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Polyethylenimine-coated Magnetic Nanoparticles with Improved Biocompatibility for Hyperthermia

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Abstract – Biocomfortable polyethylenimine (PEI) coated Fe_3O_4 nanoparticles (NPs) have been successfully fabricated for their magnetic hyperthermia performance. After coating with PEI, the sample reveals a tiptop performance of magnetic property and an enhanced biocompatibility. Compared with Fe_3O_4 NPs, PEI-coated Fe_3O_4 NPs do not induce notable cell cytotoxic effect at their higher concentration, which indicates that PEI-coated Fe_3O_4 NPs has improved biocompatibility. The results show that PEI-coated Fe_3O_4 NPs not only possess effective magnetic property for cancer hyperthermia treatment or gene delivery, but also demonstrates an improved biocompatibility for various biomedical applications.

Keywords - Fe_3O_4 Nanoparticles, Polyethylenimine (PEI) Hyperthermia, Biocompatibility, Magnetic field.

1. Introduction

Recently, magnetic nanoparticles (MNPs) have been applied in diverse fields mainly because of their low cost of production, ease surface modification, and appealing magnetic properties [1]. Besides, the chemical property of the MNPs is stable than the other NPs so that can be used as magnetic resonance imaging (MRI) agents, bio-probe, drug or gene delivery cargo or hyperthermia agents for the treatment of cancer. Although MNPs offer rapid growth and therapeutic benefits, at the same time, there are risks and concerns related with their exposure to cell [2-7]. Therefore, there is a considerable need to address biocompatibility and safety concerns associated with their usage in diverse biomedical applications.

The mechanism of toxicity induced from MNPs is mainly because of the generation of reactive oxygen species (ROS), which could indirectly damage proteins, DNA, lipids and result in cell death [8, 9]. Until now, significant improvements to the cell toxicity of MNPs have been made. Lots of surfactants or polymers, which are biocompatible, have been used for the surface modification of magnetic NPs to reduce its toxicity. For example, albumin-derived superparamagnetic NPs appeared more biocompatible property than the uncoated magnetic NPs [10]. Uncoated superparamagnetic NPs showed more toxicity compared to that of NPs that coated with biocompatible polyvinyl alcohol (PVA) [11]. Cyclodextrin, polyethylene glycol (PEG) and folic acid can be also used to modify magnetic NPs to enhance biocompatibility and cellular uptake of nanomaterials [12].

Although the magnetic NPs' biocompatibility and solubility was improved, they still had lower magnetic property for hyperthermia therapy. What was worse, the coated nanoparticles do not well dispersed, which may also influence the performance of the magnetic property. In summary, it is important to optimize magnetic NPs' property for appearing high biocompatibility and colloidal stability in aqueous solution and high heat generation efficiency by an alternating magnetic field. Thus, it is essential to design magnetic NPs to meet the needs of the rapidly growing field of magnetic application.

Recently, many researches have been reported to design biocompatible materials using polyethylenimine (PEI) [7, 12, 13]. PEI as a synthetic cationic polymer has a high efficiency for gene delivery due to its unique "proton sponge effect". It is normally used to modify nanoparticles positively for gene delivery. To date, there are a few cases about the PEI modified magnetic NPs to be used as magnetic hyperthermia agents for cancer treatment or drug delivery cargo for gene therapy. In this study, we employ highly mono-dispersed Fe_3O_4 NPs and modify the surface of Fe_3O_4 NPs using PEI coating. Fe_3O_4 NPs tend to aggregate because of Vander Waals interactions and generate reactive oxygen species (ROS) which indirectly damage the cells. However, Fe_3O_4 NPs coating with PEI not only prevents aggregation via electrostatic repulsion, but also prevent the Fe_3O_4 NPs directly exposure to the cell so that improvement of the NPs' biocompatibility[6]. Finally, their magnetic performance and cell toxicity are characterized and evaluated.

2. Materials and Methods

2.1 Materials

Iron (III) chloride (reagent grade, 97%), iron (II) chloride (98%), polyethylenimine, branched (PEI) were purchased from Sigma-Aldrich. Sodium hydroxide (NaOH) was purchased from Junsei Chemical Co., Ltd, and hydrochloric acid (HCl) was purchased from DUKSAN. Additionally, docetaxel (DTX) was supplied by Jinhe Bio-Technology (Shanghai, China).

2.2 Preparation of highly monodispersed Fe_3O_4 NPs and Fe_3O_4 NPs / PEI

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Magnetic nanoparticles were prepared through the standard co-precipitation technique in aqueous medium according to previously reported procedure [12]. Briefly, 10 mM iron (III) chloride and 5 mM iron (II) chloride were added to 12ml hydrochloric acid (1M) solution. In addition, the mixture solution was dissolved in 50 ml 1M sodium solution in a four-neck round-bottom flask equipped with a mechanical stirrer. The reaction is performed under a non-oxidizing oxygen-free environment, by bubbling N_2 in the reaction, which helps to reduce the final size of the nanoparticles, heated to 80 ºC, and then kept at that temperature for 2 hours. Then, PEI (10g) was directly poured into the Fe_3O_4 solution and heated to 90 ºC and kept at that temperature for 1 hour. The precipitate, consisting of anionic magnetite particles (Fe_3O_4), is washed 5 times by stirring for 3 min in distilled water and dispersed in aqueous solution.

2.3 Characterization

First, the size and morphology of samples were characterized by using a JEOL 100CX high-resolution transmission electron microscope (HRTEM; JEOL Ltd., Akishima-shi, Japan). The mean particle size was obtained from HRTEM images by counting more than 100 particles. Second, the dynamic light scattering (DLS) measurements were performed in a Malvern Zetasizer Nano-ZS device (Malvern, WR, UK) to determine the hydrodynamic size of Fe_3O_4 NPs before and after coating PEI in a colloidal suspension. Third, the zeta-potential of the suspensions was measured at 25°C. UV-vis absorption spectra were taken using a Shimadzu UV-1601 UV-visible spectrophotometer (Shimadzu, Kyoto, Japan). Finally, the magnetization properties of the samples were characterized by a LakeShore Model 7407 vibrating sample magnetometer (VSM; Lake Shore Cryotonics Inc., Westerville, OH, USA).

2.4 Magnetic performance

Fe_3O_4 NPs before and after coating with PEI were dispersed in water and thermally insulated plastic bottles containing 2 mL samples were placed within a water cooled copper coil driven by an Inductelec A.C. generator (SPG-10AB-II; Shenzhen Magtech Company Limited, Shenzen, China). The applied frequency was 312 kHz, and the currency is 290.4 A. The FLIR E60 Compact infrared thermal imaging camera was used to measure the sample's temperature. The specific absorption rate (SAR) of the samples was calculated from the following equation [14]:

$$SAR = C\frac{\Delta T}{\Delta t}\frac{1}{m_{Fe}}$$

where C is the specific heat of the medium (C_{water} = $4.18 Jg^{-1}°C^{-1}$), $\Delta T/\Delta t$ is the maximum slope of the time dependent temperature curve, and m_{Fe} is the weight fraction of the magnetic element in the sample.

2.5 Cytotoxicity assay

The NIH3T3 mouse embryo fibroblast cells were seeded in Dulbecco's Modified Eagle Medium (DMEM, Gibco-BRL/ Invitrogen, Carlsbad, CA) added with 10% (v/v) of FBS and 1% (v/v) of antibiotic solution (Gibco-BRL/Invitrogen) in a 96-well plate. Each well contained ten thousand viable cells in 100μl DMEM. The culture wells were incubated overnight in a humidified CO_2 incubator (37 ºC, 5 % CO_2) to allow attachment of the cells. After 24h, the media in each wells were added with various Fe concentrations (50 to 300 μg/mL) for co-incubation for 24 h. Next, the cells were prepared with thiazolyl blue tetrazolium bromide (MTT, Sigma-Aldrich Chemical) in DMEM (0.5 mg/ml) and further incubated 4h. Finally, the viability of the cells was obtained using a micro plate reader (Thermo Scientific, Waltham, CA) at a wavelength 570 nm.

3. Results and Discussion

3.1 Characterizations of the Fe_3O_4 NPs and Fe_3O_4 NPs with PEI coating

Highly, monodispersed superparamagnetic Fe_3O_4 NPs were synthesized by co-precipitation technique. TEM image (Fig. 1(a)) showed the synthesized Fe_3O_4 NPs and Fig. 1(b) revealed the size distribution of Fe_3O_4 NPs. In addition, TEM image (Fig. 1(c)) showed the Fe_3O_4 NPs after coating with polyethylenimine (PEI), where the dispersion of Fe_3O_4 NPs in the water does not changed by the PEI coating. In addition, the surface charge properties (Fig. 1(d)) of Fe_3O_4 NPs before and after coating with PEI were analyzed by the zeta potential measurement. The surface charge (zeta potential) of the corresponding Fe_3O_4 NPs was measured at neutral pH values (pH =7). Before coating PEI, Fe_3O_4 NPs shows +43.49 mV and after coating PEI, it becomes 54.50 mV, which results from the attachment of positive PEI polymer on the surface. These observations clearly indicated the presence of PEI on the

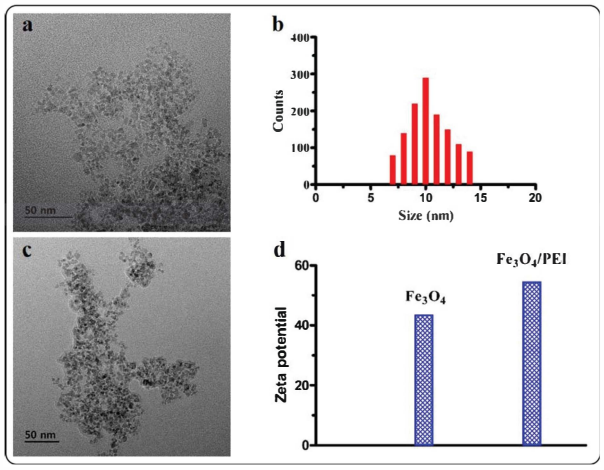


Fig 1. HRTEM, DLS, and Zeta-potential. (a) HRTEM image of Fe_3O_4 NPs before coating with PEI, (b) Size distribution histogram of the Fe_3O_4 NPs, (c) HRTEM image of Fe_3O_4 NPs after coating with PEI, and (d) The zeta-potential of Fe_3O_4 NPs before and after coating PEI.

surface of Fe_3O_4 NPs.

The PEI-coated Fe_3O_4 NPs were further characterized by UV-vis absorbance to verify the formation of the PEI coating. Fig. 2 showed the UV-vis absorption spectra of PEI and PEI coated Fe_3O_4 NPs, respectively. The absorption band is at 285 nm, and the peak is present in PEI coated Fe_3O_4 NPs which implies that PEI is capped on the surface of Fe_3O_4 NPs.

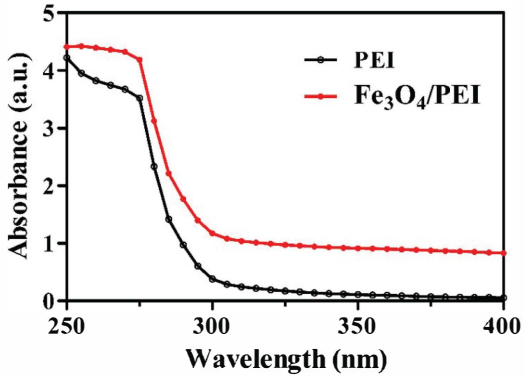


Fig. 2. UV-vis spectra of PEI and Fe_3O_4 NPs.

3.2 Magnetic performance

For the real application, PEI-coated Fe_3O_4 NPs can be used as a high performance magnetic hypertheria agent or gene deliver therapy. Therefore, it is significant that PEI-coated Fe_3O_4 should maintain their magnetic properties at room temperature. The magnetic properties of PEI-coated Fe_3O_4 NPs were charecterized by using a VSM. The Fe_3O_4 NPs exhibited magnetic properties at room temperature before and after coating with PEI. As shown in Fig. 3, the saturation magnetizations (M_s) of Fe_3O_4 NPs before and after coating with PEI are about 63 and 45 emu/g, respectively. The reduced saturation magnetization after coating with PEI is mainly attributed to the decreased effective weight fraction of the magnetic core. However, the result demonstrated that PEI coating does not significantly change the magnetic properties of Fe_3O_4 NPs.

In order to evaluate the efficacy of PEI-coated Fe_3O_4 NPs as hyperthermia mediators, the magnetic heating was characterized using an induction heating system. As shown in Fig. 4, 2ml plastic tube containing samples were placed in the center of the induction heating coil. The samples before and after coating with PEI revealed slightly different temperture rise profiles. Initially, the temperature rise profile of PEI-coated Fe_3O_4 NPs was coincided with Fe_3O_4 NPs before coating. After 20s, however, the rate of temperature rise of PEI-coated Fe_3O_4 NPs was decreased. Therefore, we expected that the decrased temperature rise profile results from the reduced M_s of PEI-coated Fe_3O_4 NPs in VSM characterization.

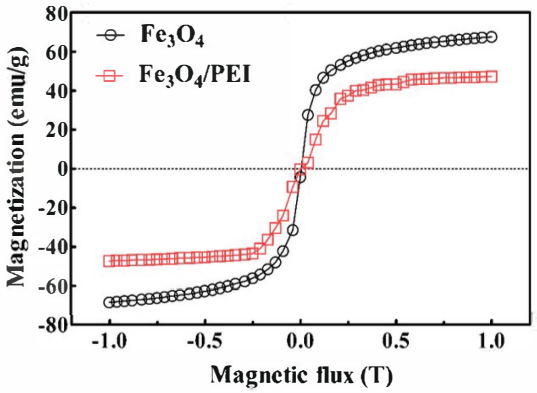
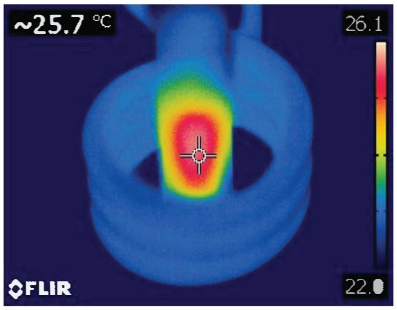


Fig. 3.VSM characterization. The hysteresis loop of Fe_3O_4 NPs before (black circle) and after (red cubic) at room temperature.

3.3 Cell viability

It is important to confirm the biocompatibility of PEI-coated Fe_3O_4 NPs for biomedical applications. The cell viabilities were determined after a 24-h incubation with NIH3T3 cells. As shown in Fig. 5, the cytotoxicity was increased with the increasing Fe_3O_4 NPs concentration. At 50 to 100 μg/ml of Fe_3O_4 NPs and PEI-coated Fe_3O_4 NPs, there is no obvious difference in the viability of the NIH3T3 cells. And, PEI-coated Fe_3O_4 NPs did not show notable cytotoxic effect between 100 to 300 μg/ml. However, at a concentration of 300 μg/ml, Fe_3O_4 NPs induced a cytotoxic effect of NIH3T3 cells.



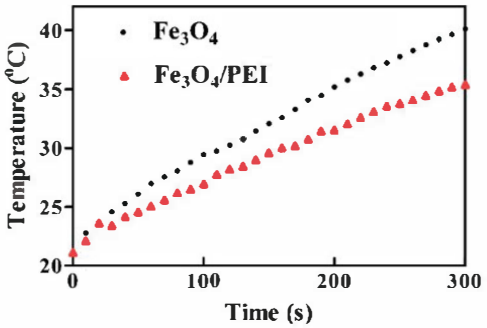


Fig. 4. Magnetic hyperthermia. Time-dependent heating 1 ml of Fe_3O_4 NPs dispersion before and after coating PEI on exposure to 290.4 current and alternating current field at 312 kHz.

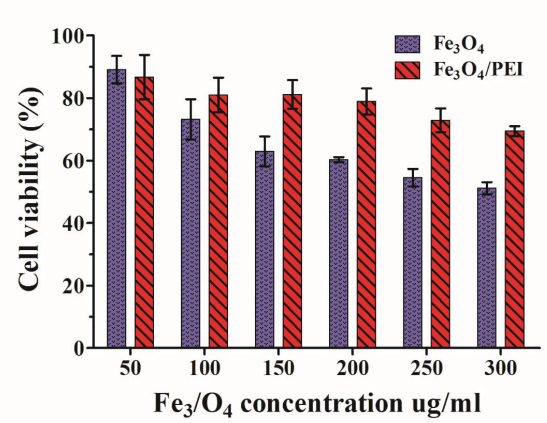


Fig. 5. Biocompatibility test of Fe_3O_4 NPs and PEI-coated Fe_3O_4 NPs

Because PEI coating has excellent biocompatibility, PEI-coated Fe_3O_4 NPs exhibited better biocompatibility than uncoated Fe_3O_4 NPs at high concentration. These results imply that PEI-coated Fe_3O_4 NPs become a prominent candidate for biomedical applications.

4. Conclusion

Fe_3O_4 NPs with PEI coating were fabricated for biocompatibility of the Fe_3O_4 NPs. The PEI coated Fe_3O_4 NPs were analyzed through the magnetic performance and cell toxicity. From the characterizations, we found that the PEI coated Fe_3O_4 NPs not only had an excellent magnetic property but also the biocompatibility was improved after coating with PEI. Therefore, the surface functionalization of Fe_3O_4 NPs with biocompatible PEI generated the improved stability, heating efficacy, and reduced cell toxicity towards normal cells. Finally, we expected that the PEI coated Fe_3O_4 NPs become a potential NPs for magnetic hyperthermia in cancer treatments.

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