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Research paper

Effect of cell media on polymer coated superparamagnetic iron oxide nanoparticles (SPIONs): Colloidal stability, cytotoxicity, and cellular uptake studies

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Abstract

The influence of the composition of the polymer coated polyvinyl alcohol (PVA), vinyl alcohol/vinyl amine copolymer (A-PVA) and polyethylenimine (PEI) coated superparamagnetic iron oxide nanoparticles (SPIONs) on the colloidal stability, cytotoxicity and cellular uptake of these particles in different cell media is reported in this paper. Although all examined polymer coated SPIONs were stable in water and PBS buffer these colloidal systems had different stabilities in DMEM or RPMI media without and supplemented with fetal calf serum (FCS). We found that A-PVA coating onto the surface of the SPIONs decreased the cytotoxicity of the polymer compared to the same concentration of A-PVA alone. As well, polyplexes of PEI-SPIONs with DNA in concentration used for transfection experiments showed no cytotoxicity compared to PEI and PEI-SPIONs. Our data show that the choice of medium largely influences the uptake of these particles by HeLa cells. The optimal medium is different for the different examined polymer coated SPIONs and it should be determined in each case, individually.

Keywords: Superparamagnetism; Iron oxide; Nanoparticles; PVA; PEI; Colloidal stability; Cytotoxicity

1. Introduction

The synthesis and characterization of nanoparticles have been a focus of intensive research for more than 10 years since they play an important role in electronics, catalysis, biology and medicine. Nanoparticles that can be biochemically functionalized are potential medical device systems that can be used in many different biological and medical fields of application. Beside the unique physical properties induced by surface or quantum effects, the size of the primary particles is with 2–30 nm comparable to the size of biological building blocks and allows investigation of the cellular functioning or direct interaction with biological targets. Well described examples are inorganic photoluminescent particles (quantum dots) as markers or nanosized superparamagnetic iron oxide particles. Superparamagnetic particles with a diameter of around 10 nm have been used for many years for e.g. non-viral gene delivery, as MRI contrast agents, or for typical separation applications [1]. Those applications are relatively well established on the market, whereas targeted particle delivery is still in the development stage. It has been shown that magnetic particles are physiologically well tolerated and that the surface

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of the particles is responsible for the biocompatibility and stability to the reticulo-endothelial system [2]. On the other hand, concerns have been raised about nanoparticle-derived adverse health effects and scientists currently aim at developing a rational, science-based approach to nanotoxicology. Recent investigations were focused on relatively simple cytotoxic tests as readily available prescreening methods, on the effect of inhaled or instilled ambient nanoparticles that can induce oxidative stress or pulmonary inflammation, or on possible consequences of particle related dysfunction of the cardio-vascular system [3]. A key question is the assessment of nanoparticles' potential toxicity due to their nanosize nature whereas the type of particle does not seem to play an important role [4,5]. The surface properties of the particles are particularly investigated in more detail since the surface seems to be the determining factor for cell uptake and cytotoxicity.

Many applications and investigations use nanoparticles in colloidal suspensions. By tailoring interactions between colloidal particles, one can design stable fluids, gels, or colloidal crystals. Long range, attractive van der Waals forces are ubiquitous and must be balanced by Coulombic, steric, or other repulsive interactions to engineer the desired degree of colloidal stability [6]. The colloidal behaviour of nanoparticles in different cell media or body fluids is almost never considered or related to particle–cell interactions. Williams et al. investigated the impact of silica, silica/iron oxide, and gold nanoparticles on the growth and activity of *Escherichia coli* and correlated the results with dynamic light scattering experiments that were performed in growth media [7]. It has been shown that submicron polymeric particles coagulate in the cell medium, whereas colloidal stability in aqueous solution was ensured for several weeks [8].

The aim of this work is to show in detail the influence of the composition of the polymer coating and surface charge of nanoparticles on the colloidal stability of these particles in different cell media. Furthermore, we tried to establish correlations between cytotoxicity or uptake rates and agglomeration behaviour of the particles. This correlation in particular is very important for the design of simplified toxicity tests and for the further development of such particles for *in vivo* applications.

2. Materials and methods

2.1. Materials

All chemicals were of analytical reagent grade and were used without further purification. Polyvinyl alcohol PVA (Mowiol® 3-83) with an average molecular weight (MW) of 14,000 g/mol and a hydrolysis degree of 83% was supplied by courtesy of CLARIANT. Vinyl alcohol/vinyl amine copolymer M12, with an average MW of 80,000–140,000 was supplied by courtesy of ERKOL.

Polymer solutions were prepared by dissolving the powders in water followed by rapidly heating the solutions for 15 min (Mowiol® 3-83) to 4 h (M12) at 90 °C, and filtering the hot solutions over paper filters (Schleicher & Schuell AG). Ultra-pure deionized water (Seralpur delta UV/UF setting, 0.055 μS/cm) was used in all synthesis steps. D-9527 Sigma cellulose membrane dialysis tubing with a molecular weight cut-off at 12,000 was used for dialysis.

2.2. Iron oxide nanoparticles

Superparamagnetic iron oxide nanoparticles (SPIONs) were prepared by alkaline co-precipitation of ferric and ferrous chlorides in aqueous solution as described elsewhere [9–11]. The obtained brown suspension was dialyzed against 0.01 M nitric acid for two days, and stored at 4 °C.

2.3. Polymer coated particles

In order to obtain SPIONs coated with either polyvinyl alcohol (Mowiol® 3-83) or with a mixture of polyvinyl alcohol (Mowiol® 3-83) and vinyl alcohol/vinyl amine copolymer (M12, vinyl alcohol/vinyl alcohol copolymer mass ratio = 45) the nanoparticle dispersion was mixed at various ratios with the different polymer solutions. The products will be referred to as PVA-SPION and A-PVA-SPION in this work.

For PEI coating the iron oxide nanoparticles were mixed at a PEI:Fe mass ratio of two ($R = 2$) with 25 kDa polyethylenimine (Aldrich). The samples will be referred to as PEI-SPION in this work. DNA-PEI-SPIONs were prepared at a N/P ratio (ratio of nitrogen-containing groups of the polymer to phosphate groups of the nucleic acid) of 7.5, assuming the DNA was entirely complexed. The nitrogen content of PEI has been measured and calculated according to Harpe et al. [12], and the phosphate content was calculated from the size of the plasmid (4.7 kb).

The iron content of the suspensions was determined by redox-titration, essentially as described [13].

2.4. Transmission electron microscopy

Transmission electron microscopy (TEM) was performed using a Phillips CM-20 microscope operating at 200 kV. For sample preparation, dilute drops of suspensions were allowed to dry slowly on carbon-coated copper grids.

2.5. Colloidal stability

The colloidal stability of coated particles in different environments was investigated by turbidity measurements. Therefore, the particle dispersions were mixed with commonly used cell media such as RPMI and DMEM, both in presence and absence of 10% fetal calf serum (FCS) thereby setting the iron concentration to 100 μg/ml. After rapid homogenization, the turbidity was measured by light absorption at a wavelength of 500 nm as a function of time (t) [14].