MAGNETIC NANOPARTICLES FOR CANCER CELLS CAPTURE

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Introduction

Metastasis is the leading cause of cancer-related deaths; thus, its early detection is crucial for patient's prognosis and optimal treatment strategy. The detachment and migration of the cells from the primary tumor is enabled by the change in their phenotype, known as the epithelial-mesenchymal transition (EMT). EMT permits the epithelial cells to go through a series of biochemical and morphological processes, shifting them to the more invasive, mesenchymal phenotype with enhanced migratory properties [1]. Such cells, known as circulating tumor cells (CTC), can spread cancer to distant locations. Capturing, and analysing the captured CTC allows for a better estimation of patient's prognosis, and may allow to prevent, limit, or at least slow down metastasis. Among various approaches to this problem the use of inorganic or hybrid magnetic nanoparticles seems quite promising. Here we report on two different systems: targeted superparamagnetic iron oxide nanoparticles (SPION) stabilized with chitosan derivative and decorated with anti-N-cadherin antibodies and halloysite nanotubes modified with SPION. Both systems were designed to bind CTC, allowing for their magnetic capture, removal from the bloodstream and subsequent analysis.

Materials and Methods

Chitosan was modified using glycidyltrimethylammonium chloride (GTMAC, ≥ 90%, Sigma-Aldrich) according to the procedure developed earlier [2]. SPION were obtained by co-precipitation of Fe^{2+} and Fe^{3+} salts with ammonia in the presence of cationic derivative of chitosan (CCh). For the flow cytometry studies nanoparticles were fluorescently labelled with fluorescein isothiocyanate (BioReagent, Sigma-Aldrich). Halloysite nanotubes (nanopowder; 30-70 nm dia × 1-3 μm length) were modified with SPION also using the co-precipitation method, however, the OH^- ions were produced *in situ* by urease (from *Canavalia ensiformis*, type C-3, powder, ≥600,000 units/g solid) in the presence of urea. The enzyme was entrapped inside the nanotubes' lumen before the modification step.

The systems obtained were characterized using DLS, ATR-FTIR, and SEM/STEM. The magnetic properties of the nanoparticles were measured using magnetometry and Mössbauer spectroscopy. The targeted SPION were also studied using confocal microscopy, AFM and flow cytometry. Human prostate cell lines (American Type Culture Collection—ATCC, Manassas, Virginia, USA) - LNCaP PC-3 and DU 145 were cultured in supplemented RPMI 1640 medium with 10% (v/v) of FBS in the incubator (37ºC, 90% humidity with 5% CO_2) according to previously described procedure [3]. Cytotoxicity measurements were performed using MTT and Alamar Blue assays.

Results and Discussion

SPION/CCh nanoparticles were decorated with anti-N-cadherin antibodies, which were specific to N-cadherin present on the surface of cancer cells which underwent EMT. The nanoparticles obtained had an average size of ca. 30 nm, as measured by AFM. They also had a moderate tendency to form aggregates, which had an average hydrodynamic diameter of 223 nm and were colloidally stable (zeta potential of – 45 mV). The magnetic properties of the nanoparticles were excellent, providing the superparamagnetic character of the system. Flow cytometry experiments allowed us to find out if SPION/CCh-N-cad bind to cancer cells expressing N-cadherin and how fast the binding process is. Confocal microscopy allowed us also to see the distribution of the nanoparticles within the PC-3 monoculture and the co-culture with LNCaP cells (before EMT, low N-cadherin expression). Magnetic capture experiments in quasi-static system (specially designed and 3D-printed cuvette) were performed. To enhance the magnetic effect, we have also obtained halloysite nanotubes loaded with SPION. The synthesis was based on the procedure proposed by Zheng et al. [4]. Urease/urea system was used as a source of hydroxyl groups, allowing to form SPION in situ. near the inner surface of the nanotubes. To obtain smaller particles with more uniform size the halloysite was pre-treated using high-power sonication in the presence of a surfactant (hexadecyltrimethylammonium bromide, CTAB, ≥99.0%, Sigma-Aldrich). The pre-treated halloysite had an average hydrodynamic diameter of 315 nm and relatively uniform size (PDI = 0.183). Magnetic halloysite was easily separated from the suspension with a magnet. The preliminary microscopic studies (SEM/EDX) confirmed the formation of the nanoparticles of iron oxide.

Conclusions

SPION/CCh decorated with anti N-cadherin antibody were found to be a colloidally stable system. The magnetic properties of the SPION were not affected by the coating and modification with antibodies. Flow cytometry confirmed the effective magnetic capture of PC-3 cells with bound SPION/CCh/N-cad particles. The incubation time as short as 1 minute was sufficient for SPION/CCh/N-cad to effectively bind to cancer cells. Confocal microscopy images of co-cultures confirmed the specificity of interactions of SPION/CCh/N-cad system with N-cadherin expressing PC-3 cells. SPION were also successfully deposited on the surfaces of halloysite nanotubes, rendering them magnetic and prospecting for CTC capture.

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