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Hydrophilic chlorin-conjugated magnetic nanoparticles—Potential anticancer agent for the treatment of melanoma by PDT

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ABSTRACT

This Letter reports the synthesis and the characterization of two new water-stable and soluble photosensitizer-conjugated magnetic nanoparticles (PS-MNPs) composed of an iron oxide magnetic core coated with a biocompatible dextran shell bearing polyaminated chlorin *p6*. Designed to improve cancer cell targeting, these photosensitizers were assayed for their antitumour activity against two variants of B16 mouse melanoma cell line (B16F10 and B16G4F, with or without melanin, respectively). Cell viability measurements demonstrated that PS-MNPs were more phototoxic than PEI-chlorin *p6* making these photosensitizers promising for further in vitro and in vivo investigations.

Malignant melanoma is the most aggressive form of skin cancer. Indeed, it accounts for 4% of total cancer prevalence and is responsible for 80% of deaths related to skin cancers.[1] Although surgical excision of malignant melanoma in the early stages has a good prognosis, it often fails to completely avoid tumour progression and metastasis. Metastatic melanoma is refractory to existing therapies. As a consequence, median survival time for patients with stage IV melanoma is approximately 9 months, and 3 year survival rates are less than 15%. Dicarbazine, temozolomide, high-dose interleukin-2 and paclitaxel (with or without carboplatin) are in current use for the treatment of melanoma.[2] Nevertheless, therapeutic tools are weakly efficient and among new approaches for treatment of skin melanoma, photodynamic therapy (PDT) could be potentially pertinent.[3] Indeed, PDT is an established modality for the treatment of neoplastic diseases which involves accumulation of a photosensitizer (PS) into cancer cells, followed by in situ photoactivation of the PS by visible light which leads to production of singlet oxygen ($^1O_2$) and reactive oxygen species (ROS) then subsequent death of the treated cells.[4] Nevertheless, lack of selectivity is the main drawback with this technique and considerable efforts are being done to improve tumour targeting. For example, PS can be covalently attached to sugars, peptides, folic acid, hormones or polyamines, leading to receptor-mediated accumulation of drugs into malignant cells.[5–10] Another strategy exploits enhanced permeability and retention of solid tumours, a specific behavior explained by effect their leaky blood vessels and poor lymphatic drainage systems.[11] Recent observations have shown that nanoparticle-carried PDT agents are accumulated in significant amounts by a variety of tumour cells.[12,13] Magnetic nanoparticles (MNPs) have gained significant attention due to their intrinsic properties, which enable tracking through magnetic resonance imaging.[14,15] This class of nanoparticles includes in particular super-paramagnetic iron oxide nanoparticles (SPIONs) which present an inoffensive toxicity profile, and a surface which is easily modified by biocompatible polymers.[16–18] It occured that the use of porphyrins grafted on metallic nanoparticles to target tumour by EPR effect could appear as promising new therapeutic platform for an application in PDT against melanoma.[19–21] We report the design, synthesis and characterization of a new photodynamic agents **1a**,**b**-MNPs constituted of chlorin *p6* **1a**,**b** grafted on dextran-(MNPs) **3** to target tumour by EPR effect via polyethyleneimine (PEI) internalization agents. The dextran biopolymer is assumed to increase plasmatic life time and PEI is a coupling agent that increases drugs internalization into cancer cells.[22,23] Phototoxicities of this nanoplatform **1a,b**-MNPs were evaluated against B16F10 and B16G4F malignant melanoma cell lines .

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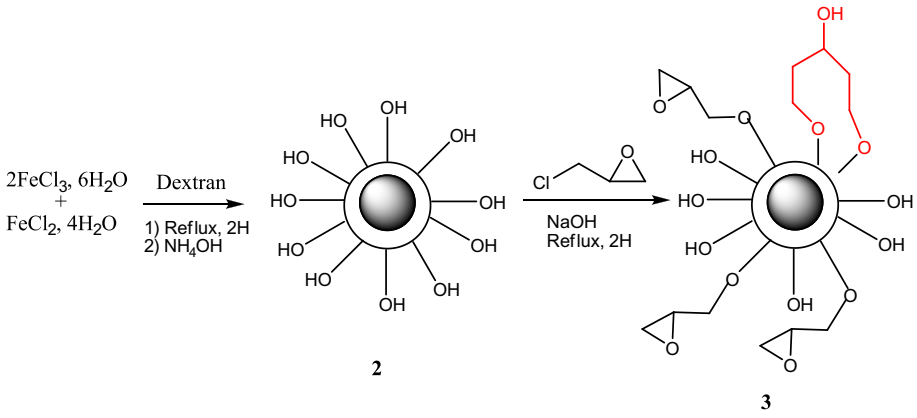

Scheme 1. Synthesis of native dextran T10-coated iron oxide nanoparticles **3**.

PEI-chlorin *p6* **1a,b**, were prepared from purpurin-18 (Pp-18) in two steps as previously described.[24] Light absorption spectra and mass spectrometry of these compounds showed the expected signals.[25] In parallel, magnetic nanoparticles were obtained by the process of Molday and Mackenzie.[26] Nanoparticles **2** were formed by addition of iron(III) chloride hexahydrate (1 g, 3.7 mmol) and iron(II) chloride tetrahydrate (0.5 g, 2.5 mmol) to a solution of dextran (10 000 Da) (2 g in 35 mL of water) (Scheme 1). After 2 h of reaction at reflux, the solution was neutralized (pH ~6–7) with ammonium hydroxide (9.34 mL, 66.6 mmol). Ultrafiltration through Sephadex LH20 column was performed in order to obtain uniform size nanoparticles; the average diameter of nanoparticles determined by TEM was around 22.4 nm (Fig. 1). However the weak interaction between iron particles and dextran, can sometimes cause desorption of the polymer. To avoid this drawback, we cross-linked macromolecules with epichlorohydrin (72 mmol/g of dextran) in the presence of 1 M NaOH (400 mL/g of dextran) creating bridged alkyl ethers, which resulted to mechanically trapping of the iron oxide particle inside the dextran crown (Scheme 1). Epoxidation of dextran and suspension in water resulted in a yellow fluid (Fig. 2). This colloidal suspension, was purified by dialysis to remove salts and unreacted reagents, and then freeze–dried. A feature of ferrofluids synthesized is their superparamagnetic behavior shown in the magnetization curve (Fig. 3) characterized by an absence of hysteresis loop at 300 K. The magnetization appears here as a symmetrical sigmoid well described by the Langevin formalism. These synthetized nanoparticles had an overall paramagnetic behavior at 300 K.

In order to covalently attach PEI-chlorin *p6* **1a,b** to the surface of magnetic nanoparticles **3**, epoxidized glucose units were reacted with the primary amine functions of PEI 600 (**1a-**MNP) or 2000 (**1b-**MNP) grafted on chlorin *p6* (Scheme 2). After synthesis, chlorin-bearing magnetic nanoparticles **1a**,**b-**MNPs were purified by dialysis against distilled water through a 6–8 kDa cut off membrane.

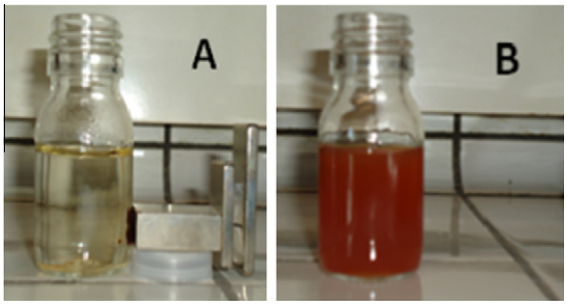

Figure 2. (A) MNPs suspension in the presence and (B) absence of a magnet.

The amount of polyaminated chlorin *p6* attached to magnetic nanoparticles was evaluated by UV–vis absorption at 665 nm (Fig. 4). Therefore, the grafting yield of PEI-chlorin *p6* on magnetic nanoparticles was estimated to about 43% for each one of the two photosensitizers.

In order to demonstrate the covalent binding of MNPs–PEI–chlorin *p6*, we performed an IR spectrum (Fig. 5). We have observed the bands of dextran (3350 and 1646 cm^{-1}), those of grafted chlorin *p6* (1500, 1250 and 810 cm^{-1}) and finally a band at 1629.6 cm^{-1} which corresponds to C–N amine functions of PEI.

The potential cytotoxicity and photocytotoxicity of these photosensitizers were assessed on two B16 murine melanoma variants (B16F10 and B16G4F, with or without melanin, respectively). Cells were seeded at 10^5 cells/mL in 96-well plates in complete culture medium. After 24 h, cells were washed twice with PBS and incubated with PS for 3 h in culture medium, in darkness and in a humidified 5% CO_2 atmosphere at 37 °C. The cells were then irradiated with an Aktilite Lamp™ at room temperature (25 °C). The exposure time was adjusted to obtain 37 J/cm^2 fluence (corre-

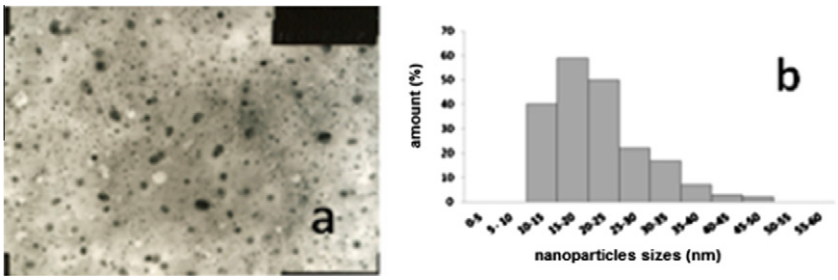

Figure 1. (a) TEM image of nanoparticles coated with dextran T10. (b) Histogram size of the nanoparticles coated iron oxide dextran T10. Size distribution was unimodal. The most probable diameter dp was around 22.4 nm. The histogram showed a size distribution as a Gaussian curve.