Synthesis, characterization and evaluation of hyaluronic acid-functionalized biomimetic-magnetoliposomes as drug delivery systems

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Nanotechnology has become very attractive for its applications in several fields, comprising biology, medicine, especially oncology offering new therapeutic approaches that could overcome the limits of the current conventional treatments.
In this context, the concept of drug delivery systems (DS), which exploit the peculiar properties of tumor cells or their milieu, is highly attractive, as it recapitulates the advantages of topical treatments.
Nanoparticles (NPs) have become very attractive, as they represent the realization of the old “magic bullet” concept. This being to selectively carry a therapeutic molecule to a specific target (tumor cells), while sparing the healthy cells, reducing the systemic exposure and adverse effects.
Besides magnetic nanoparticles (MNPs), the most used are the ones composed of iron oxide and they can be obtained by synthetic chemistry, but they are also produced in nature as magnetosomes by magnetotactic bacteria. Unfortunately, culturing these bacteria is cumbersome and difficult to be scaled up so, a possible solution is to synthetize inorganically the MNPs with magnetosome membrane associated proteins (MAPs) which play a crucial role in the mineralization process of the nanocrystals[1]. One of these MAPs is MamC protein that directs the *in vitro* nucleation and growth of MNPs, eliminating the requirement of bacterial cultures and improving the production of magnetosome-like biomimetic magnetic NPs (BMNPs) with high yields[2].
Recently, BMNPs synthetized in presence of MamC protein from *Magnetococcus marinus MC-1* and covered by a lipid layer of 1,2-distearoyl-sn-glycero-3-phosphocholine [LP(BMNPs)] have been used as DS and become particularly interesting[3]. Indeed, MamC protein also confers a negative charge to BMNPs that can be functionalized by electrostatic interactions with chemotherapeutic drug as doxorubicin (DOXO)[4]. Moreover, exploiting their inner properties, BMNPs can be manipulated by an external gradient magnetic field (GMF), besides being multifunctional highly biocompatible platforms[5].
Among targeting agent such as monoclonal antibodies directed against tumor associated markers, hyaluronic acid (HA) is an anionic glycosaminoglycan that has gained special attention since it interacts with CD44 receptor that is overexpressed on a variety of solid cancers (colon, ovarian, breast, lung, and pancreatic)[6] and its aberrant expression and dysregulation contributes to tumor initiation and progression. So, the pleiotropic roles of CD44 in carcinoma potentially offer new molecular target for therapeutic intervention[7]. In this context, 14800 Da HA has been linked to an aminated phospholipid (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine, DPPE) by reductive amination[8] and used in the synthesis of HA-LP(BMNPs) nanoformulations.
Herein, BMNPs were synthetized, functionalized with DOXO, and then encapsulated in +/-HA conjugated liposomes obtaining +/-HA-LP[(+/-DOXO)-BMNPs].
The obtained nanocomplexes were extensively characterized by transmission electron microscopy analysis (TEM), hydrodynamic radius, ζ-potential, Fourier-transform infrared (FT-IR), and colloidal stability. The crystals inside the nanoformulations showed a rhombic shape, with a size lower than 200 nm, a negative ζ-potential value and their effective functionalization with the different moieties was confirmed. *In vitro* biological tests were performed in red blood cells (hemolysis test) and with several cell lines of human breast cancer (MDA-MB-231 and MCF7) and human ovarian cancer cell line (A2780). Biocompatibility was analyzed by ROS production and MTT assay and +/-HA-LP(BMNPs) were not found to have cell toxicity.
The targeting ability of HA-LP(BMNPs) was evaluated on three cancer cell lines with high (MDA-MB-231), middle (MCF7) and low (A2780) CD44 expression and compared to that of LP(BMNPs). To this aim, the cellular interactions of the nanocomplexes were evaluated by iron quantification and Prussian Blue staining. The results showed that the uptake of HA-LP(BMNPs) was significantly higher in MDA-MB-231 cells, suggesting that the entry of HA-conjugated formulations is likely receptor-mediated.
Moreover, when LP(DOXO-BMNPs) were incubated with different cell lines, the nanocomplexes exerted cytotoxic activity which increased when HA was conjugated to the nanoformulations.
These promising results show the suitability of the HA-LP(DOXO-BMNPs) as magnetic nanocarriers for local targeted chemotherapy and for future agents for hyperthermia and photothermia paving the way for the development of powerful new approaches for cancer therapy suggesting a tumor multiple attack by combined strategies.

References

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Figures

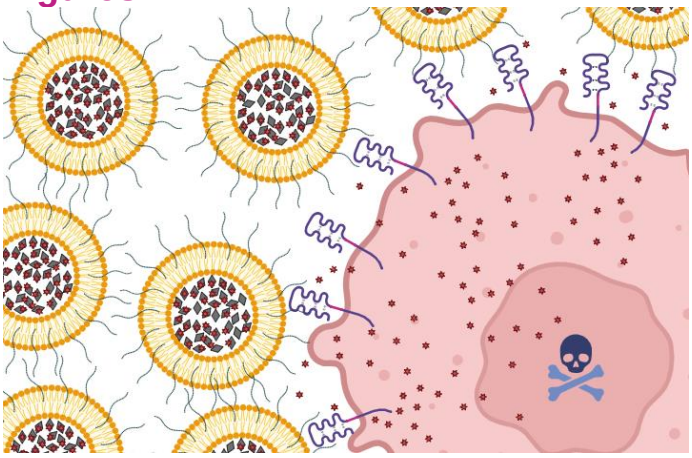

Figure 1. Schematic model of interactions of the nanocomplexes with cells: HA-LP(DOXO-BMNPs) nanoformulations interact with cancer cells overexpressing CD44 receptor where DOXO is delivered acting as cytotoxic agent.