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# Self-assembled fluorescent magnetic nanoprobes for multimode-biomedical imaging

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ABSTRACT

We fabricated multimode nanoprobes for acquisition of biological information at different object levels, i.e., *in vivo* detection and *ex vivo* validation for characterizing tumor angiogenesis. Fluorescent magnetic nanoprobes (FMNPs) were synthesized by using amphiphilic pyrenyl polyethylene glycol (Py-PEG) and superparamagnetic $MnFe_2O_4$ nanocrystals (MNCs). Py-PEG, which is synthesized by conjugation of hydrophilic PEG with hydrophobic and fluorescent 1-pyrenebutyric acid through an esterification process, is capable of self-assembly and maintaining a high UV fluorescent intensity in aqueous phase. Py-PEG can be used as a fluorescent surfactant that simultaneously and efficiently encapsulates MNCs to exhibit fluorescent and magnetic properties as well as maintaining high water-solubility. Consequently, we proved that our biologically non-toxic FMNPs were prominent multimode imaging probes by showing not only excellent MR sensitivity but also high illumination intensity with strong signal strength under short exposure time of UV light from the extensive imaging studies of *in vitro/vivo* and *ex vivo* using orthotopic and xenograft mice models.



## 1. Introduction

Molecular nanoprobes using well-tailored superparamagnetic nanocrystals are of great interest for detecting various biological objects via magnetic resonance (MR) imaging due to their high-sensitivity and specificity gifted from a nanoeffect [1–4]. Further, the superparamagnetic nanocrystals as an MR contrast agent are hybridized with organic/inorganic fluorescent materials to create a single nanoplatform of multimodal imaging nanoprobes, which are utilized to measure *in vivo* biological events via MR and optical imaging at the same level of biological objects. In particular, both fluorescent and magnetic properties from conjugated multimodal imaging nanoprobes have been majorly used to render accurate appreciation of clinically significant events of cells, tissues and organisms via dual monitoring. Furthermore, a challenge for acquisition of biological information at different object levels, such as *in vivo* detection and *ex vivo* validation by using multimodal imaging methods to characterize tumor angiogenesis has been emerged [5–15]. Ultraviolet (UV) imaging has been recently introduced to provide higher spatial resolution with detailed insight into cellular events compared to visible light due to their shorter wavelength illumination. Thus, it would be of great significance to integrate MR imaging (*in vivo*) with UV imaging (*ex vivo*) for more robust acquisition of biological event with higher spatial resolution. However for UV imaging, to acquire high spatial intra-cellular resolution while keeping the cells alive and undamaged as long as possible, higher illumination intensities and longer exposure times over which cells are observed before they exhibit signs of damage should be necessary to provide sufficient signal strength [16–18]. Herein, we presented a fluorescent magnetic nanoprobe (FMNP) as a multimodal imaging agent. The fabrication of FMNP began with synthesizing pyrenyl polyethylene glycol (Py-PEG) with methoxy poly(ethylene glycol) (PEG) and 1-pyrenebutyric acid (Py) exhibiting amphiphilic and UV fluorescent properties. Then a simultaneous self-assembly of hydrophobic magnetic nanocrystals and Py-PEG as a fluorescent surfactant formed FMNPs utilized for UV and MR imaging capability (Fig. 1).

## 2. Materials and methods

### 2.1. Materials

The following materials were purchased from SigmaeAldrich: iron (III) acetylacetonate, 1,2-hexadecanediol, dodecanoic acid, dodecylamine, benzyl

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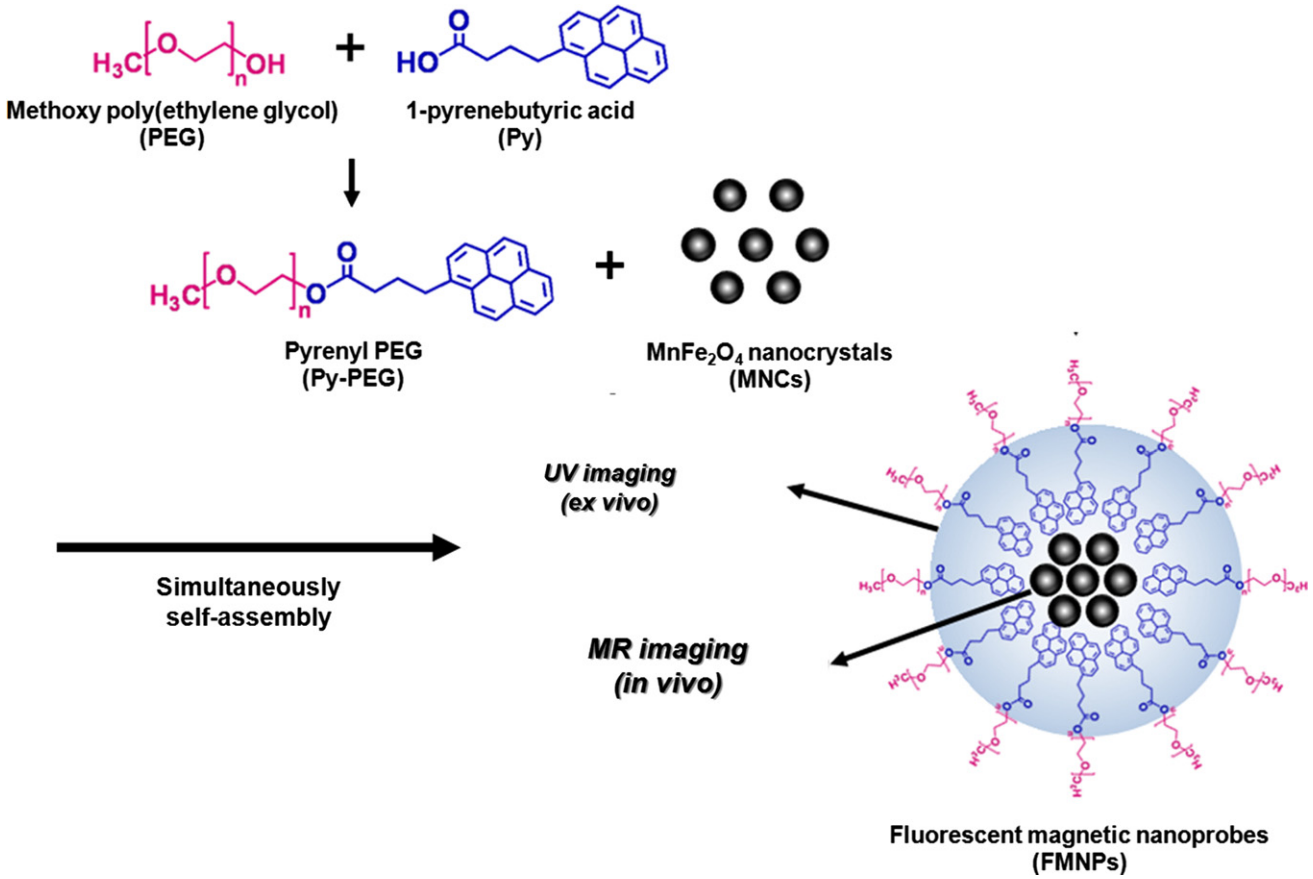


**Fig. 1.** Schematic illustration of simultaneously self-assembled fluorescent magnetic nanoprobes (FMNPs) as multimodal biomedical imaging probes.

ether, 1-pyrenebutyric acid, 1,3-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), anhydrous dichloromethane, and triethylamine (TEA). Monomethoxy polyethylene glycol (MW: 5000 Da) was obtained from Fluka Chemical Co. Phosphate buffered saline (PBS; 10 mM, pH 7.4) and DMEM were purchased from Gibco. All other chemicals and reagents were of analytical grade.

### 2.2. Synthesis of $MnFe_2O_4$ nanocrystals (MNCs)

Using a previously reported method, we synthesized monodispersed $MnFe_2O_4$ nanocrystals (MNCs) that are soluble in non-polar organic solvent [19–21]. Briefly, 2 mmol of iron (III) acetylacetonate, 1 mmol of manganese (II) acetylacetonate, 10 mmol of 1,2-hexadecanediol, 6 mmol of dodecanoic acid, and 6 mmol of dodecylamine were dissolved in 20 mL of benzyl ether under ambient nitrogen atmosphere. The mixture was then pre-heated to 200 °C for 2 h and refluxed at 300 °C for 30 min. After the reactants were cooled at room temperature, the products were purified with an excess of pure ethanol. Approximately 12 nm of MNCs were synthesized using the seed-mediated growth method.

### 2.3. Synthesis of pyrenyl polyethylene glycol (Py-PEG)

Pyrenyl polyethylene glycol (Py-PEG), an amphiphilic fluorescent surfactant, was formed by conjugating the hydroxyl group of monomethoxy polyethylene glycol (Mw: 5000 Da) with the carboxyl group of 1-pyrenebutyric acid (Mw: 288.34 Da) using DCC and DMAP (Fig. 1) [20,21]. First, 1 mmol of 1-pyrenebutyric acid and 1 mmol of monomethoxy polyethylene glycol were added into a flask containing 100 mL of anhydrous dichloromethane, 9 mmol of DCC, 9 mmol of DMAP, and 9 mmol of triethylamine (Sigma Aldrich Chemical). After reacting for 48 h at room temperature under a nitrogen atmosphere, the reactants were filtrated through a 200 nm pore size cellulose acetate filter (Advantec) to remove the by-product (dicyclohexyl urea). Subsequently, the solvent was rapidly removed using a rotary evaporator (50 Hz, EYELA) and the products were washed using dichloromethane and an excess of ethyl ether. The purified precipitates were lyophilized and stored under vacuum for later use.

### 2.4. Characterization of Py-PEG

The synthesis of Py-PEG was performed as follows (Fig. 1). After the conjugation process, the chemical structure of the synthesized Py-PEG was confirmed by FT-IR (Varian, ExcaliburTM series) and $^1$H NMR (400 MHz, Varian INOVA400 NMR spectrometer) spectra using $CDCl_3$ as a solvent. In addition, the change in molecular weight of Py-PEG compared with naked PEG was evaluated by gel permeation chromatography analysis. Fluorescence intensities of Py-PEG compared with pyrene and critical micelle concentration (CMC) of Py-PEG were determined by fluorescence spectrometer readings (SL55, Perkin Elmer) [22,23].

### 2.5. Preparation of fluorescent magnetic nanoprobes (FMNPs)

FMNPs were prepared by the nano-emulsion method utilizing the hydrophobic interaction of MNCs with the hydrophobic portion of Py-PEG [9,24,25]. First, 50 mg of MNCs were dissolved in 4 mL of hexane (as organic phase). This organic phase was added to 20 mL of deionized water (DW) as an aqueous phase containing 200 mg of Py-PEG. After mutual saturation of the organic and water phases, the emulsion was ultra-sonicated in an ice-cooled bath for 10 min at 450 W. The resulting suspension was stirred overnight at room temperature to evaporate organic solvent and was subsequently centrifuged for 30 min at 18,000 rpm in triplicate. After the supernatant was removed, the precipitated FMNPs were re-dispersed in 10 mL of DW. The size distribution and the zeta-potential of FMNPs were analyzed using laser scattering (ELS-Z, Otsuka electronics). The morphologies of FMNPs and MNCs were confirmed using high resolution transmission electron microscope (HR-TEM, JEM-2100F, JEOL Ltd.). The colloidal stability of the prepared FMNPs was determined based on the resistance ability against the addition of a wide range of salt (sodium chloride, NaCl) solution concentrations (up to 1 M) and pH conditions (pH 4–9) at room temperature. After 24 h, the stability of the FMNPs was evaluated using laser scattering. The residual weight (%) of the MNCs in the FMNPs was analyzed with a thermo-gravimetric analyzer (SDT-Q600, TA instrument). We also confirmed the crystallinity of MNCs in the FMNPs using X-ray diffraction (Rigaku, X-ray Diffractometer Ultima3) at 298 K [2,4]. The magnetic properties of MNCs and FMNPs were measured using vibration sample magnetometer (MODEL-7300, Lakeshore) at 298 K. The relaxivity (R2) and fluorescence properties of the FMNP solution were measured by magnetic resonance (MR) imaging analysis and fluorescence