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In vitro genotoxicity and cytotoxicity of polydopamine-coated magnetic nanostructures

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ABSTRACT

Synthesis of magnetic nanoparticles and magnetic nanoclusters was performed by the co-precipitation method or solvothermal synthesis, respectively, followed by oxidative polymerization of dopamine, resulting in a polydopamine (PDA) shell. The nanomaterials obtained were described using TEM, FTIR and magnetic measurements.

For the first time, cyto- and genotoxicity studies of polydopamine-coated nanostructures were performed on cancer and normal cell lines, providing in-depth insight into the toxicity of such materials. The tests conducted, *e.g.* ROS, apoptosis and DNA double-break of the nanomaterials obtained revealed the low toxicity of these structures. Thus, these results prove the biocompatibility and low genotoxicity of these materials and provide new data on the toxicity of PDA-coated materials, which is of great importance for their biomedical application.

1. Introduction

Nanotechnology is a modern and intensive developing branch of science and has attracted the attention of many laboratories. The main utility of this technology is that it uses the unique properties of nanoparticles (NPs) with respect to their macroscale counterparts. These changes significantly improve their mechanical, optical, and electrical properties. Due to their size, which are similar to that of biomolecules, nanoparticles can pass through the cellular lipid bilayer and potentially be used in biological systems (Zhang et al., 2008). Thus, over the last decade there has been dynamic growth in research in nanotechnology for biomedical applications. One of the most frequently investigated types of nanoparticle in this field of science is magnetic nanoparticles (MNPs). MNPs are characterized by excellent superparamagnetic properties, a large surface area and the possibility of surface chemical modifications and biofunctionalization. The processes of synthesizing magnetic nanoparticles offer the possibility of modification, which produces nanomaterials in of different size and purity. Applying magnetic nanoparticles to biomolecules or drug delivery increases the efficiency of such systems. Combining various properties of MNPs provides multimodal nanocarriers that can be used in targeted therapy, theranostics or hyperthermia.

One of the most important factors determining the utility of such types of nanoparticles in biomedicine is their toxicological profile. Nanotoxicology concerns the cytotoxicity and genotoxicity of nanoparticles on living cells or organisms. Exposure of *in vitro* systems to magnetic nanoparticles may lead to them being damaged and/or to their death. The most commonly investigated morphological and biochemical changes in cells include cell membrane permeability, enzyme activity, proliferation studies, free radical production and changes in genetic material (Tang et al., 2015). The majority of the changes described may lead to cell death. Reactive Oxygen Species (ROS) is well known to play an important role in many cellular processes, such as cell signaling and regulation, activation of signaling cascades and apoptosis, and alternate death pathways (Hancock et al., 2001; Burhans and Heintz, 2009; Li et al., 2003; Chen et al., 2009). A highly regulated and controlled process of apoptosis induces changes in the content and location of proteins or other surface structures, activates proteases (caspases) and destroys DNA and cellular body. Genotoxicity is also an emerging subject in nanotoxicological research. Fragmentation of nucleic acids is the final product of genetic material degradation, but it is a key issue in determining DNA damage *via* cell signaling pathways, as well as epigenetic interactions (Thongkumkoon et al., 2014; Tanaka et al., 2007).

In general, nanoparticle toxicity is highly complex and may depend on the purity, type of synthesis, coating, shape, concentration and

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biological system being tested. Therefore, toxicity tests should be performed on every type of nanoparticle and after every step of synthesis with the use of different compositions and under various conditions. The lack of strict regulations concerning physicochemical and biological evaluation of nanoparticles leads to a great need to provide extensive, careful analysis, in particular for biomedicine.

One of the most important issues that influence the toxicity of magnetic nanoparticles is surface chemistry. Bare nanoparticles often are hydrophobic. However, functionalization with functional groups, ligands or cationic polymers increases their dispersion properties, biocompatible profile, and cellular uptake, due to electrokinetic interaction (Jin et al., 2011). Coating the surface of MNPs with a polymer not only improves the biocompatible profile of magnetic nanoparticles, but also provides functional groups for further modifications with biomolecules or drugs. However, differential cellular responses were described due to various surface modifications (Yang et al., 2013).

It was proved that poly(vinylalcohol)-coated SPIONs (Superparamagnetic iron oxide nanoparticles) decreased antigen processing and the CD4 + T cell stimulation capability of human monocyte-derived dendritic cells. Yu et al. showed that dextran and polyethylene glycol coatings reduced iron oxide NPs' cytotoxicity in aortic endothelial cells compared to bare ones. They investigated MNPs of 5 and 30 nm in diameter using a Live/Dead assay, ROS and Cell Length and Actin Cytoskeleton (Yu et al., 2012). Yang et al. also examined MNPs of different sizes (10, 100–150 nm) with different functional groups (−OH, $-NH_2$) and coated with tetraethyl orthosilicate (TEOS), 3-aminopropyltrimethoxysilane (APTMS) or TEOS/APTMS. They tested those nanoparticles with a CCK-8 assay, LDH assay and tail content of DNA and concluded differential cellular responses (normal fibroblasts, fibrosarcoma cells) (Yang et al., 2013). A careful study of the literature also revealed data describing the extremely low level of cytotoxicity and genotoxicity of thiolated (SH) and *S*-nitrosated (S-NO) iron oxide superparamagnetic nanoparticles on healthy (3 T3, human lymphocytes cells, and Chinese hamster ovary cells) and cancer (MCF-7) cell lines (Seabra et al., 2014).

These research papers presented the toxicity of magnetic nanoparticles in contexts of biosafety that indicate good biocompatible properties, depending on the surface coating. However, there is a need to develop a modern/novel coating which will possess a hydrophilic and stabilization function and will also be able to serve as a donor of a functional group for further biomolecule (drugs, nucleic acid, antibody, peptides) attachment, as well as a nanostructure compound with physicochemical properties enabling hyperthermia or phototherapy. Due to PDA's unique and specific properties, there is a possibility to bind biologically active biomolecules in a highly efficient and effective way (Mrówczyński et al., 2016). It is also possible to achieve a local toxic effect by controlled NIR radiation and heating (Chen et al., 2016).

Polydopamine (PDA) is one of the most promising bioinspired coating polymers that allows to straightforward modification of coated objects, and exhibits adhesive properties towards various materials. PDA is formed by the oxidation of dopamine and is composed of dihydroxyindole, indoledione, and dopamine units (Liebscher et al., 2013). Other structures were also proposed in the literature, where units of polymer linked covalently or *via* hydrogen bonding. The recently exploited features of polydopamine in biomedicine are photothermal properties, which allows the use of mere polydopamine or polydopamine nanomaterials in photothermal therapy, where cancer cells are destroyed by an irradiating photothermal agent with a laser beam, resulting in temperature elevation. What is of greater importance, polydopamine has been used to increase the photothermal properties of magnetic nanoparticles which exhibit such features but are not big enough for application. Thus, combining magnetic nanomaterials with a polydopamine coating provides a versatile nanoplatform which can be applied in cancer thermal therapy and after the functionalization of such composites can be used for preparing multifunction nanomaterials for targeted drug and nucleic acid delivery, guided photothermal therapy and MRI.

Even though magnetic nanomaterials coated with polydopamine have already been successfully applied *in vivo*, proving their effectiveness, there still exists a significant need to provide complex cytotoxicity and genotoxicity analysis of such nanostructures to determine their wide biocompatibility profile for biomedical applications. Consequently, for the purpose of this study, the two most commonly used magnetic nanomaterials - magnetite nanoparticles (MNP 2) and magnetic nanoclusters (MNP 3), both coated with polydopamine - were chosen due to their promising application as nanocarriers in biomedicine and the lack of information regarding their comprehensive cytotoxic data. To our best knowledge, so far no report has been produced which deals with intense *in vitro* studies of polydopamine-coated nanomaterials and their influence and cytotoxicity on cancer cells and normal human fibroblasts.

2. Materials and methods

2.1. Synthesis of nanoparticles

2.1.1. MNP 2

Firstly, magnetic nanoparticles were obtained according to the literature protocol with a slight modification as follows: $FeCl_2{\cdot}4H_2O$ (1.72 g, 8.65 mmol) and $FeCl_3{\cdot}6H_2O$ (4.7 g, 17.38 mmol) were dissolved in water (80 mL) and degassed. The temperature was then elevated at 85 °C and an ammonia solution (20 mL) was added. Heating was continued for 30 min, followed by the addition of citric acid (2 g, 10.4 mmol) in 8 mL of water. Stirring continued for 1.5 h at 95 °C. The mixture was cooled down to RT and the resulting nanomaterial was washed with distilled water (3 × 200 mL) and finally redispersed in 100 mL of water.

Next, the magnetic nanoparticles that had been obtained (1.4 g) were redispersed in 70 mL of H_2O and 70 mL of Tris buffer (pH = 8.5, 10 mmol) followed by the addition of dopamine hydrochloride (140 mg, 0.74 mmol, c = 1 mg mL^{-1}). Stirring under air access continued for 6 h and then the nanoparticles were collected by an external magnetic field and washed with water (3 × 200 mL). Finally, they were redispersed in water (250 mL).

2.1.2. MNP 3

Magnetic clusters were synthesized *via* the solvothermal method. $FeCl_3{\cdot}6H_2O$ (0.54 g) was dissolved in ethylene glycol with the addition of sodium acetate, sodium acrylate and then transferred into a sealed Teflon autoclave and heated at 200 °C for 10 h. After cooling down to RT, the particles were washed with water and EtOH and dried in a vacuum at 40 °C. In the next step 25 mg of the result material was redispersed in 25 mL of water and 25 mL of Tris buffer using ultrasounds. Then dopamine hydrochloride (25 mg, 0.13 mmol) was added and continued to stir for 2 h. Then nanoparticles were collected by an external magnetic field and washed with water.

Preparation of MNP 2 and MNP 3 is presented in Scheme 1.

2.2. Physicochemical characterization

Transmission electron microscopy (TEM) images were recorded on a JEM-1400 microscope made by JEOL (Japan). The accelerating voltage was 120 kV. A small amount of the sample was placed on a copper measuring grid (Formvar/Carbon 200 Mesh made by TedPella (USA)) after 5 min of sonication in deionized water. Next, the sample was dried in a vacuum desiccator for 24 h. FTIR spectra were recorded on a Bruker Tensor 27 spectrometer in KBr pallets. Magnetization measurements were performed using a MPMS-XL SQUID magnetometer. Temperature-dependent magnetization was studied in the temperature range 2–300 K. Magnetization, as a function of the magnetic field ± 30 kOe, was performed at 5 and 300 K. The zeta (ζ) potential of the MNPs suspension in water was determined by electrophoretic light