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Accumulation and biological effects of cobalt ferrite nanoparticles in human pancreatic and ovarian cancer cells

Vita Pašukonienė [a,*], Agata Mlynska [a], Simona Steponkienė [b], Vilius Poderys [b], Marija Matulionytė [b,c], Vitalijus Karabanovas [b], Urtė Statkutė [b], Rasa Purvinienė [a], Jan Aleksander Kraśko [a], Arūnas Jagminas [d], Marija Kurtinaitienė [d], Marius Strioga [a], Ričardas Rotomskis [b,c]

[a] Laboratory of Immunology, National Cancer Institute, Vilnius, Lithuania
[b] Biomedical Physics Laboratory, National Cancer Institute, Vilnius, Lithuania
[c] Biophotonics Group of Laser Research Centre, Faculty of Physics, Vilnius University, Vilnius, Lithuania
[d] Laboratory of Nanostructures, State Research Institute Centre for Physical Sciences and Technology, Vilnius, Lithuania

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ABSTRACT

Background and objective: Superparamagnetic iron oxide nanoparticles (SPIONs) emerge as a promising tool for early cancer diagnostics and targeted therapy. However, both toxicity and biological activity of SPIONs should be evaluated in detail. The aim of this study was to synthesize superparamagnetic cobalt ferrite nanoparticles (Co-SPIONs), and to investigate their uptake, toxicity and effects on cancer stem-like properties in human pancreatic cancer cell line MiaPaCa2 and human ovarian cancer cell line A2780.

Materials and methods: Co-SPIONs were produced by Massart's co-precipitation method. The cells were treated with Co-SPIONs at three different concentrations (0.095, 0.48, and 0.95 μg/mL) for 24 and 48 h. Cell viability and proliferation were analyzed after treatment. The stem-like properties of cells were assessed by investigating the cell clonogenicity and expression of cancer stem cell-associated markers, including CD24/ESA in A2780 cell line and CD44/ALDH1 in MiaPaCa2 cell line. Magnetically activated cell sorting was used for the separation of magnetically labeled and unlabeled cells.

Results: Both cancer cell lines accumulated Co-SPIONs, however differences in response to nanoparticles were observed between MiaPaCa2 and A2780 cell. In particular, A2780 cells were more sensitive to exposition to Co-SPIONs than MiaPaCa2 cells, indicating that a safe concentration of nanoparticles must be estimated individually for a particular cell type. Higher doses of Co-SPIONs decreased both the clonogenicity and ESA marker expression in A2780 cells.

* *Corresponding author at:* Laboratory of Immunology, National Cancer Institute, Santariškių 1, 08660 Vilnius, Lithuania.
E-mail address: vita.pasukoniene@nvi.lt (V. Pašukonienė).

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Conclusions: Co-SPIONs are not cytotoxic to cancer cells, at least when used at a concentration of up to 0.95 μg/mL. Co-SPIONs have a dose-dependent effect on the clonogenic potential and ESA marker expression in A2780 cells. Magnetic detection of low concentrations of Co-SPIONS in cancer cells is a promising tool for further applications of these nanoparticles in cancer diagnosis and treatment; however, extensive research in this field is needed.

1. Introduction

Nanotechnologies have a great potential for various biomedical applications, including cancer diagnostics and treatment [1–4]. Superparamagnetic iron oxide nanoparticles (SPIONs) were the first nanoscale materials approved for clinical use as contrast agents for magnetic resonance imaging (MRI) of liver [5] and lymph nodes [6]. SPIONs also allow a targeted delivery of drugs, proteins and genes [7], cell bioimaging [8], and induction of cell death by the hyperthermia effect [9].

Different types of SPIONs, varying in their composition and/or surface coating, can be synthesized, depending on the requirements for their application [10–12]. Considering current limitations in the field in terms of SPION application in routine clinical practice, we aimed to synthesize and investigate a specific type of SPIONs – superparamagnetic cobalt ferrite nanoparticles (Co-SPIONs) – that are attractive candidates for magnetic resonance imaging (MRI), magnetic field-assisted drug delivery and magnetothermal therapy of cancer and other diseases. Owing to a mixed spinel structure [13], the properties of Co-SPIONs depend not only on their size and shape, but also on the cobalt content [14,15]. Due to high saturation magnetization, low coercivity, excellent chemical and thermal stability [16], Co-SPIONs possess a great potential for a wide application both in biomedical research and clinical practice.

However, there are no data about biological activity of Co-SPIONs, including their cellular uptake, toxicity, effects on cell proliferation, phenotype and functional activity. Therefore, these aspects remain a subject of particular interest.

In this study, we aimed to investigate the accumulation and biological effects of Co-SPIONs, originally synthesized by our group. Two well-characterized cancer cell lines (human pancreatic cancer cell line MiaPaCa2 and human ovarian cancer cell line A2780) served as biological models in our in vitro experiments. Both cell lines are poorly differentiated, highly tumorigenic and heterogeneous, with certain phenotypic subsets attributable to cancer stem cell-like properties [17–19]. We sought to evaluate the potential of using Co-SPIONs in clinical applications, since pancreatic and ovarian cancers are among the most aggressive and intractable oncological diseases, which could benefit from improved means of diagnosis and treatment [20,21].

2. Materials and methods

2.1. Synthesis and characterization of Co-SPIONs

Co-SPIONs were synthesized in the thermostated glass reactor by Massart's co-precipitation method [22] from the alkaline solutions of Co(II) and Fe(III) metal salts at 70 °C for 5 h. All reagents used for Co-SPION synthesis were at least of analytical grade, thus they were not additionally purified, except for NaOH, which was purified by preparation of a saturated solution resulting in the crystallization of other sodium salts. $CoCl_2$, $Fe_2(SO_4)_3$ and citric acid were purchased from Aldrich Chemicals Inc. For preparation of the working solutions 0.12 mol/L $CoCl_2$, 0.06 mol/L $Fe_2(SO_4)_3$, 5.0 mol/L NaOH, and 0.3 mol/L citric acid solutions were prepared and deoxygenated with argon before mixing. Ultrapure water was used throughout all procedures. Molar ratio of cobalt(II) and iron(III) salts in the reactor was 1:1.2 at their total concentration of 40 mmol/L. pH of solutions was maintained at 11.5. The required amount of 5.0 mol/L NaOH solution was predetermined in a blank experiment. In the subsequent experiments, the estimated amount of NaOH was added to the reactor, containing all other components, in several seconds under vigorous stirring. The synthesis in the thermostated reactor was conducted under a continuous argon gas bubbling. Crude products were centrifuged at 2800 × *g* for 5 min and rinsed several times. The supernatants obtained from the last three centrifugations were mixed, neutralized by the addition of citric acid solution up to pH 6.0, and used as a stable ferrofluid within the following week. The composition of the synthesized products was investigated by energy dispersive X-ray spectroscopy and nanoparticle dissolution in HCl (1:1) solution by inductively plasma coupled optical emission spectrometry, using an OPTIMA 7000DV analyzer (Perkin Elmer, USA).

The morphology of Co-SPIONs was investigated with an atomic force microscope Veeco-dilnnova (Veeco Inc., USA), using a tapping mode. A small amount (40 μL) of Co-SPION solution was dropped on a freshly cleaved mica surface, spinning at 50 × *g*. Zeta potential of the particles was measured using a Brookhaven ZetaPALS zeta potential analyzer (Brookhaven Instruments, USA).

Magnetic measurements of Co-SPIONs were carried out on a vibrating sample magnetometer. A gauss-/teslameter FH-54 (Magnet Physik, Germany) was applied to measure the