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Magnetic iron oxide nanoparticles as drug delivery system in breast cancer

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ABSTRACT

Present work was focused on producing improved iron oxide nanoparticles for targeted drug delivery in breast cancer. Nanometric-sized iron oxide particles were synthesized by laser pyrolysis and were morphologically/structurally characterized. These new nanoparticles were compared with some commercial, chemically prepared iron oxide ones. Cytotoxicity and the anti-proliferation effects of nanoparticles were tested in vitro on the breast adenocarcinoma cell line MCF-7. Nanoparticles were further coated with the antracyclinic antibiotic Violamycine B1 and tested for the anti-tumor effect on MCF-7 cells. The nanoparticles produced by us seem more effective in vitro than the commercial ones, with respect to cellular uptake and VB1 delivery. Violamycine B1 bound on nanoparticles is as efficient as the free form, but is better delivered into tumor cells.

1. Introduction

A major problem in cancer therapy is the lack of specificity of chemotherapeutic drugs for tumor cells; large doses of drug with serious side-effects need to be injected for achieving efficient concentration in the tumor. Therefore, current efforts are focused on developing strategies for targeted drug delivery, including molecular and magnetic systems.

In the recent development of nanobiotechnology, magnetic nanoparticles (NPs) have gained increasing attention for use in biomedical applications. NPs are particularly advantageous for in vivo drug transport, due to their small size and large surface area, while their magnetic properties are relevant for targeted drug delivery. Maghemite (γFe_2O_3) is one of the most suitable materials for the core of magnetic NPs because it is least likely to cause major health hazards: iron(III) ions are widely found in the human body, so leaching of metal should not cause significant side-effects [1]. The alternative use of Fe_3O_4 magnetite NPs can be detrimental due to the release of Fe(II) ions which generate toxic hydroxyl radicals via Fenton reaction [2]. As such, maghemite NPs became intensively investigated due to their low nanotoxicity and their good magnetic properties [3–9].

Antracyclinic antibiotics are effective in solid tumors, particularly in the case of breast cancer, but also in acute leukemia and malignant lymphomas [10]. All anthracyclines contain a planar aglycone ring coupled with amino-sugar groups and thus exhibit positive electrostatic charges [11]. The planar ring (Fig. 1) intercalates between DNA base pairs and the amino-sugar moiety and interacts with the negatively charged phosphate groups in the DNA major groove [12]. The intercalation causes changes in the shape of the DNA helix, hinders DNA replication and RNA transcription [13]. Violamycine B1 (VB1) has a high binding efficiency to DNA but its clinical use is not allowed due to severe side-effects, such as cardiotoxicity [14]. VB1 is the only antracyclinic antibiotic with two amino-sugar groups which are supposed to play a role in the coating process on the NP surface. Accordingly, VB1 is a perfect candidate for targeted delivery by magnetic iron oxide NPs.

The aim of our work was to produce and characterize new magnetic NPs with improved drug delivery properties, when compared to some commercially available NPs. We produced iron oxide NPs by laser pyrolysis, with uniform size and high magnetic properties , characterized them from morphological and structural point of view, and investigated their cellular uptake and VB1 delivery to breast adenocarcinoma tumor cells.

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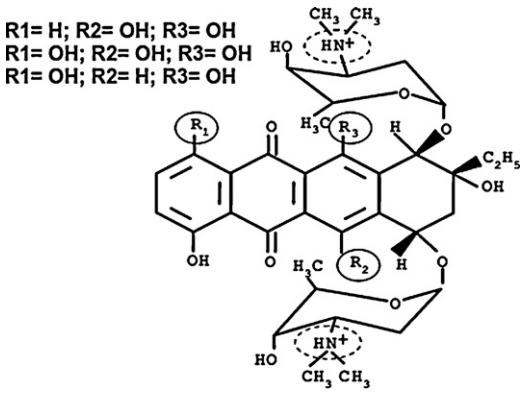

Fig. 1. VB1 structure.

2. Experimental systems and procedures

2.1. NPs fabrication

The reactive gas mixtures [$Fe(CO)_5$ vapors and oxidative medium] and the sensitizer laser absorbent gas (C_2H_4) were admitted through the central tube and interacted orthogonally with a focused CW CO_2 laser beam (Fig. 2). The laser-induced reaction occurred due to photon – thermal energy transfer provided by ethylene in the reactive gas mixture. The admission of gases through the reaction chamber implied a nozzle with two concentric tubes. An Ar stream through the external tube was used to preserve the laminar flow, especially for the central trajectory, before the reactive gases crossed the laser irradiation zone. Main experimental gas debits: ethylene 100 sccm, $Fe(Co)_5$ 8.5 sccm, synthetic air 32 sccm and Ar for mixture confinement, 1500 sccm. The global pressure was 60 kPa, laser incident power 250 W/cm^2, while the resulting temperature in the plasma was 620 °C. More details on the laser pyrolysis technique and its particularities for iron oxide NPs synthesis were described elsewhere [15–17]. NPs produced by us were morphologically characterized by scanning electron microscopy, and transmission electron microscopy (TEM), while structural investigation was performed by X-ray diffraction (XRD) and Mossbauer spectroscopy. Magnetic properties were investigated using a superconducting quantum interference device (SQUID). Commercial NPs used in our experiments were pure γFe_2O_3 (from MIT Corporation) with a diameter of 20 nm and purity above 99%.

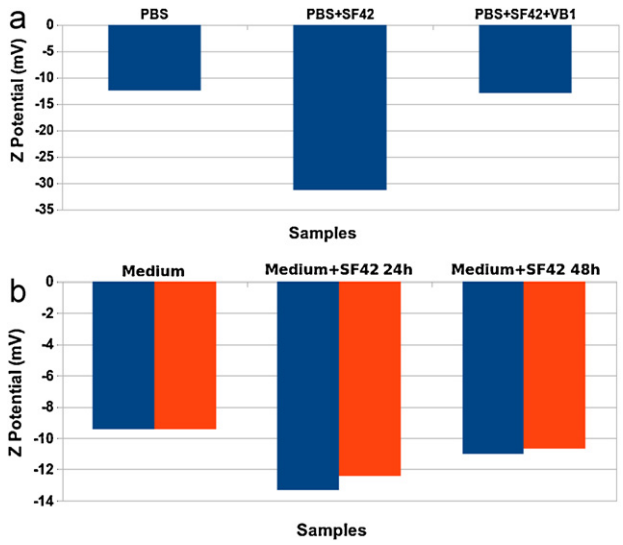

Fig. 3. Zeta potential analysis of loaded and unloaded nanoparticles in (a) PBS and (b) FBS containing medium.

2.2. VB1-coated NPs

VB1 from *Streptomyces violaceus* [18] was coated on NPs by dispersion. 10 mg NPs were suspended into a 1 ml of 1.0×10^{-4} M VB1 solution. The suspension was kept in dark for 4 days, at room temperature. The VB1 loaded NPs were separated by centrifugation at $4000 \times g$ and the uncoated VB1 concentration in the supernatant was measured spectrophotometrically at 500 nm [19]. The VB1-coated NPs were washed 3 times with sterile phosphate buffered saline (PBS), until no detectable VB1 was found in the washing solutions. The concentration of VB1 coated on each type of NPs was calculated as difference between the initial VB1 concentration and the remaining VB1 concentration in the supernatant collected immediately after NPs loading. The VB1-coated NPs were dispersed in sterile PBS at a concentration of 1 mg/ml.

The coating mechanism is assumed to rely on the electrostatic interaction of NPs negative charges with VB1 positive charges. *Z*-Potential measurements in PBS (Fig. 3a), indicate that, by introducing the nanoparticles, the solution potential decreases, while introducing nanoparticles loaded with VB1, the potential tends to return to the original PBS solution potential. From this experiment we could assume that the nanoparticles load the "maximum" quantity of VB1 while "annihilated whole surface charge

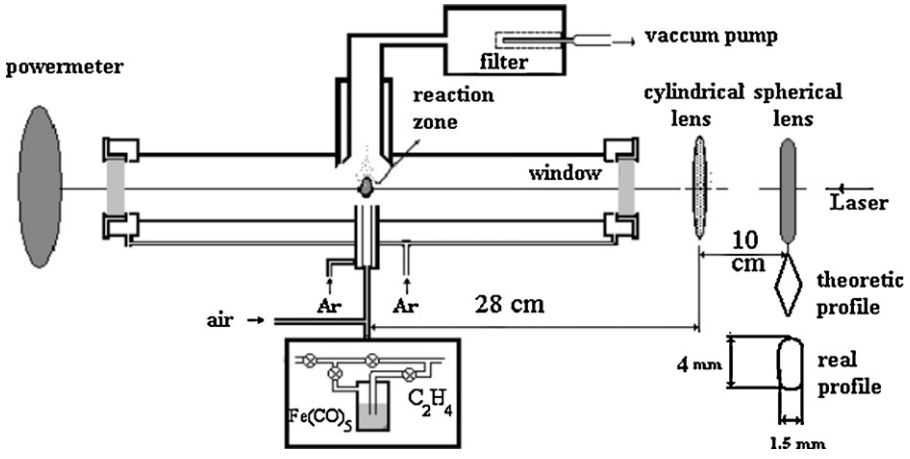

Fig. 2. Experimental setup of laser pyrolysis.

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