SURFACE-MODIFIED SPION SYSTEMS FOR CANCER THERAPY

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Introduction

About 90% of all cancer-related deaths are due to metastasis – a process in which circulating tumor cells (CTC) detach from the primary tumor site, and through the bloodstream travel to the other, sometimes distant organs, where they form secondary tumor. Epithelial-mesenchymal transition (EMT) enables cancercells to suppress their epithelial features and activate mesenchymal ones, allowing them to migrate from the primary tumor as CTC. As a result of EMT process E-cadherin on the surface of cancer cells is replaced by N-cadherin. Anti-N-cadherin antibodies can thus be used to effectively target CTC. Superparamagnetic iron oxide nanoparticles (SPION) exhibit a number of unique properties, which make them a subject of growing interest of scientific community. SPION are small crystals of iron oxide, usually magnetite (Fe_3O_4) or its oxidised form, maghemite (γ-Fe_2O_3). Many properties of SPION can be controlled by their coating, as well as by a further modification of their surface.

Our aim was to obtain SPION stabilized by cationic derivative of chitosan and decorated with anti N-cadherin antibodies, which could be used as a targeting system able to selectively bind to CTC. We have also planned to introduce methotrexate to the surface of SPION. Such systems may be used either to deliver an anticancer drug (methotrexate) or to magnetically capture CTC - either for diagnostic or therapeutic purposes.

Materials and Methods

The cationic derivative of chitosan (CCh) was obtained in the reaction between chitosan and GTMAC (glicydyltrimethylammonium chloride). SPION stabilized with CCh (SPION/CCh) were obtained *via* co-precipitation of Fe^{2+} and Fe^{3+} salts with ammonia in the presence of CCh. Magnetic chromatography was used to purify the nanoparticles. SPION were then tosylated. in reaction with p-toluenesulfonyl chloride. Tosylated SPION/CCh (SPION/CCh-Tos) were reacted with anti-N-cadherin antibodies in borate buffer (pH = 9.5) in the presence of ammonium sulfate at 37°C [1]. Methotrexate (MTX) was attached to SPION/CCh using EDC/NHS chemistry.

CCh was characterized using ^{1}H NMR, ATR-FTIR and elemental analysis. SPION/CCh were characterized by dynamic light scattering (DLS), nanoparticle tracking analysis (NTA) and zeta potential measurements. Tosylation was confirmed using ATR-FTIR. MTX attachment was verified using UV-Vis absorption spectroscopy. The success of the antibodies attachment to SPION/CCh was confirmed using immunostaining with fluorescent secondary antibodies (NorthernLights™ anti-sheep IgG-NL557)

Human prostate cell lines (American Type Culture Collection): LNCaP (androgen-dependent cell line derived from lymph nodes metastasis) and PC-3 (androgen independent cell line derived from bone metastasis) were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin. MTT test was used to evaluate the cytotoxicity of SPION modified with anti-N-cadherin antibodies and MTX. The unbinding force AFM measurements were performed using atomic force microscope equipped with a ''liquid cell'' setup, in culture medium, at room temperature. Cells lysis and Western Blot were carried out as previously described [2-4]. Confocal microscope was used to visualize the interaction between cells and specific antibodies or SPION/CCh with bound anti-N-cadherin antibodies (SPION/CCh-N-cad). SPION/CCh-N-cad were also stained with secondary fluorescent antibodies, incubated with CTC and studied by flow cytometry.

Magnetic properties of various SPION systems, were determined using Vibrating Sample Magnetometer. 57Fe Moessbauer measurements were carried out in the transmission mode at a constant acceleration spectrometer with 50 mCi 57Co/Rh source.

Results and Discussion

CCh was successfully synthetized and used to obtain SPION/CCh. The average size of the obtained nanoparticles was 143 ± 21 nm, and their zeta potential was high (37.7 ±1.8 mV), confirming they were colloidally stable. The surface of SPION/CCh was successfully decorated with anti-N-cadherin antibodies and MTX. Magnetic studies confirmed superparamagnetic character of the studied SPION systems. Confocal microscopy revealed that SPION/CCh are effectively taken up by cancer cells. MTT assay showed that SPION/CCh system is cytotoxic to PC-3 prostate cancer cells after 24 h of incubation. Western Blot analysis gave an insight in the differences in protein expressions in the untreated cells and cells exposed to SPION/CCh. Flow cytometry studies and AFM analysis allowed to confirm the specific binding of the targeted system to PC-3 prostate cancer cells. Preliminary studies showed effective magnetic capture of cancer cells with attached SPION/CCh-N-cad.

Conclusions

We have synthetized SPION nanoparticles stabilized with CCh, and successfully decorated their surface with anti-N-cadherin antibodies and MTX. The physicochemical, biological and magnetic properties of the obtained systems were studied. Preliminary studies on the SPION/CCh-MTX and SPION/CCh-N-Cad systems confirmed their potential in targeted therapy of cancer.

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References

[1] A. Kaczyńska, K. Guzdek-Zając et al., RSC Adv. 6 (2016) 72586-72595.
[2] J. Dulińska-Litewka, J.A. McCubrey *et al*., Curr. Med. Chem. 20 (2013) 144-157.
[3] E. Górowska-Wójtowicz, A. Hejmej *et al.,* Toxicol. In Vitro 40 (2017) 324-335.
[4] D. Gil, M. Zarzycka *et al*., Hum. Cell 32 (2019) 379-389.