

Multifunctional Stable Fluorescent Magnetic Nanoparticles

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Materials and Methods

Materials

Dextran with an average molecular weight of 5,000, sodium periodate, potassium cyanide, poly-L-histidine (molecular weight of 5,000-25,000), and poly(ethylene oxide) (6-arm, anthracene-terminated) with an average molecular weight of 12,000 were purchased from Sigma-Aldrich. Oleic acid (90%) and 1-octadecene were purchased from Aldrich. Oleyl alcohol was purchased from TCI. N-hexane was purchased from Samchun Chem.

Methods

The preparation of the carboxyl-dextran

The carboxylated-dextran was prepared according to the procedure reported elsewhere.¹ First, the hydroxyl groups in the dextran were oxidized to aldehyde groups by sodium periodate.² Then, sodium periodate was dissolved in deoxygenated DI water and introduced to the dextran solution (4 g in 30 mL of deoxygenated DI water). The obtained solution was homogenized for 2 hr at room temperature and then dialyzed for 4 days in a membrane bag with a 1,000 molecular weight cut-off. The solution was then reacted with potassium cyanide to prepare the cyanohydrin intermediate. Finally, the carboxylic acid group was created on the terminal units of dextran by hydrolysis of the obtained cyanohydrin intermediate. The prepared carboxylated dextran was lyophilized and stored at -80°C.

The preparation of the SPIONs

To obtain nanoparticles with a narrow size distribution, the polyol route was employed. The SPIONs were synthesized according to the previously reported procedure.³ Iron-oleate complexes were prepared by reacting sodium oleate and iron(III) chloride.³ For the synthesis of 13 nm SPIONs, 18 g (20 mmol) of iron-oleate complex and 5.7 g (20 mmol) of oleic acid were dissolved in 100 g of 1-octadecene at room temperature. The reaction mixture was

degassed at 80°C for 2 hr. The mixture was heated to a reflux temperature at a heating rate of 3°C/min and then kept for 30 min under an inert atmosphere. After the reaction, the reaction vessel was rapidly cooled to room temperature, and 500 mL of acetone was added to precipitate the SPIONs. The SPIONs were separated by centrifugation and dispersed in hexane.

The preparation of the carboxyl-dextran-coated SPIONs

To coat the prepared hydrophobic nanoparticles with carboxyl-dextran, the ligand exchange process was used.⁴ SPIONs with an iron concentration of 1 mg/mL were prepared and mixed with the dextran ligands in DMSO. It is notable that DMSO is a dipolar solvent, and the reactions of the nanoparticles and polymers were performed at room temperature for 72 h under shaking conditions. DMSO can form homogeneous solutions with both aqueous polymer solutions and organic solvents.⁴ A stock SPION solution (1 mL) was mixed with dextran in 30 mL of DMSO. After the completion of the reaction, the SPIONs were magnetically collected through a strong magnetic field using a magnetic-activated cell sorting (MACS[®]) system and were redispersed into 1 mL of DI water. These water-soluble SPIONs were completely stable at room temperature.

The preparation of gold-coated SPIONs with a fluorescent polymeric gap

The smooth gold-coated SPIONs were prepared as follows (see Figure 1 for details). The prepared carboxyl-dextran-coated SPIONs were mixed for 10 hours with poly(ethylene oxide) (6-arm, anthracene-terminated) in DI water in a shaking incubator. The obtained materials were collected with a strong magnet and washed several times with DI water. PLH was added to the solution of SPIONs, and the pH was adjusted to 5-6 by using 0.1 N HCl. After a 60 min incubation, the magnetic NPs were collected with a magnet and washed several times with DI water. The obtained solution was mixed with H₂AuCl₄ (1% w/w) for 20 min, and the pH was adjusted to 9-10 with NaOH. Afterward, NH₂ OH· HCl was added to

the solution, which was mixed well until the color of the colloidal suspension turned dark blue. The obtained solution was washed several times, redispersed in DI water with a sonicator, and kept between 2 and 8°C.

The characterization methods

The dynamic light scattering (DLS) measurements were performed with a Malvern PCS-4700 instrument equipped with a 256-channel correlator. The 488.0 nm line of a Coherent Innova-70 Ar ion laser was used as the incident beam. The laser power that was used was 250 mW. The scattering angles, θ , that were employed ranged from 40°-140°. The temperature was maintained at 25°C with an external circulator. The data analysis was performed according to standard procedures and interpreted through a cumulant expansion of the field autocorrelation function to the second order. To obtain a distribution of the decay rates, a constrained regularization method, CONTIN, was used to invert the experimental data.

The size and shape of the NPs were evaluated with a Phillips CM200 transmission electron microscope (TEM) equipped with an AMT 2 x 2 CCD camera at an accelerating voltage of 200 kV. To prepare the samples for the TEM, a drop of the suspension was placed on a copper grid and dried.

The magnetization measurements were performed on the solid (dry powder) samples with a Quantum Design Superconducting Quantum Interference Device (SQUID) MPMS-XL7 magnetometer. Hysteresis experiments in the range of $-5\text{T} \leq H \leq +5\text{T}$ were performed at $T=300\text{ K}$.

The *in vitro* MRI experiments were performed at 8.5 MHz with a 0.2 Tesla Artoscan Imager by Esaote S.p.A. We used a Spin Echo (SE) T_2 pulse sequence with $\text{TR/TE/NEX}=2000\text{ ms}/80\text{ ms}/1$, $\text{matrix}=256*192$, and $\text{FOV}=180*180$ as the imaging parameters.

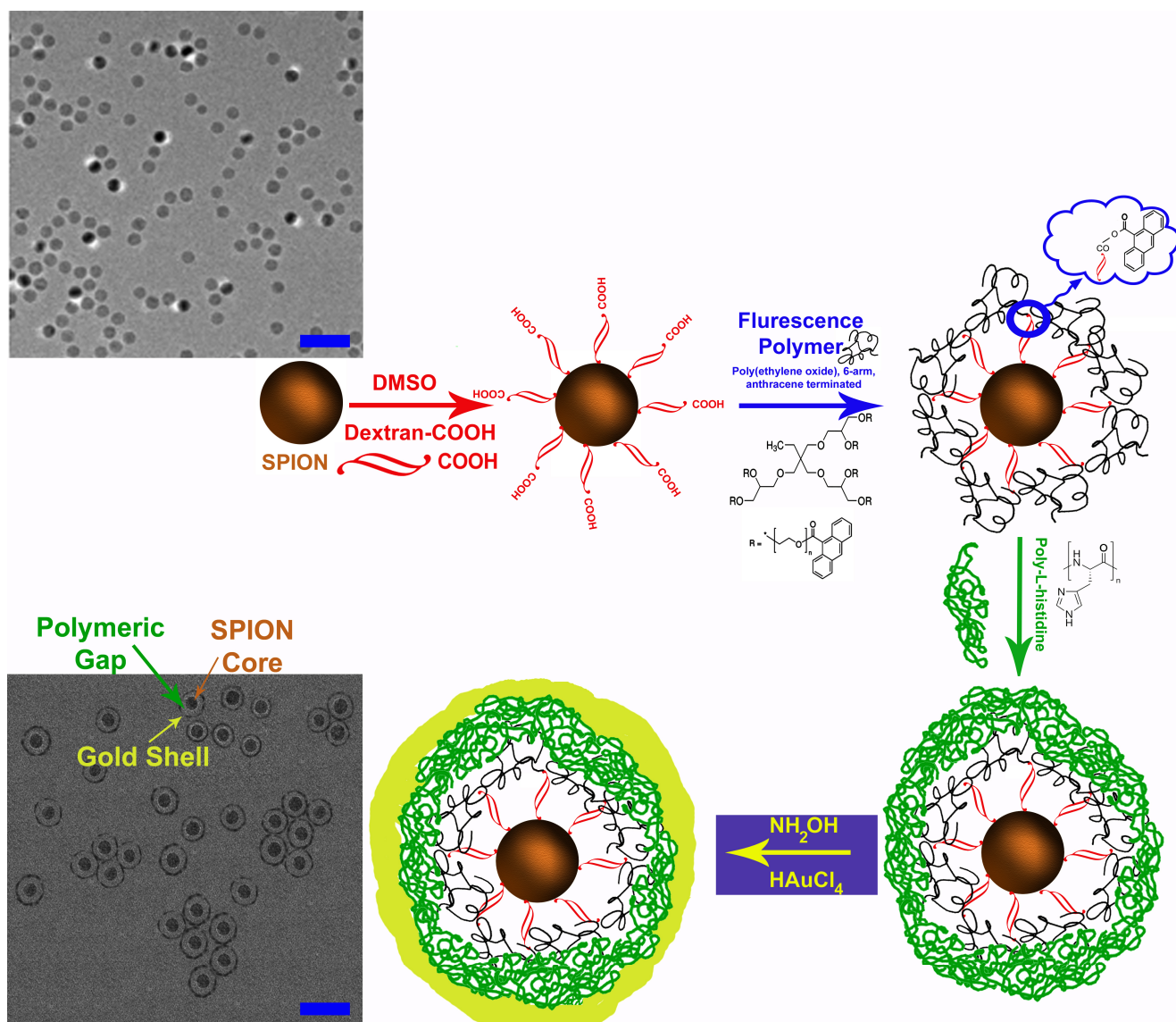


Figure 1 of paper for better vision.

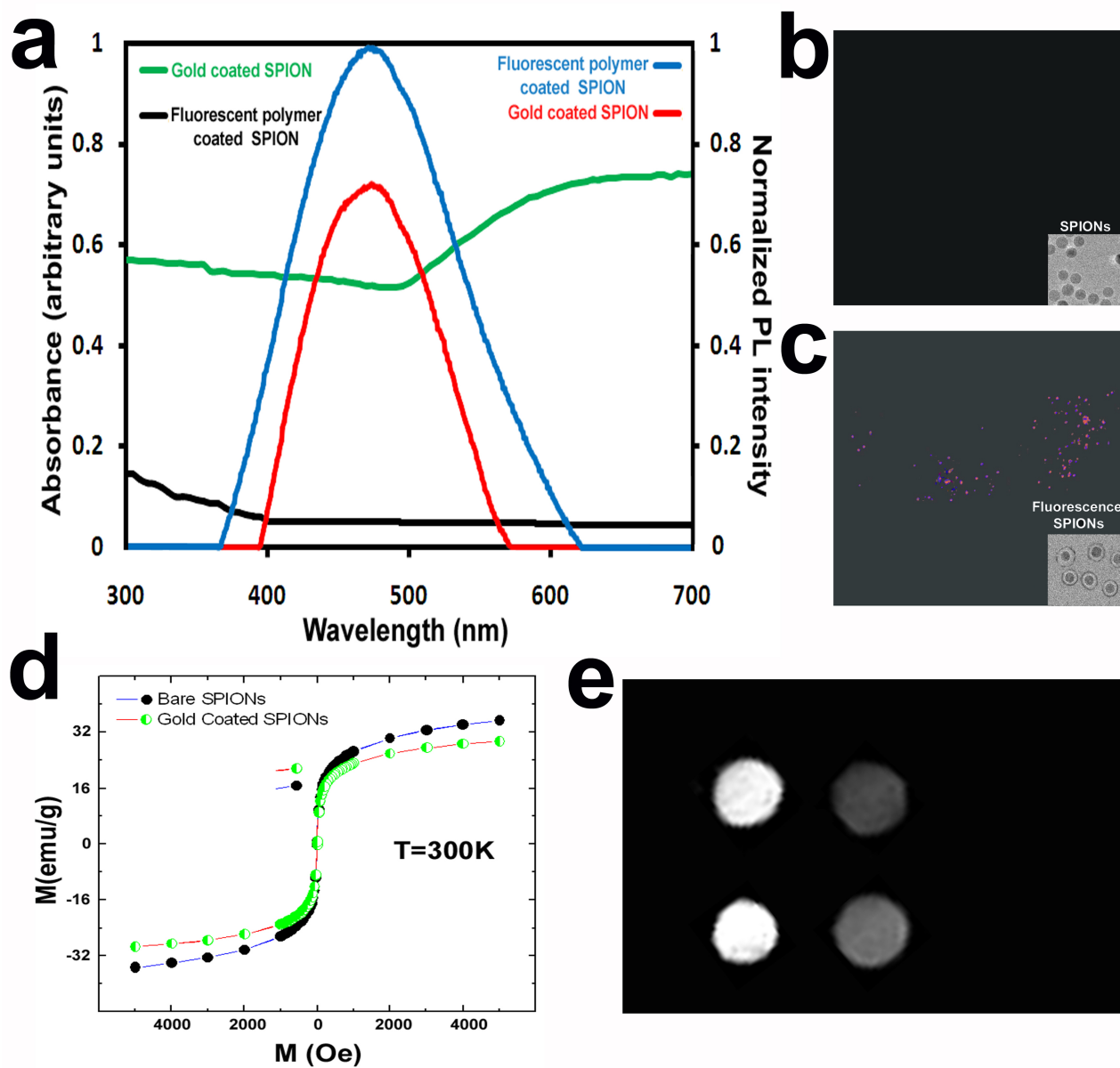


Figure 2 of paper for better vision.

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