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Advanced Lipid-Based Nanotransporters for Encapsulation of Cytochrome C

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Extended Abstract

Therapeutic proteins are an exciting and growing, yet complicated research area [1]. Achieving any therapeutic success can be an issue, mainly due to their short half-life, low permeation and possible immunogenicity [2]. Therefore, to overcome these challenges, the aim of this project is to develop a pH-sensitive liposomes (LNPs) suitable for cytochrome c encapsulation. Cytochrome c is a relatively small protein with a molecular weight of about 12 kDa. It is located in mitochondria and is mostly known as an electron carrier between complexes III and IV of the respiratory chain [3]. However, cytochrome c is a protein with a dual function. In the presence of the right stimuli, cytochrome c is released from mitochondria in a process called mitochondrial outer membrane permeabilisation, which in turn induces apoptosis [4].

In order to trigger apoptosis using extracellular cytochrome c, we designed and optimised two liposomal formulations (EM-LNPs and EMC-LNPs). These two formulations differed in their sensitivity to pH, with EMC-LNPs being pH-sensitive and EM-LNPs non-pH sensitive. Both types of LNPs were surface-coated with 5 mol.% of polyethylene glycol (PEG) to ensure their stability and minimal cytotoxicity. In EM-LNPs, a phospholipid with PEG attached to it, DSPE-PEG2000, was used. However, when producing the pH-triggered EMC-LNPs, mPEG2000 was attached to LNPs pH-responsive chemical bond. This bonding occurred in pH 4, and the whole PEGylation process took about 18–22 hours. PEGylation was terminated when pH was changed back to 7.

Cytochrome c was successfully encapsulated into both EM and EMC-LNPs. Average size of EM-LNPs loaded with cytochrome c was 128.1 nm, with polydispersity index (PdI) of 0.174 and ζ-potential–4,7 mV. EMC-LNPs had an average size of 144.2 nm, with PdI of 0.124 and ζ-potential –7.6 mV. After optimisation, the EE% of EM-LNPs was ~33.0%, and EE% of EMC-LNPs was ~81.5%. Biophysical characterisation included long-term stability measurements at room temperature and 4°C. In terms of size and PdI, only small changes occurred, therefore, both EM and EMC-LNPs can be considered stable for up to 1 month. We compared changes in secondary structure caused by the encapsulation process using circular dichroism. Obtained data suggested that encapsulation into EMC-LNPs caused only minor changes in cytochrome c's secondary structure. However, encapsulation into EM-LNPs significantly changed the representation of secondary structures in the sample. In order to determine the sufficiency of washing away of unencapsulated cytochrome c, samples were analysed via fast protein liquid chromatography. It was found that the majority of unencapsulated protein was indeed washed away using diafiltration/ultracentrifugation. Our next steps will focus on biological experiments, to understand the cytotoxicity, intracellular fate of cytochrome c and determination of possible use as anticancer agent.

Our LNPs offer a platform for successful protein encapsulation. We believe that using liposomes as nanocarriers for therapeutic proteins can vastly increase their effectiveness, which can change key strategies of cancer treatment in the near future.

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