Toxicity studies of polymer based superparamagnetic iron oxide nanoparticles

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Abstract

Superparamagnetic iron oxide nanoparticles (SPIONs) have been of great interest since the last decades due to their important contributions to nanomedicine [1, 2]. These inorganic nanomaterials can be useful as a diagnostic tool (e.g. magnetic resonance image contrast agent), a therapeutic tool (e.g. hyperthermia), or a theranostic tool. Stable biocompatible suspension of these nanoparticles is mandatory for efficient application, which is achieved by an adequate polymeric coating. Our model consists of iron oxide nanoparticles (γ-Fe_2O_3) embedded within a hydrophobic poly(vinylpyridine) (P4VP) polymer and coated with a hydrophilic polyethylene glycol (PEG). A fraction of coating PEG can also be functionalized for the conjugation of fluorescent dyes (dual reporter nanoparticles), antibodies and drugs Fig1. These nanoparticles are dispersed in phosphate buffer saline (PBS) at pH 7.4 to mimic physiological conditions. The resulting ferrofluids have core diameter (ferric oxide nanoparticles diameter) ranging between 4 to 15 nm, with 10% size dispersion, and hydrodynamic diameter ranging between 50 to 164 nm.

Since the in vivo delivery of these nanoparticles for biomedical applications ends at the cell, studies pertaining to the toxicological effect on the cell (cytotoxicity), and nanoparticles cellular uptake and uptake kinetics are of utmost importance.

Cytotoxicity studies of the ferrofluids have been carried out on two different cell lines, opossum kidney cells (OK) and vascular smooth muscle cells (VSMS). The activity of the lactate dehydrogenase in culture media was determined as a function of the dose. LC50 has been also calculated.

As the nanoparticles uptake by the cell is depending on several factors [3], this work focused on the effect of the nanoparticle size and cell type on the cellular uptake. Sub cellular tracking studies have been carried out using fluorescent nanoparticles. Results show the localization of the nanoparticles after 24h of incubation with the cells inside the lysosomes Fig 2. By using the pharmacological inhibitor we found that the nanoparticles uptake takes place by clathrin-dependent endocytosis.

These nanoparticles are developed for intravenous administration; therefore, studies pertaining to their haematological behaviour are of outmost importance and should be included in the toxicity and compatibility tests to be made in the development of these nanoparticles. We studied the effect of the nanoparticles and their polymers on the blood coagulation process. Results show that P4VPg-PEG-coated SPIONs in PBS act as non-specific circulating anticoagulant agents in vitro. While PEG component does not seem to have any effect on the coagulation process, the coating copolymer P4VP-g-PEG shows strong anticoagulant behaviour indicating that P4VP is at the origin of the effect.

References

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Figures

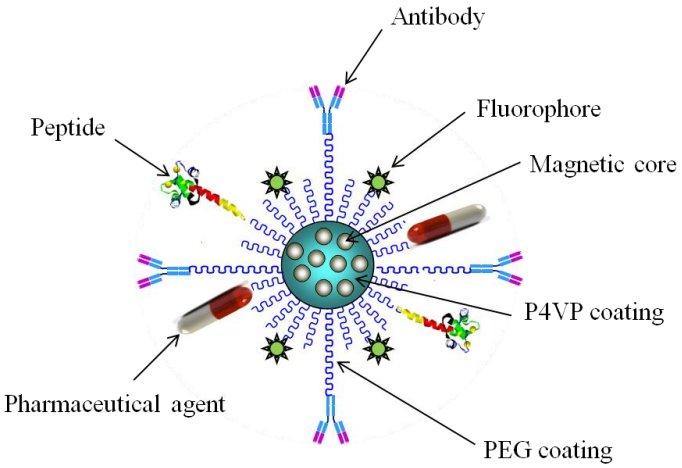

Fig 1. Polymer based superparamagnetic iron oxide nanoparticle model.

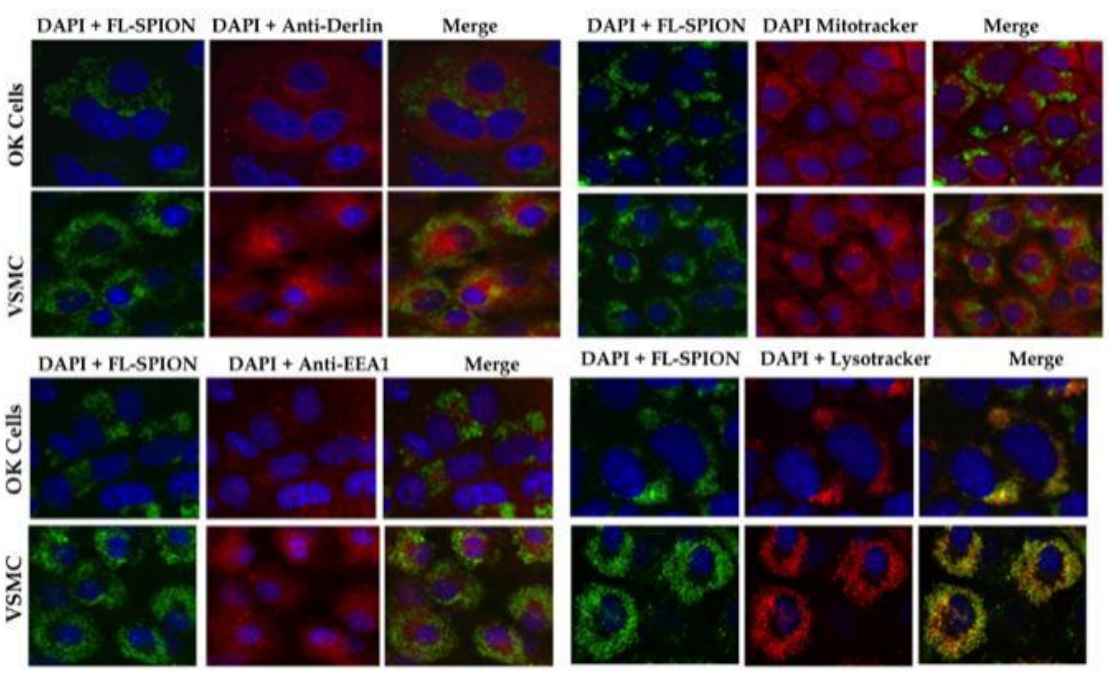

Fig 2. Subcellular localization of nanoparticles using markers of the endoplasmic reticulum (Anti-Derlin Ab), mitochondria (Mitotracker), early endosomes (Anti-EEA1), lysosomes(lysotracker) by fluorescence microscopy. Cells were treated with fluorescent nanoparticles (R8) at 0.007 g/L Fe_2O_3 for 24 h.