Conference paper

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Near-IR absorbing Bodipy functionalized SPIONs: a potential magnetic nanoplatform for diagnosis and therapy

Abstract: Photodynamic therapy (PDT), especially with the recent advances in photosensitizer (PS) design, has already been established as a noninvasive technique for cancer treatment. Recently, near-IR-based absorbing PSs that have a rising potency to implement light-triggered tumor ablation have attracted much attention since near-IR light in the 650–850 nm range penetrates more deeply in tissues. Up to now, numerous nanomaterials tailored to suitable sizes have been studied for effective delivery of PSs. In this study, four different types of Bodipy-based PSs were covalently attached to magnetic resonance imaging (MRI) active, biocompatible, and nontoxic nanocarriers and generation of singlet oxygen capabilities were evaluated. It was demonstrated that these core-shell nanoparticles are promising delivery vehicles of PSs for use in diagnosis and therapy.

Keywords: Bodipy; core-shell nanoparticles; IUPAC Congress-44; magnetic resonance imaging; photodynamic therapy; photosensitizer; superparamagnetic iron oxide nanoparticles.

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Introduction

Biocompatible nanosized structures with multiple functionalities are highly sought after due to their recognized potential as versatile therapeutic agents [1–3]. The overlap of imaging and therapeutics within a single type of agent is possible through a number of distinct avenues [4, 5]. For the last couple of years, our group [6–8] and others [9–11] have been actively involved in transforming Bodipy dyes into efficient photosensitizers with a potential in photodynamic therapy [12, 13]. The versatility of Bodipy chemistry [14, 15] allows straightforward access to a wide range of dyes with fast intersystem crossing rates [10], even without the incorporation of heavy atoms [16, 17]. The absorption band corresponding to S_0-S_1 transition can be tuned as well, anywhere from 450 nm to 850 nm [18, 19].

In this work, we targeted a series of long wavelength absorbing Bodipy derivatives which can be covalently attached to iron oxide core-silica shell nanoparticles. Iron oxide nanoparticles (maghemite or magnetite) are often referred to as superparamagnetic iron oxide nanoparticles (SPIONs) for their impressive

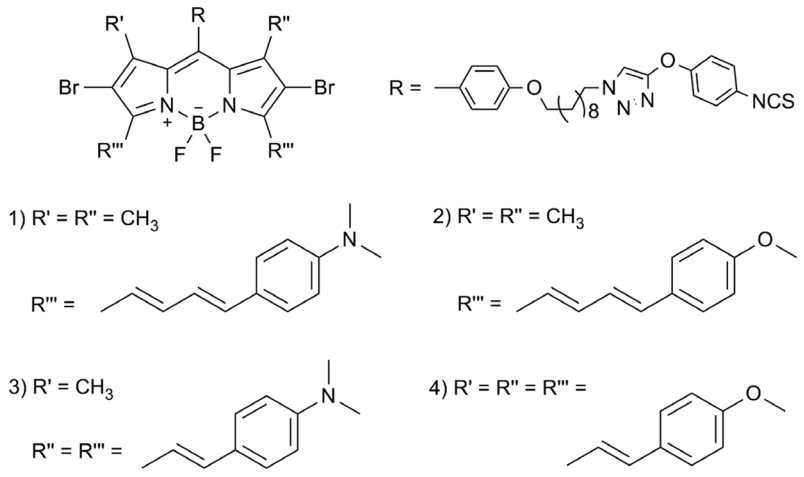

Fig. 1 Structures of Bodipy-based photosensitizers designed for conjugation to SPIONs.

magnetic properties [20–22], and there is ample literature precedence for the preparation of iron oxide core-silica shell (Fe_3O_4@SiO_2) nanoparticles [23–25]. The silica shell can be prepared with functional groups for further modification. MRI imaging of SPIONS have been reported [26–28], offering a non-invasive methodology for tracking the trafficking and localization of such particles together.

The structure of the reactive Bodipy dyes synthesized to confer sensitization potential is shown in Fig. 1. Detailed synthesis procedures can be found in the ESI. Long wavelength absorption is achieved by the extension of conjugation [16] or tetrastyryl substitution [18] of the core Bodipy structure. The *meso* (8) position carries an amine reactive isothiocyanate moiety for efficient functionalization of amino-terminated silica shells. The absorption band peaks vary in the region with any active of 700–770 nm. This wavelength is optimal for excitation through mammalian tissue as this range is safely within what is typically referred to as the therapeutic window [29].

Iron oxide core-silica shell (Fe_3O_4@SiO_2) nanoparticles were prepared by the reaction with tetraethylorthosilicate (TEOS) in the presence of catalytic amount of NH_4OH in aqueous citrate solution (Fig. 2) [30]. A silica layer covering a few smaller iron oxide nanoparticles was apparent on the TEM images (Fig. 3). The citrate stabilized nanoparticles were then functionalized by the reaction with 3-(aminopropyl)triethoxysilane (APTES) in ethanol solution at 80 °C. Amino functionalized nanoparticles were lyophilized before reactive

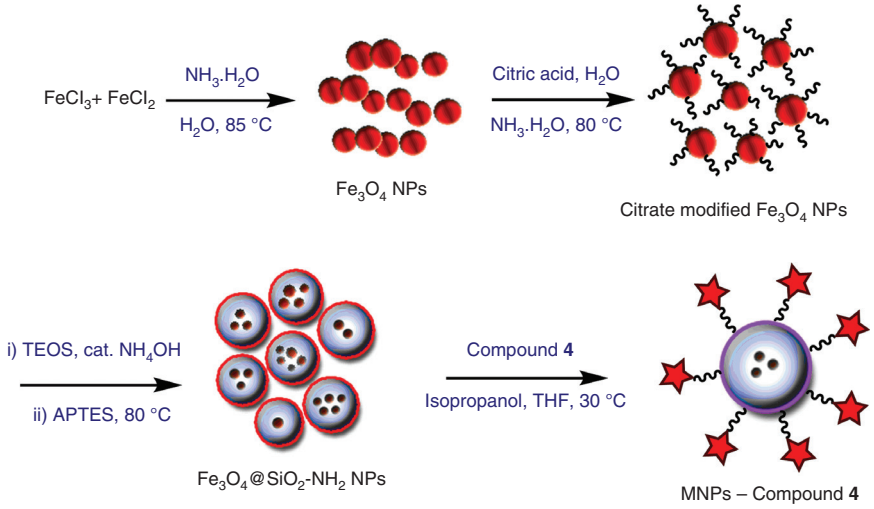

Fig. 2 Schematic representation of Fe_3O_4@SiO_2-NH_2 preparation and functionalization with corresponding Bodipy dyes.

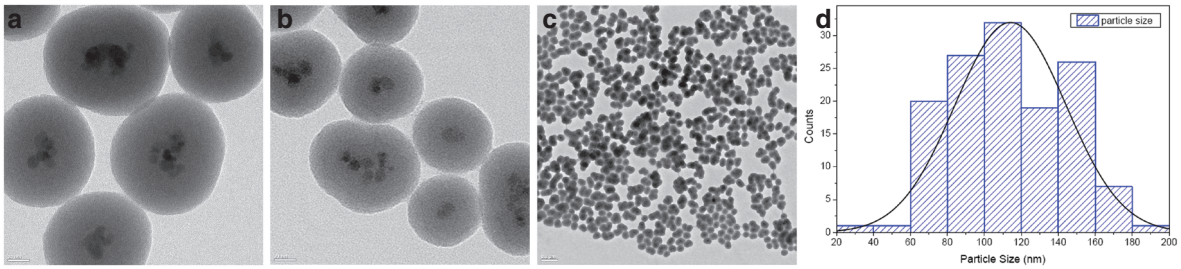

Fig. 3 TEM images of Fe_3O_4@SiO_2 NPs (a), Fe_3O_4@SiO_2-NH_2 NPs (b) and (c). Particle size distribution histogram of Fe_3O_4@SiO_2 NPs with an average diameter of 124.5±40.1 nm (d).

dye treatment. Amino functionalization was confirmed by zeta potential measurements before and after APTES reaction and XPS measurements (ESI).

As expected, on amino modification, the isoelectric point of the particles moves from 4.8 to 9.6. In the conjugation step, nanoparticles prepared in this manner (Fe_3O_4@SiO_2-NH_2) were treated in THF/isopropanol mixture with the isothiocyanate derivative of the long wavelength absorbing dyes (**1**–**4**). The functionalized nanoparticles were then separated by centrifugation, and then washed with $CHCl_3$ and lyophilized.

The extent of the reaction was estimated for each dye, by absorption spectra, with the assumption of unchanged extinction coefficients before and after conjugation. Bodipy functionalization was further evidenced by the energy-filtered transmission electron microscopy (EFTEM) studies (Fig. 4) where the presence of boron and bromine on the nanoparticles were unequivocally demonstrated.

The halogenated dyes were remarkably active (ESI). High singlet oxygen generation ability was also present in the dye-modified nanoparticles (Fig. 5 and ESI). Singlet oxygen generation capacity of the dyes before and after conjugation to the nanoparticles was studied using trap molecule 1,3-diphenylisobenzofuran (DPBF) in dichloromethane (DCM) (for the molecules **1**, **2**, **3** and **4**) and in isopropanol (for **NP** + **1**, **NP** + **2**, **NP** + **3**, and **NP** + **4**). The absorbance of DPBF was adjusted around 1.0 and photosentizers' absorbance was around 0.2-0.3 in air saturated dichloromethane or isopropanol. Following the control measurements in the dark, the cuvette was exposed to 725 nm emitting LED light source for different time intervals for each solution. (The light intensities were calculated to be 0.13 mW/cm^2 for compounds **1**, **2**, **3**, and **4**, and 0.6 mW/cm^2 for **NP** + **1**, **NP** + **2**, **NP** + **3** and **NP** + **4**.) Comparative singlet oxygen experiments with reference to methylene blue (MB) in DCM and isopropanol are provided in the ESI.

At very low concentrations of the nanoparticles and with relatively weak LED irradiation, we observed efficient transformation of the singlet oxygen trap, accompanied with a drop in the absorption peak at 411 nm. As control, nanoparticles without conjugated dyes were also studied, and they showed no activity. Singlet oxygen generation capacity is clearly due to covalently attached Bodipy dyes on the nanoparticles. We noted that compound **4** was particularly active in singlet oxygen generation (Fig. 5).

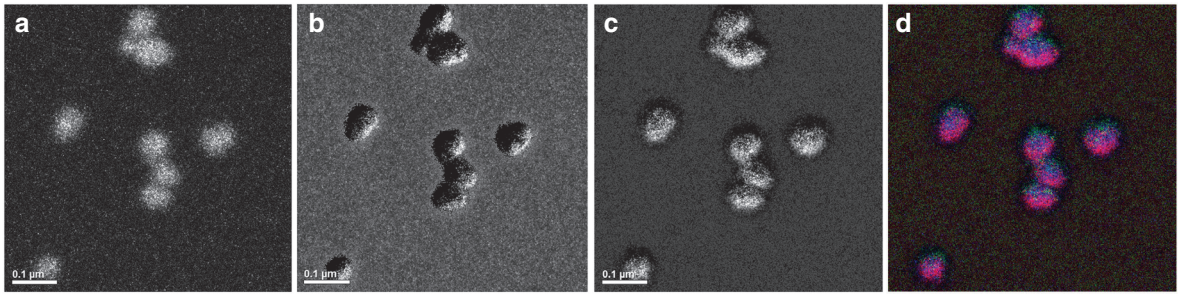

Fig. 4 Elemental maps obtained by EFTEM for Fe_3O_4@SiO_2-NH_2 particles reacted with compound **4** nanoparticles from EFTEM images: (a) boron map, (b) bromine map, (c) silicon map, (d) the RGB image created by superimposing the elemental EFTEM maps of B (green), Br (blue), and Si (pink).

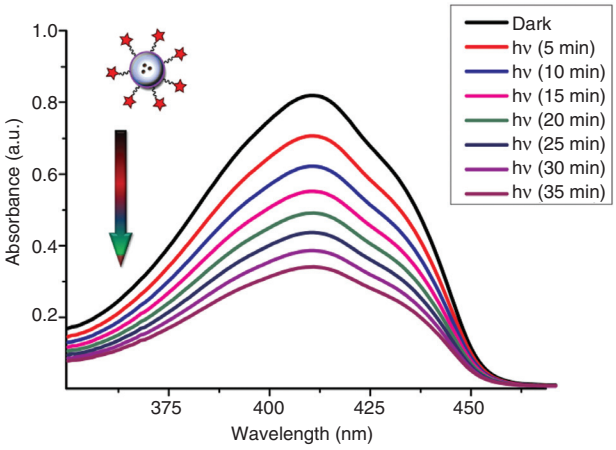

Fig. 5 Decrease in absorption of the trap molecule DBPF in the presence of 7.46 μM compound **4** attached to Fe_3O_4@SiO_2 in isopropyl alcohol at various time points on irradiation at 725 nm LED source.

We also investigated the magnetic properties of the functionalized nanoparticles, through vibrating sample magnetometer (VSM) analysis. For the most active nanoparticles with the dye **4** conjugation (Fe_3O_4@SiO_2-NH-dye **4)**, the hysteresis loop of nanoparticles was registered at room temperature and the high field of 30 kOe (ESI). The hysteresis loop demonstrated that there was no coercive force, thus demonstrating superparamagnetic behavior. The saturation magnetization of the nanoparticles was determined to be 2.93 emu/g (2.68 emu/g at a field of 10 kOe) which is comparable to literature values for citrate and silica coated superparamagnetic nanoparticles [30].

Conclusion

In this work, novel near-IR absorbing Bodipy dyes were successfully conjugated to SPIONS. Both magnetic and photosensitization properties were safely carried over to the nanoparticles. We are confident that promising photosensitizers for photodynamic therapy, together with MRI potential will make similarly prepared nanoparticles highly useful theranostic tools in the near future. Our work along that line is in progress.

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