A study of kidney parameters in mice induces by super magnetic Fe_3O_4 nanoparticles capped with PEG

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Abstract
The magnetite nanoparticles Fe_3O_4 capped with polyethylene glycol PEG were prepared by hydrothermal method. Due to the successful coating of PEG molecules on the surface of Fe_3O_4, these nanoparticles exhibited excellent dispersibility and dissolvability in physiological condition. The obtained nanoparticles were characterized by transmission electron microscopy (TEM), Fourier transform infrared (FTIR) spectroscopy and vibrating sample magnetometer (VSM). The Fe_3O_4 nanoparticles size is 17 nm while PEG–Fe_3O_4 size is 4nm and exhibited superparamagnetism and high saturation magnetization at room temperature.

However, the available data on their in vivo toxicity are limited. The present study was aimed to evaluate the toxicity of Fe_3O_4, PEG–Fe_3O_4 using both morphological and functional criteria. The biochemical parameters were assessed in-vivo using animal model. MNPs were injected intraperitoneal for 7, 14, 21 and 28 day. Furthermore, the histological structure of kidney samples was examined. Our results show no significant changes in biochemical parameters. The histological remained unchanged through the entire observation period time. The body weight was non-significant change through long period after administration of MNPs.

Keywords: PEG-Fe_3O_4, Hydrothermal synthesis, MNPs side effect, Superparamagnetic.

Introduction
The nanoparticles have more of specific physical and chemical characters in relation to their shape, size and high proportion in the surface to volume. Where these characters are present, make them appropriate for the use in multiple biology and medical applications like contrast media to drug delivery[17], tissue repair, cellular therapy[19], immunoassays[1], hyperthermia[4] and magnetic resonance imaging[20].The nanoparticles consist of elements like nickel, iron and cobalt exhibiting magnetic properties and are called magnetic nanoparticles. The developments and works on novel process in fields of tumors hyperthermia are be more studied. For the development, the nanoparticles composite use such as core-shell superparamagnetic particles have combined advantage from hetero-materials. Polyethylene glycol (PEG) coating nanoparticles and Fe_3O_4 nanoparticles all of particulars interest for having not only magnetism in Fe_3O_4.

Fe_3O_4 and Fe_2O_3 magnetic nanoparticle are used as satisfactory thermal therapeutic on cells line and animals model of kidney cancer and liver cancer in magnetic fluid hyperthermia (MFH), not with histological toxicity in the tissues or organs[5,21]. In these present studies, high crystalline and monodispersed core _shell multifunction PEG-Fe_3O_4 composite magnetic nanoparticles were synthesized and used in magnetic fluid hyperthermia as combined with near-inferred hyperthermia. Formation of the core-shell structure is achieved in two consecutive steps, where the Fe_3O_4 core is coated by the PEG shell. Good engineering PEG-Fe_3O_4 composite magnetic nanoparticles tend to aggregate in the tumors due to the unorganized nature of malignancy vasculature.

The nature in the composite PEG-Fe_3O_4 (MNPs) can show to have biocompatibility. The biocompatibility and the toxicity in both (*in vivo* and *in vitro*) are needed to evaluate. Thus, hemolysis test, the cytotoxicity assay, micronucleus assay and detection of very toxicity in mice are introduced to evaluate the biocompatibility in self-assemble PEG-Fe_3O_4 composite magnetic nanoparticles in these researches.[6,22]

Material and Methods
Chemicals and Materials: Anhydrous sodium acetate (NaOAc), ethylene glycol (EG), polyethylene glycol (PEG-4000), ethanolamine (ETA), ethanol and ferric chloride hexahydrate ($FeCl_3·6H_2O$) were supplied by Beijing Chemicals (China) while Calcein AM was procured from Sigma–Aldrich (Shanghai, China).

Preparation of Fe_3O_4-PEG MNPs: Fe_3O_4 – PEG MNPs were synthesized by hydrothermal method. 3gm of $FeCl_3·6H_2O$ was dissolved in 40ml of solvent containing EG 20ml and ETA 20ml to form a stable orange solution. NaOAc 4.0g and PEG-4000 2.0g were added into the above solution under magnetic stirring. The homogeneous solution was transferred to a Teflon-lined stainless-steel autoclave 100ml and sealed to heat at 200°C. After 10 hrs., the autoclave was cooled to ambient temperature naturally. The magnetic nanoparticles were washed with ethanol and DW in sequence and then dried in vacuum at 60°C overnight.

Characterisation of PEG-Fe_3O_4 MNPs: The prepared magnetite nanoparticles (MNPs) were characterized via optical and structural techniques. The TEM samples were prepared by placing a drop of the MNP solution on a gold-coated Cu grid containing about 200 meshes. Transmission electron microscope (TEM; Philips) was used to examine the morphological features of the MNPs. The molecular vibrations of the samples were studied using 8000 Series Shimadzu FTIR spectroscopy system. The magnetic properties of Fe_3O_4 NPs were determined using a BVH-55 vibrating sample magnetometer (VSM).

Toxicity of PEG-Fe_3O_4 MNPs

Animal body weight Examination: The mice were of age between 6-8 weeks and have weight from 25-33 gram. The mice were divided in to three groups: First group: the mice injected with the physiological phosphate solution (PBS) as a negative control. Second group: injected with 1 mg/kg with bare Fe_3O_4. Third group: injected with 1 mg/kg with Fe_3O_4 -PEG MNPs, all animals group were left for 1, 2, 3 and 4 weeks. After end of experiment time, the body weight of animal lab was measured.[15]

Assessment of Biochemical Serum Markers: Blood was drawn from the heart through the heart puncture to obtain the largest quantity of blood. Whole blood was collected using universal tubes and then centrifuged at 3500rpm for 10min in order to separate serum. Using a biochemical analyzer (Chemistry Analyzer, Automated, Abbott Laboratories and Architect c4000), serum biochemical analysis was carried out to measure blood urea and creatinine in order to evaluation kidney function.

Histopathological Examination: The kidney samples were fixed in buffered (10%) par formaldehyde, embedded in paraffin, cut into (5µm) sections and stained with hematoxylin and eosin (H and E) for histological examination using standard techniques. After H and E staining, the slides were observed and photos were taken using an optical microscope at 100×.

Statistical analysis: The obtained data were statically analyzed by unpaired t.test with GraphPad Prism 6. The values were shown as the Mean ± SEM of triplicate of each experiment.[14]

Results and Discussion

Morphological properties of PEG-Fe_3O_4 MNPs

Transmission Electron Microscopy (TEM): The particles size and morphology of magnetite nanoparticles samples were investigated using TEM as shown in fig. 1. The particles are approximate spherical shape and the average diameter for Fe_3O_4 was 17 nm while PEG–Fe_3O_4 size was 4nm. Our results show that the PEG-Fe_3O_4 MNPs are less agglomerated from Fe_3O_4. The less agglomerated texture of the PEG-Fe_3O_4 can be related to the effect of polymer layer during the particle formation and using more concentration of ($FeCl_3 \cdot 6H_2O$). Then, PEG coating reduced in size and aggregation and the particles dispersion got better.

Chemical properties of PEG-Fe_3O_4 MNPs: The FTIR spectra of the Fe_3O_4-PEG MNPs are shown in fig. 2. Our results showed the broad peak near 3431.48 or 3450.77 cm^{-1} in all FTIR spectra belong to attached hydroxyl groups.[22] The absorption bands around 1620.26cm^{-1} originate from stretching and deformation vibration hydroxyl groups connected to the surface of nanoparticles. Also, the C-O-C ether stretch and vibration bands exist at 976.01 and 1043.52 or 1039.67cm^{-1} respectively. Also, the bands around 2922.25cm^{-1} correspond to -CH stretching vibration and its out-of-plane bending vibration respectively.

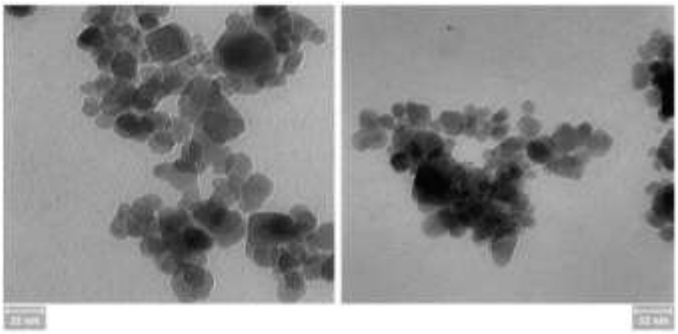

Fig. 1: TEM images of Fe_3O_4 and Fe_3O_4-PEG magnetic NP

The –CH-groups bending are also observed at 1462 or 1464.02cm^{-1}. All these bands confirmed the existence of PEG in the product. The results exhibit metal-oxygen band at 580.59 or 584.45cm^{-1} corresponding to intrinsic stretching vibrations of the metal at tetrahedral site (Fetetra–O) whereas metal-oxygen band observed at 426.28cm^{-1}, is assigned to octahedral-metal stretching (Feocta–O).

Vibrating sample magnetometer (VSM): The magnetic properties for the Fe_3O_4-PEG MNPs were characterized by vibrating sample magnetometer (VSM). Figure 3 shows the typical at room temperature magnetization curves of PEG coated Fe_3O_4MNPs. Reduced magnetization in magnetic nanoparticles is often observed due to the increase of surface to volume ratio. With reduction of particle size and agglomeration, spin relaxation of domain increases and consequently, magnetization decreases. The absence of hysteresis and remanance implies that our nanoparticles have superparamagnetic features.

The saturation magnetization (Ms) values of samples Fe_3O_4-PEG are 42.5 and 51emu/g respectively. The significant softening in magnetic properties occurred by PEG-coating onto the Fe_3O_4 MNPs. The coercively (Hc) and the saturation magnetization (Ms) of the MNPs increased with increasing particle size. The soft magnetic material is especial important in the biomedical fields, these PEG-coated soft magnetic Fe_3O_4 MNPs may find good applications in the biological studies.

Toxicity of PEG-Fe_3O_4 MNPs: The body weight of mice and the total white blood cell (WBCs) count was unaffected after intraperitoneal exposure to Fe_3O_4, PEG-Fe_3O_4 MNPs for 4 weeks (fig. 4). Our results show no significant differences in the urea concentration as well as creatinine in serum of injected for period of 1 week to 4 weeks (fig. 5).

However, histopathological examination of mouse tissue from control and treated groups has been done. The tissues of control and treated groups showed no significant pathological changes in the kidney (fig. 6). Our results show no changes in the kidney nodules. The kidney tissue also appeared as normal when compared with control untreated animals. No atrophy of the glomerular and renal tubular epithelial cells has been found in the kidneys.

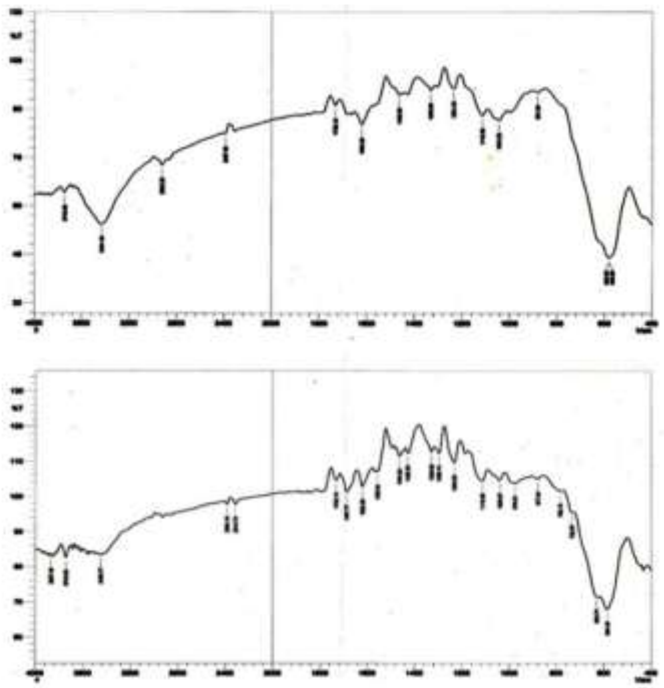

Fig. 2: FTIR spectra of Fe_3O_4 and Fe_3O_4-PEG magnetic NPs

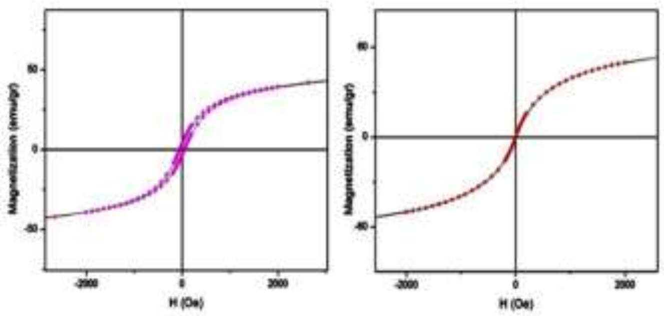

Fig. 3: VSM properties of Fe_3O_4, Fe_3O_4-PEG magnetic NPs

The exposure to Fe_3O_4, PEG-Fe_3O_4 MNPs did not induce any histopathological changes in treated lab animals. The effects of injection of Fe_3O_4 -PEG MNPs on blood urea and creatinine concentration are shown in fig. 6, the results showed no significant differences between the injected group and the control group during the 4 weeks. In conclusion, the PEG coated magnetite are biocompatible magnetic nanoparticles. The intravenous administration of PEG-Fe_3O_4 MNPs is done at a dose of 1 mg/kg, where any changes in serum biochemical markers or Histological on long period are not observed in 28 days after infusion. Body weights were not different after PEG-Fe_3O_4-treated animals. All these results are taken into consideration during the design of novel drug delivery systems.

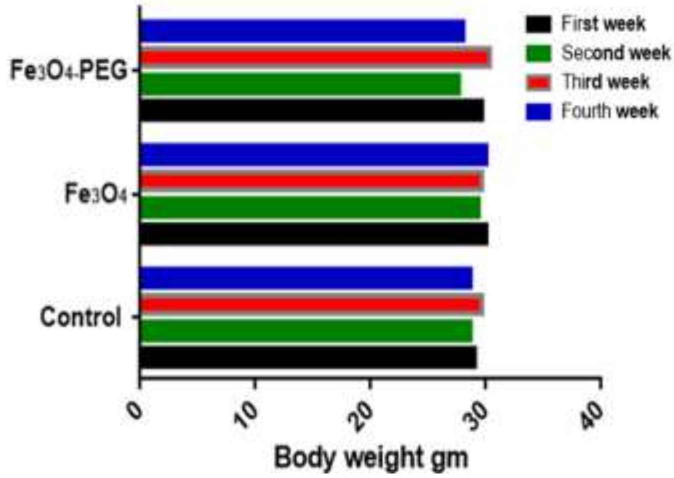

Fig. 4: Animals body weight after intraperitoneal injection of Fe_3O_4, Fe_3O_4-PEG MNPs.

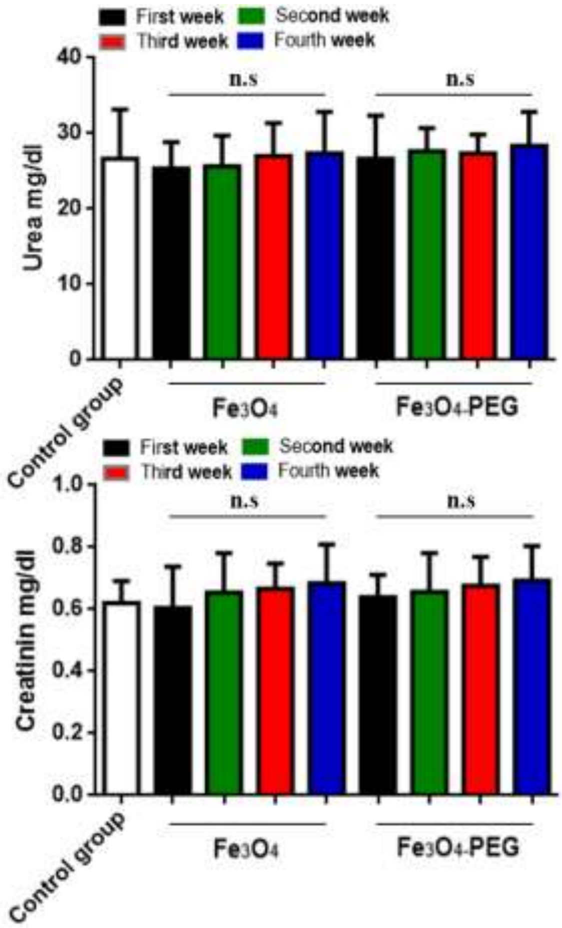

Fig. 5: Urea and creatinine level after intraperitoneal injection of Fe_3O_4, Fe_3O_4-PEG MNPs

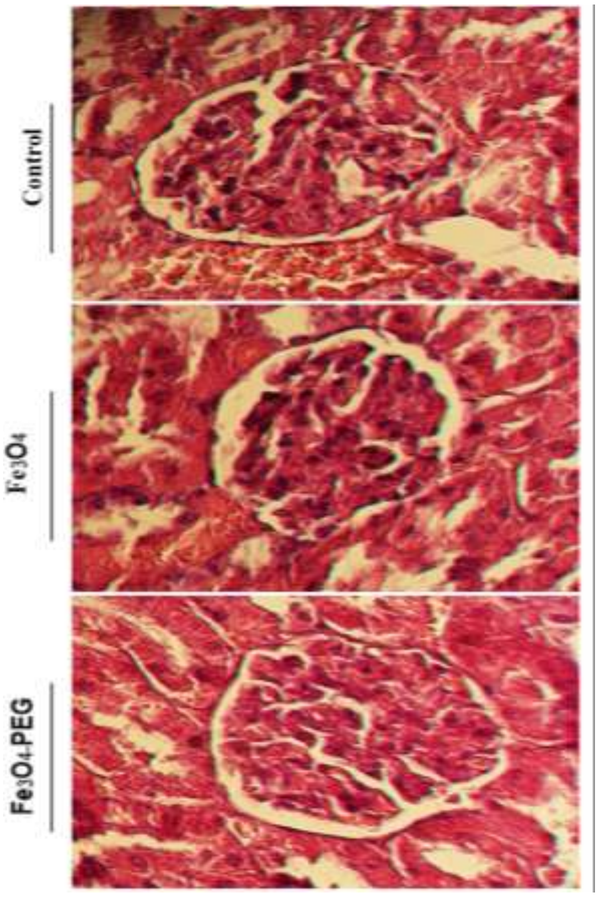

Figure 6: Kidney histological section after intraperitoneal injection of Fe_3O_4, Fe_3O_4-PEG MNPs

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