Development of Innovative Nano-Systems for the Targeted Treatment of Cancer

Alexandra PUSTA[1], Mihaela TERTIȘ[1], Rodica TURCU[2], Victor DICULESCU[3], Cecilia CRISTEA[1,*]

[1] *Department of Analytical Chemistry, Iuliu Haţieganu University of Medicine and Pharmacy, 400349 Cluj-Napoca, Romania*

[2] *National Institute for Research and Development of Isotopic and Molecular Technologies, 400293 Cluj-Napoca, Romania*

[3] *National Institute of Materials Physics, Atomiștilor 405A, 077125 Măgurele, Romania*

ccristea@umfcluj.ro

Chemotherapy represents the mainstay of treatment for a wide variety of cancers that cannot benefit from surgical procedures. Despite its efficiency, chemotherapy presents many drawbacks such as systemic side effects and lack of specificity for tumour tissue. For this reason, it is crucial to develop novel methods that can ensure the targeted delivery of the active compound to the tumour site. This can be achieved using two different strategies: passive transport, which relies on the specific properties of the tumour tissue, or active transport, in which delivery systems are functionalized with structures that show specificity towards tumour tissues (antibodies, enzymes, aptamers) [1].

The present work proposes four different nano-systems for the targeted delivery of several chemotherapeutics: nanosomes loaded with (*i*) doxorubicin and (*ii*) carboplatin for passive delivery and magnetic (*iii*) nanoparticles or (*iv*) nanoclusters for the active delivery of sorafenib.

In the case of passive approaches, the nanosomes were incubated with the corresponding drug solutions and drug loading was confirmed by UV-Vis spectrophotometry. Subsequently, drug release was carried out in media of different pH values and the maximum release was obtained in acidic pH for both chemotherapeutics. This represents an advantage, as the pH of the tumour microenvironment is more acidic compared to that of healthy tissues [2]. To evaluate the release of doxorubicin and carboplatin from the nano-systems, electrochemical methods were developed for the detection of these substances. Direct electrochemical detection was performed on bare in-house produced electrodes in the case of carboplatin and on in-house produced electrodes modified with gold nanostrctures in the case of doxorubicin. The results obtained were compared to those obtained by UV-Vis spectrophotometry and good correlations were observed. A schematic representation of the protocol used for the development and testing of doxorubicin-loaded nanosomes is represented in Fig. 1.

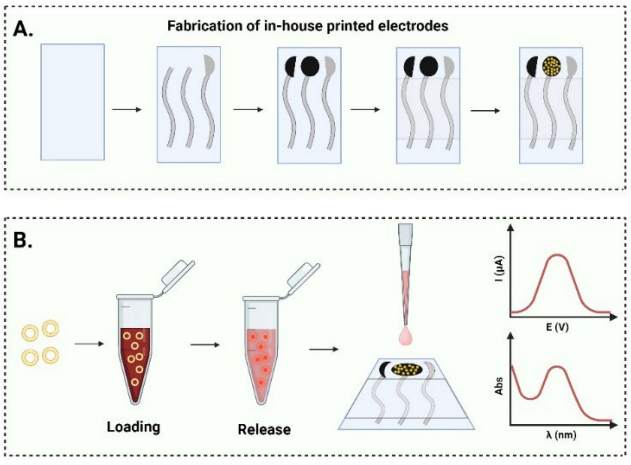

Fig. 1. *Schematic representation of the protocol used for the development and testing of doxorubicin-loaded nanosomes.*

In the case of active delivery, two types of magnetic nano-carriers were used: azelaic acid functionalized magnetic nanoparticles and poly-tartaric acid functionalized magnetic nanoclusters.

Aptamer TLS11a, a DNA aptamer with high affinity for hepatocellular carcinoma HepG2 cells, was used for the functionalization of the magnetic carriers. The functionalization was performed in two

steps: first, the carboxyl groups on the surface of the nano-carriers were activated using a mixture of N-hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl)carbod iimide and next, the amino-terminated aptamer was added. This ensured the formation of an amidic bond between the amino group in the aptamer structure and the activated carboxyl groups. Aptamer functionalization was confirmed using UV-Vis spectrophotometry and electrochemical impedance spectroscopy in the case of magnetic nanoparticles. The next step consisted in loading sorafenib onto the modified magnetic nano-carriers and performing sorafenib release studies in buffers of different pH values. Both steps were confirmed using UV-Vis spectrophotometry and a better release profile was observed at pH 5.5 compared to pH 7.4. A schematic representation of each step in the development of the magnetic nano-carriers is represented in Fig. 2.

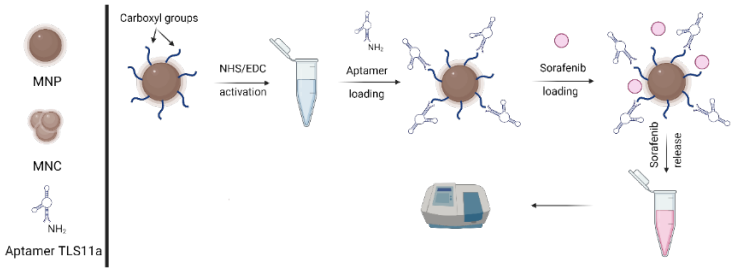

***Fig. 2**. Schematic representation of the development of aptamer modified, sorafenib loaded magnetic nanocarriers.*

Cell internalization tests were performed in the case of magnetic nanoparticles on two different cell lines: HepG2 hepatocellular carcinoma cells and fibroblasts. A higher internalization in HepG2 cells was observed in the case of aptamer-modified nanoparticles compared to unmodified nanoparticles at all concentrations tested. In the case of fibroblasts, the presence of the aptamer led to lower internalizations at all concentrations. These results demonstrate the positive impact of the aptamer functionalization on the specificity of the nano-carriers for tumour cells. An electrochemical method for the detection of sorafenib was also developed based on commercially available screen-printed electrodes. The electrochemical detection of sorafenib was used to determine the drug loading and good correlations with the control UV-Vis method were observed. The limit of detection of the developed method did not allow the quantification of sorafenib from release studies.

In conclusion, drug delivery systems for the passive transport of doxorubicin and carboplatin and active transport of sorafenib have been successfully developed. Their characterization was carried out using spectral methods as well as electrochemical methods. All carriers demonstrated better drug release profiles at acidic pH compared to neutral pH, which represents an advantage for cancer therapy. Moreover, in the case of active delivery, it was demonstrated that the aptamer functionalization increases the internalization of drug carriers in tumour cells, while decreasing the internalization in healthy cells. Direct electrochemical detection was performed for all three chemotherapeutics and applied in loading and release studies in the case of doxorubicin and carboplatin and for loading studies in the case of sorafenib, demonstrating the applicability of electrochemical methods in the development of pharmaceutical formulations.

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