to evaluating the therapeutic efficacy and applicability of selected vesicular systems. Firstly, photostability, as well as the ability of encapsulated and native PSs to generate reactive oxygen species (ROS), were determined. Research confirmed that the nanoengineered bilosomes, negatively and positively charged, prevent rapid photobleaching of the loaded compounds, thereby increasing their photoactivity compared to their native form. In the final step, cyto- and photocytotoxicity evaluations were performed on selected bacterial (*Staphylococcus aureus*) and fungal (*Candida albicans*) strains, as well as human melanoma cell lines (A375, Me45) and, for comparison, normal human cutaneous (HaCaT) keratinocytes. Cellular uptake and localization studies were also conducted using flow cytometry (FC), fluorescence microscopy (FM), and holotomographic microscopy (HTM). The experiments showed that the designed nanostructures do not induce toxicity against cells without irradiation and, unlike the native form of the active cargo, do not cause morphological changes in the analyzed cell cultures. Significantly, the results of biological activity studies not only proved the high bactericidal, fungicidal, and anticancer efficacy of the proposed formulations (after irradiating them with the light of the appropriate wavelength) but also confirmed their safety against normal skin cells.

In conclusion, as a result of the experiments, novel bilosomal-type vesicular NCs with negative and positive surface charges were developed, which, due to their appropriate size and high biocompatibility with the human skin, make them an excellent base formulation in particular for transdermal delivery of pharmaceuticals. The proposed therapeutic approach

(i.e., combining the action of plant-derived compounds with classical PSs and their encapsulation in bilosomal systems) may address the challenges of antibiotic resistance and treatment of patients with metastatic melanoma. Given the above, the results presented show the high potential of the nanosystems, which can become the basis for further *in vivo* studies on the application of the developed bilosomal-derived nanophotosensitizers in the complementary treatment of skin cancer, as well as other dermatological conditions.