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Biocompatibility of magnetic iron oxide nanoparticles for biomedical applications

Abstract: Magnetic nanoparticles are highly promising for the usage in various biomedical applications including magnetic particle imaging (MPI), cancer hyperthermia treatment or as drug carriers. The present study aims at assessing *in vitro* biocompatibility of two commercially available magnetic iron oxide nanoparticle formulations: dextran-based magnetic nanoparticle synomag-D and bionized nanoferrite BNF-starch. Biological performance of both nanoparticle formulations were studied in human endothelial cells by analyzing cell viability and nanoparticle internalization in order to judge their suitability as theranostics.

Keywords: magnetic nanoparticle, magnetic particle imaging, human endothelial cells, biocompatibility, internalization, cellular uptake

<https://doi.org/10.1515/cdbme-2019-0144>

1 Introduction

Magnetic nanoparticles have attracted high consideration as potent theranostics such as for diagnostic magnetic resonance imaging, thermal cancer therapy, cell tracking and drug delivery [1,2]. They offer unique properties comprising superparamagnetism, surface-to-volume ratio, high surface area, and easy separation methodology, disclosing their great potential in the biomedical field. However, magnetic response and suitability for biomedical applications strongly

depends on nanoparticle's physico-chemical properties among them size, morphology, core composition or coating [3].

Most nanoparticles are internalized endocytically that may lead to corrosion events of the core due to the presence of acidic lysosomes. Released metal ions could be putatively cytotoxic [4]. Hence, it is of basic importance to assess the biological performance of magnetic iron oxide nanoparticles, including cytotoxicity, cellular uptake and alteration of distinct cellular functions prior to their utilization in biological environments.

The present study is focussed on the evaluation of the suitability of two types of magnetic iron oxide nanoparticles for biomedical applications by analyzing their biocompatibility with human endothelial cells.

2 Materials and methods

2.1 Magnetic iron oxide nanoparticles

Two magnetic iron oxide nanoparticle formulations: (i) dextran-based magnetic nanoparticle synomag-D and (ii) bionized nanoferrite BNF-starch were examined. Synomag-D particles possess a maghemite (γ - Fe_2O_3) core of nanoflower-shaped nanocrystallites with a dextran shell and a hydrodynamic particle diameter of 50 nm. Whereas, BNF-starch is composed of cubic magnetite (Fe_3O_4) crystals coated with hydroxyethyl starch while reaching a hydrodynamic particle diameter of 100 nm. For fluorescence imaging, the surface of the particles was functionalized with amino groups for covalent binding of the red fluorescent (redF) dye DY-555-*N*-hydroxysuccinimide ester (Dyomics, Germany).

2.2 Cell viability assay

In order to assess biocompatibility of synomag-D and BNF-starch nanoparticles, cell viability analysis was performed

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according to ISO 10993. Therefore, human endothelial EA.hy926 cells (ATCC, CRL-2922) were seeded with a density of 1.5×10^4 cells/cm² into a 96-well plate and incubated at 37°C and 5%CO₂. After 24 h, cells were loaded with either synomag-D or BNF-starch nanoparticles with Fe-concentrations of 10, 25, 50 or 100 µg/ml. After 24 h, cell viability was determined by using the CellQuanti-Blue™ assay (BioAssay Systems, Hayward, CA, USA) according to the manufacturer's instructions. Briefly, cell viability was assessed by measuring metabolic activity by the reduction of the substrate resazurin to resorufin by cellular reductases. The resulting fluorescence of resorufin was measured at an emission wavelength of 590 nm with an excitation wavelength of 544 nm using a microplate reader (FLUOstar OMEGA, BMG Labtech, Germany). Three independent biological replications were performed. Data were

compared to unloaded control (mean + SD, n=3, One-way ANOVA, **p < 0.01).

2.3 Cellular nanoparticle uptake and F-actin formation

Confocal laser scanning microscopy was carried out in order to determine cellular uptake of synomag-D and BNF-starch nanoparticles as well as actin cytoskeleton formation. Human endothelial EA.hy926 cells were incubated with red fluorescent-labelled synomag-D and BNF-starch nanoparticles with a Fe-concentration of 100 µg/ml for 24 h. Actin cytoskeleton was stained with 2.5 µg/ml phalloidin-FITC for 1 h at room temperature. Cell nuclei were stained with 2 µg/ml Hoechst 33342 (Sigma-Aldrich Chemie GmbH, Germany) for 3 min at room temperature. Cells were washed in phosphate-buffered saline and embedded in mounting medium. Image acquisition was performed with confocal laser scanning microscope Olympus FV 1000 (Olympus, Japan).

2.4 Statistical analysis

Data were reported as mean value + standard deviation and analyzed by one-way ANOVA carried out with GraphPad®Prism 5 software (La Jolla, CA, USA). Statistical significance was assumed at p < 0.05.

3 Results

3.1 Biocompatibility of magnetic nanoparticles

Cell viability of human endothelial EA.hy926 cells in response to synomag-D nanoparticles with Fe-concentrations of 10 to 100 µg/ml ranged between 94.0 – 109.3% and was not significantly altered compared to the unloaded control (Figure 1). Also, no dose-dependent effect was observed for synomag-D. Exposure to BNF-starch at 10-50 µg Fe/ml also demonstrated no significant alteration in cell viability compared to the unloaded control, while at 100 µg Fe/ml, cell viability was significantly decreased to 58.1%. Thus, for synomag-D and BNF-starch, at all Fe-concentration levels (except for 100 µg Fe/ml BNF-starch) relative viability of human endothelial EA.hy926 cells was more than 70% (as critical threshold for the biocompatibility of medical products according to ISO 10993) and thus assuming biocompatibility.

normalized to cell viability of an unloaded control.

Figure 1: Relative viability of human endothelial EA.hy926 cells loaded with synomag-D and BNF-starch nanoparticles for 24 h with doses ranging from 10, 25, 50 and 100 µg Fe/ml, respectively

3.2 Nanoparticle internalization and actin cytoskeleton organization

Confocal laser scanning microscopy was carried out to determine cellular uptake of synomag-D and BNF-starch nanoparticles (Figure 2). For synomag-D, results showed no significant cellular uptake of nanoparticles in EA.hy926 cells at a Fe-concentration of 100 $\mu\text{g/ml}$.

In contrast, for BNF-starch nanoparticles an obvious uptake in EA.hy926 cells could be observed. In particular, BNF-starch nanoparticles were shown to be concentrated near the cell nuclei, whereas in the cytoplasm no obvious accumulation of BNF-starch particles was detected.

Regarding the organization of the actin cytoskeleton in response to the treatment with synomag-D or BNF-starch nanoparticles, no obvious morphological alterations in the actin formation in human endothelial EA.hy926 cells could be observed (Figure 2). The formation of the actin cytoskeleton in EA.hy926 cells was similar among cells treated with either synomag-D or BNF-starch nanoparticles or without nanoparticles (unloaded control).

4 Discussion and conclusion

Magnetic nanoparticles are promising theranostics for the usage in several biomedical applications like magnetic imaging, hyperthermia cancer treatment, stem cell tracking or thermosensitive drug release. Assessing their putative effect on biological response and cellular functions is of substantial importance to judge their suitability for biomedical usage.

Synomag-D and BNF-starch nanoparticles were found to exhibit excellent biocompatibility according to ISO 10993, however, for BNF-starch, cell viability of human endothelial cells was significantly decreased at a concentration of 100 $\mu\text{g Fe/ml}$. Studies reported that labelling with superparamagnetic iron oxide nanoparticles can interfere with the organization of F-actin [5]. However, in the present study F-actin staining of nanoparticle-labeled cells did not reveal any disorganization of F-actin for synomag-D and BNF-starch nanoparticles.

Analysis of cellular uptake also showed no significant internalization of synomag-D nanoparticles. On the contrary, BNF-starch demonstrated enhanced internalization in human endothelial EA.hy926 cells at a Fe-concentration of 100 $\mu\text{g/ml}$. This might correspond to the higher cytotoxic effect of BNF-starch observed at the same Fe-concentration, probably reasoned in induced cytotoxic events by endocytic nanoparticle internalization [4,5].

Distinct traits in intracellular uptake among synomag-D and BNF-starch nanoparticles might be due to distinct particle properties, *i.e.* surface functionalization or hydrodynamic diameter [6].

Further research including *in vivo* data has to distinguish the sufficient dose since dose-dependent effects might occur when used in higher dosage. Results will provide deeper insights in cell-nanoparticle interactions towards the assessment of the biological performance of iron oxide nanoparticles and their suitability as theranostics.

Figure 2: Intracellular localization of synomag-D (A) and BNF-starch (B) (at 100 $\mu\text{g Fe/ml}$, respectively) and actin formation in human endothelial EA.hy926 cells after 24 h compared to the unloaded control (C) (blue: cell nuclei, red: synomag-D-redF, BNF-starch -redF, green: actin cytoskeleton, confocal laser scanning microscopy; bar = 50 μm).

Acknowledgment: The authors greatly acknowledge technical assistance of Martina Nerger and Gabriele Karsten.

Author Statement

Research funding: Financial support by The State Ministry of Economics, Employment and Health of Mecklenburg-Vorpommern within the project “NanoVis” is gratefully acknowledged.

Conflict of interest: Both nanoparticle types were manufactured and provided by micromod Partikeltechnologie GmbH. The authors state that there are no restrictions on sharing of data and/or materials. Authors state no conflict of interest.

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