Development and biostability evaluation of hybrid nanoconstructs for cancer therapy based on zinc oxide nanocrystals

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Title: Development and biostability evaluation of hybrid nanoconstructs for cancer therapy based on zinc oxide nanocrystals

Cancer is a leading cause of death, second only to cardiovascular diseases, and its large diffusion and incidence, together with the numerous drawbacks associated with antitumor traditional therapies, made the research of new diagnostic and therapeutic strategies of paramount importance. In recent years, promising options were offered by nanomedicine which envisages the application of nanomaterials to the medical field to formulate more selective and stable therapeutic tools.

In this context, this PhD thesis focuses on the development of a biocompatible and colloidally stable hybrid nanoconstruct for the targeted treatment of cancer cells. The nanoconstruct, evocatively named TrojaNanoHorse (TNH), would be composed by a therapeutically active core made by chemically synthesized zinc oxide nanocrystals (ZnO NCs) encapsulated in a biomimetic shell of extracellular vesicles (EVs), opportunely functionalized with specific targeting moieties in order to further enhance their selectivity. The proposed hybrid nanoconstruct would combine the intrinsic delivery features of EVs, like their great stability in biological environment and low immunogenicity, with the toxicity of ZnO nanostructures toward cancer cells and the targeting capability of opportune engineered ligands.

More in details, the first part of the experimental work concerned the optimization of ZnO NCs synthesis and their physico-chemical characterization. Indeed, a reliable and reproducible synthetic procedure together with a precise evaluation of the features of the obtained ZnO NCs are essential for the accurate study of their biological effects. In this phase, two synthesis methods (i.e. a conventional solvothermal method and a microwave-assisted synthesis) were evaluated, analyzing the internalization rate and cytotoxicity of the obtained ZnO NCs on KB cancerous cells and decreeing the major reliability of microwave-assisted synthetic method.

The colloidal and chemical stability of synthesized ZnO NCs in the biological environment were then evaluated, analyzing the aggregation and dissolution extent through long-term biostability tests. The stability and the interaction with media components, in fact, would determine the biological identity of nanoparticles, directly affecting the biological response. The assays were

performed in different biological media, evaluating the behavior of eithers pristine and functionalized nanocrystals. In particular, ZnO NCs coated with a shell constituted by synthetic phospholipids were analyzed as a preliminary model of the proposed hybrid nanoconstruct, confirming the stabilizing effect ensured by the lipid envelope.

The actual TNH hybrid nanoconstruct was then developed, combining the synthesized ZnO NCs with EVs extracted from the conditioned cell culture supernatants of KB cancerous cells. The encapsulation was performed through a co-incubation method, optimizing several operating parameters to maximize the interactions between the two components and, thus, the loading efficiency. The effect of the EVs lipid-shielding on the colloidal stability, cellular toxicity and internalization was evaluated, comparing the behaviour of the obtained TNHs with uncoated ZnO NCs. The results evidenced a great improvement of colloidal stability in biological media accompanied by a more efficient internalization in KB cancer cells of TNH nanoconstructs with respect to pristine ZnO NCs. The samples presented a comparable cytotoxicity, highlighting that EVs shielding fully preserve the intrinsic toxicity of ZnO NCs.

The construction of TNH was further optimized in order to obtain a safer and more effective product. Primarily, to overcome safety concerns related to the application of EVs derived from cancer cells, the EVs cell source was changed, extracting the biovesicles from B lymphocytes. Moreover, the loading efficiency was optimized by the application of an active loading method, based on the application of freeze-thaw cycles as active stimulus to destabilize EVs membrane and favor the encapsulation of ZnO NCs. Two different procedures were implemented and analyzed in terms of loading efficiency, colloidal stability and morphology, evidencing the suitability of freeze-thaw method to efficiently encapsulate ZnO NCs within biologically-derived EVs, while preserving their morphology and surface protein expression. Finally, to further improve the TNHs selectivity, a functionalization method to decorate the EVs surface with specific targeting antibodies was designed and preliminary validated.

The results presented in this PhD thesis follow the development of an innovative hybrid nanoconstruct for cancer treatment based on therapeutically active ZnO NCs. The main goal of this study was the biostabilization of synthesized nanocrystals obtained through their encapsulation in EVs. The biological origin of EVs would improve the biomimetic and biocompatible features while guaranteeing the efficient intracellular release and the prominent cytotoxic activity of ZnO, making the whole nanoconstruct a promising candidate for therapeutic applications against cancer cells.