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肿瘤热疗化疗联合治疗用温敏磁性复合粒子的磁热性能

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摘要：采用无皂乳液聚合法制备了 Fe_3O_4/P(*N*-异丙基丙烯酰胺–共–丙烯酰胺)[P(NIPAAm-co-Am)]温敏磁性复合粒子,分别采用透射电镜(TEM),振动磁强仪(VSM)和动态激光散射仪(DLS)对复合粒子进行表征,并着重研究了复合粒子在交变磁场作用下的磁热性能。结果表明,Fe_3O_4 纳米粒子表面包裹了一层 P(NIPAAm-co-Am)温敏性聚合物,其最低临界溶解温度(LCST)约为 40 ℃,利用磁性粒子在交变磁场下的磁热性能可使复合粒子的温度升高至 LCST 以上,可触发复合粒子发生体积收缩。另外,复合粒子在交变磁场下的磁热性能可通过改变磁性粒子的浓度或调节磁场来调控。

关键词：磁性纳米粒子；温敏性水凝胶；热疗；化疗；药物控释

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Heat Generation Abilities of Magnetic Thermosensitive Composite Particles for Hyperthermia and Chemotherapy Application

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Abstract: Fe_3O_4/P(*N*-isopropylacrylamide-co-acrylamide) [P(NIPAAm-co-Am)], magnetic thermosensitive composite particles were prepared by emulsifier free polymerization. The structure and morphology of the as-synthesized composite particles were characterized by using TEM, and the magnetic and thermosensitive properties were investigated by Vibrating Sample Magnetometer (VSM) and Dynamic Light Scattering (DLS). In addition, the heat generation ability of the composite particles was investigated under alternating magnetic field. The results show that Fe_3O_4 nanoparticles are coated by a thermosensitive P(NIPAAm-co-Am) with an LCST about 40 ℃. The composite particles exhibit relatively high magnetic property and heat generation ability. Furthermore, the heat generation ability of the composite particles under alternating magnetic field can be adjusted by controlling the concentration of composite particles and/or the magnetic field.

Key words: magnetic nanoparticles; thermosensitive hydrogel; hyperthermia; chemotherapy; drug release

Anticancer drugs are toxic and can cause undesirable side effects on human body during therapy, so specific targeting to tumor cells and drug controlled release is an appealing approach to

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minimize the systemic toxicity and to increase drug concentrations in tumor [1-3]. Smart hydrogels have gained considerable attention due to their ability to response to external environment changes such as temperature, pH, etc[4-5].

They are considered as one of the ideal future for controlled drug release carrier[6]. Meyer et al[7] reported the use of a copolymer of *N*-isopropylacrylamide (NIPAAm) and acrylamide (Am) as a thermally responsive drug carrier to tumor. Their work demonstrates that the copolymer exhibits a lower critical solution temperature (LCST) slightly above physiological temperature, but less than the temperature in a region of local hyperthermia. They examined the effect that an LCST transition had on the accumulation of polymer-bound drug in locally heated tumor. Although this can be an advantage on drug release trigged by temperature, polymeric carriers do not get a specific site. Magnetic drug delivery system using magnetic nanoparticle carriers targeted by an external magnetic field is a promising alternative to avoid the problems associated with conventional chemotherapy[3].

The tumor cytotoxicity of chemotherapy is enhanced by the application of hyperthermia [8]. Heat may be directly cytotoxic to tumor cells or inhibit repair of sublethal damage after chemotherapy. Additionally, hyperthermia increases the permeability of tumor vasculature over normal vasculature, which can further enhance preferential delivery to tumors.

The objective of the present work is to explore the synthesis process of Fe_3O_4/P (NIPAAm-co-Am) magnetic thermosensitive particles, and investigate their heat generation ability to aid in the design of system that could combine hyperthermia and chemotherapy treatment of cancer. Fe_3O_4/P(NIPAAm-co-Am) composite particles are prepared by emulsion free polymerization of NIPAAm and Am in the presence of oleic acid modified Fe_3O_4 nanoparticles. The LCST of composite particles is investigated by DLS measurement. Heat generation is investigated under various magnetic field frequencies and different amounts of magnetic particles.

1 Experimental

1.1 Materials

The monomer *N*-isopropylacrylamide (NIPAAm) was purchased form J&K Chemica (99%), and recrystallized from hexane before use. Acrylamide (Am) was purchased from Sinopharm Chemical Reagent Co., Ltd. (China), and recrystallized from chloroform. All other materials [*N*,*N*-methylenebisacrylamide (MBAM), ammonium persulfate (APS), oleic acid, ferrous chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) and iron sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$)]were of analytical grade, all purchased from Sinopharm Chemical Reagent Co., Ltd. (China) and used without further purification.

1.2 Preparation of oleic acid-modified Fe_3O_4 Nanoparticles

The oleic acid-modified Fe_3O_4 Nanoparticles were synthesized using co-precipitation method. 2.36 g $FeCl_3 \cdot 6H_2O$ and 1.21 g $FeSO_4 \cdot 7H_2O$ were dissolved in 100 mL distilled water. Then, 5 mL of concentrated ammonia were added to the solution rapidly under vigorous stirring under nitrogen atmosphere. To this solution, 1.2 g oleic acid in 5 mL ethanol was added. Then the solution was heated to 70 ℃ and kept at that temperature for 30 min. The magnetic particles were separated by magnetic decantation and washed with hot ethanol several times to remove excess surfactant. The particles were finally dispersed in 50 mL distilled water and pH was adjusted to 10 to obtain a stable magnetic fluid for further use. Then the oleic acid modified Fe_3O_4 (marked as OA-Fe_3O_4) suspension was obtained.

2.3 Preparation of Fe_3O_4/P (NIPAAm-co-Am) composite particles

Fe_3O_4/P (NIPAAm-co-Am) composite particles were synthesized by emulsifier free polymerization. NIPAAm and Am mixtures were prepared in molar ratio of 7∶1 and the combined concentration of monomer was 0.02 mol·L^{-1}, MBAM was then added at 5% weight of the monomer and stirred for 10 min. A certain amount of Fe_3O_4 suspension was then added to the mixtures and purged with nitrogen for 30 min to remove any oxygen. The solution was heated to 70 ℃.

Then, redox initiator APS (3% of the weight of monomer) was added to the solution. The reaction was continued for 7 h. and then the composite particles were separated by magnetic decantation. The resulting particles were immersed in 1 mol·L^{-1} HCl solution for 12 h and then purified by repetitive magnetic decantation. The amounts of Fe_3O_4 in this experiment were 5%, 10% and 15% weight percents of the monomer, and the resulting samples were denoted as F5, F10 and F15, respectively.

1.4 Characterization

Samples for transmission electron microscopy (TEM) measurement were prepared by placing a drop of an aqueous Fe_3O_4 nanoparticles and Fe_3O_4/P (NIPAAm-co-Am) composite particles onto a carbon-coated copper grid. The dried grid was then transferred to take TEM photographs at 200 kV on a Hitachi H-800 electron microscope (Japan).

Magnetic property measurement was carried out at room temperature using a vibrating sample magnetometer (VSM, Model 4 HF, Nanjing University Instrument Plant, China) with a maximum field of 7×10^6 A·m^{-1}.

Dynamic light scattering particle size analyzer (DLS, Autosizer 4700, Malvern Co.) was used to investigate particle size change of the composite particles, and to characterize the thermo-sensitive property of the magnetic thermosensitive composite particles.

The heat generation ability of as-prepared nanoparticles was determined using a homemade experimental setup as reported elsewhere [9-10]. A magnetic generator with frequencies from 55 to 75 kHz and maximum amplitude of 7×10^3 A·m^{-1} (Nanjing University Instrument Plant, China) was used in this experiment. A certain amount of samples was placed into a thermally insulating glass container filled with 1.0 mL distilled water and then exposed to the alternating magnetic field for a given time. The temperature of water was monitored using an alcohol thermometer and was recorded with time.

2 Results and discussion

Fe_3O_4 magnetic nanoparticles are synthesized by typical co-precipitation method. There are many hydroxyl groups on the surface of iron oxide prepared by this method. So oleic acid can chemically adsorbed on the surface of magnetic nanoparticles through the reaction between hydroxyl groups of oleic acid and the carboxyl acid groups on the as-synthesized magnetic nanoparticles, and then form the first layer of surfactant. Excess oleic is then adsorbed to the first layer surfactant by hydrophobic interaction and form the second layer surfactant. The outer layer of oleic acid on the Fe_3O_4 surface reacts with $NH_3\cdot H_2O$ solution and transformed into an ammonium salt, resulting in well dispersibility of Fe_3O_4 nanoparticles in aqueous solution. The TEM image of oleic acid modified Fe_3O_4 (Fig.1 (a)) shows that the size of particles is about 10 nm which is lower than the critical particles size of ferromagnetism of magnetite due to a superparamagntism[11].

The bilayer oleic acid coated Fe_3O_4 nanoparticles were used as the seed of polymerization of organic

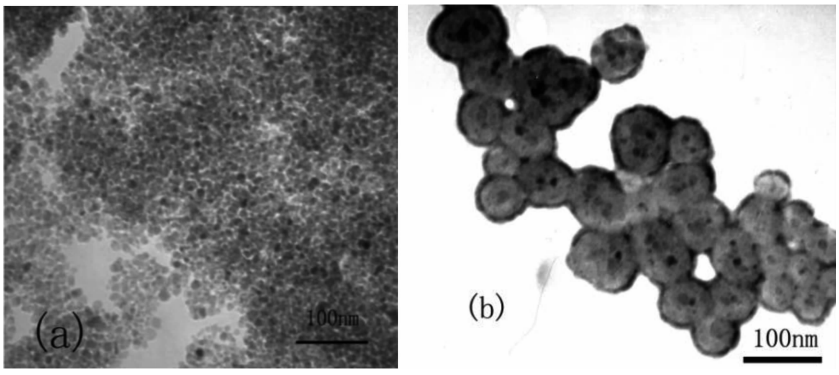

Fig.1 TEM images of oleic acid-modified Fe_3O_4 nanoparticles (a) and Fe_3O_4/P(NIPAAm-co-Am) composite particles (b)

monomer. Polymerization process was initiated by APS solution at 70 ℃, which is much higher than the LCST of copolymer. So thermosensitive copolymer chains transfer to hydrophobic and then precipitate on the magnetic particles. Furthermore, they further copolymerize with other organic monomer and MBA leading to a compact shell on the surface of magnetic particles, forming a core/shell structure. This is confirmed by TEM images of Fe_3O_4/P(NIPAAm-coAm) thermosensitive magnetic particles as shown in Fig.1 (b). The particle size is about 100 nm, but there is a little agglomeration. This may be due to a cross-link among particles. So we need to optimize our preparation process to avoid this agglomeration in our further work, such as adjusting the concentration of monomer and cross linker.

To investigate the LCST of thermosensitive magnetic particles, a plot of the particles diameter versus temperature are obtained by measurement of DLS at various temperatures. As shown in Fig.2, the particles exhibit a LCST of ~40 ℃, which is the desired temperature for hyperthermia and chemotherapy treatment of cancer because it is higher than the physiological temperature and slighter lower than the hyperthermia temperature (42~47 ℃)[14-15]. This composite particles consisting of iron oxide core and temperature-responsive polymer shell can be loaded with anti-cancer drug, injected into the blood stream, and targeted to the tumor using an external magnetic field gradient. When an alternating magnetic field is applied after localization, the iron oxide magnetic cores of the composite particles generate heat, raising the temperature of the tumor and resulting in hyperthermia. At the same time, the heat generated is conducted from the core to the surrounding polymer and raises the temperature of the polymer shell above the LCST, triggers the release of the drug loaded in the polymer. In this way, the multi-modal cancer therapy, including drug targeting, drug delivery and hyperthermia, will be achieved.When temperature increases to higher than the LCST, the hydrophilic thermosensitive copolymer on the surface of magnetic nanoparticles transfers to hydrophobic, resulting in the color of suspension changes from transparent to milky. And the diameter of particles decreases from 235 nm to 198 nm. Considering the spherical morphology of particles, the volume of thermosensitive magnetic particles can be calculated by the equation:

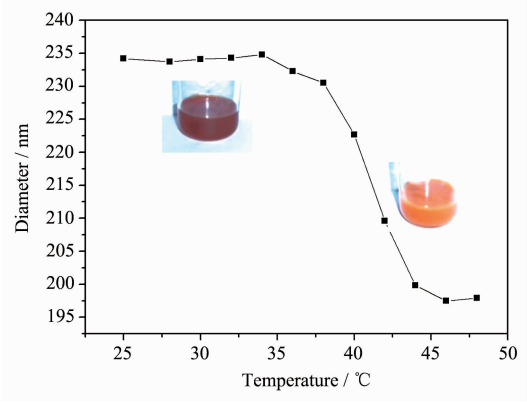

Fig.2 Results of variable temperature DLS: diameter of the composite particles vs. temperature

$$V = \frac{4}{3}\pi\left(\frac{D}{2}\right)^3 \quad (1)$$

where D is the diameter of the particle.

There is about 40% volume contraction when temperature is raised to higher than LCST. When drugs are loaded on the thermosensitive magnetic particles, the release of drugs can be triggered by raising temperature to higher than LCST.

The particles size obtained by DLS is doubled the ones by TEM as shown in Fig.1, It results from cross-link of the composite nanoparticles to form big aggregates. Additionally, the evaporation from water minimizes the particles size of the samples for TEM.

Fig.3 shows the hysteresis loops of the Fe_3O_4 nanoparticles (Fig.3 (d) and Fe_3O_4/P (NIPAAm-co-Am) composite particles with various magnetic nanoparticles concentrations (Fig.3 (a) ~(c)) at room temperature. Clearly, all samples show superparamagnetic behavior. Saturation magnetization (M_s) of Fe_3O_4 nanoparticles is 75 emu·g^{-1}, and it decreases when coating thermosensitive P (NIPAAm-co-Am) copolymer. The M_s of samples F5, F10 and F15 are 9, 20 and 23 emu·g^{-1}, respectively. The M_s of samples is determined by the concentration of Fe_3O_4 nanoparticles because it is raised from the magnetic

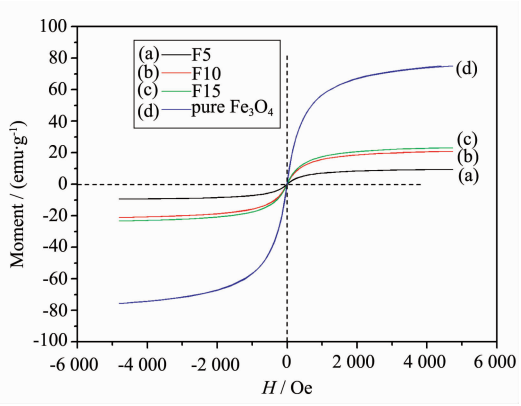

Fig.3　Hysteresis loops of the Fe_3O_4/P(NIPAAm-co-Am) composite particles ((a)~(c)) and Fe_3O_4 nanoparticles (d) at room temperature

nanoparticles. So we can infer that the concentrations of Fe_3O_4 nanoparticles in samples F5, F10 and F15 are 12%, 26.7% and 30.7%, respectively, indicating that the concentration of Fe_3O_4 improves with the increase in amount of Fe_3O_4 nanoparticles in the solution for polymerization. But when the amount of Fe_3O_4 nanoparticles added into solution is above 10% of the monomer, Ms of the resulted samples increases much slowly with further increase of Fe_3O_4 nanoparticles, indicating that we can not prepare Fe_3O_4/P (NIPAAm-co-Am) composite particles with high Fe_3O_4 concentration by emulsifier free polymerization method.

In order to investigate the heat generation ability of thermosensitive magnetic particles, 20 mg as-synthesized samples were placed into a thermally insulating glass container filled with 1.0 mL distilled water, and then exposed to an external alternating magnetic field of 65 kHz. The heating generated by samples under the alternation magnetic field leads to an increase in the temperature of distilled water. The deionized water temperature as a function of time for samples of F5, F10, F15 and Fe_3O_4 is shown in Fig.4 (a), (b), (c)and (d) , respectively. For each sample, the temperature of distilled water is elevated rapidly in the first 10 minutes, thereafter, the temperature increase rate slows down gradually and the temperature remains constant thereafter. Additionally, the temperature increase is 4.8, 8.4, 10 and 30.5 ℃ for F5, F10, F15 and Fe_3O_4, respectively.

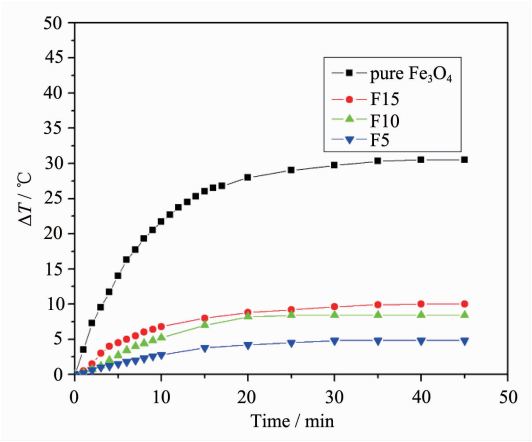

Fig.4　Deionized water temperature as a function of time for samples of F5 (a), F10 (b),F15 (c) and Fe_3O_4 (d) in AC magnetic field

The water temperature as a function of time for 10, 20, 40 and 60 mg F15 is shown in Fig.5 (a), (b), (c) and (d) , respectively. For certain amount of water (1.0 mL), higher temperature rises are shown with the increase in amount of the sample. Temperature rises (ΔT) of 10, 20, 40 and 60 mg·mL^{-1} for sample F15 are 6.5 ℃, 10 ℃, 15.7 ℃ and 18.5 ℃, respectively.

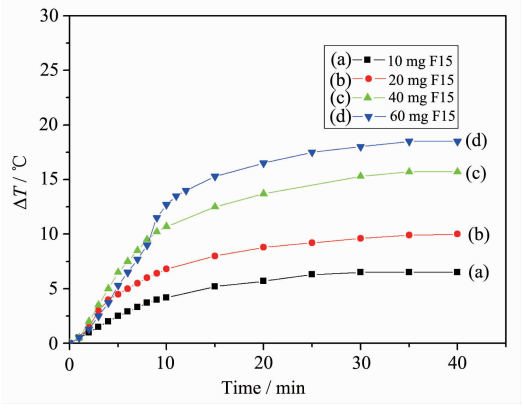

Fig.5　Deionized water temperature as a function of time for various amounts of F15 in AC magnetic field

Fig.6(a), (b), (c) show the dependence of heat generation on frequency of applied magnetic field. The frequency of applied magnetic field was varied as 60, 70, and 75 kHz. 20 mg of sample F10 in 1.0 mL distilled water was exposed to magnetic field. The measured temperatures of the suspension under magnetic field increase to reach a steady state within 40 min at all frequencies of magnetic field. The steady state temperature values are dependent on the

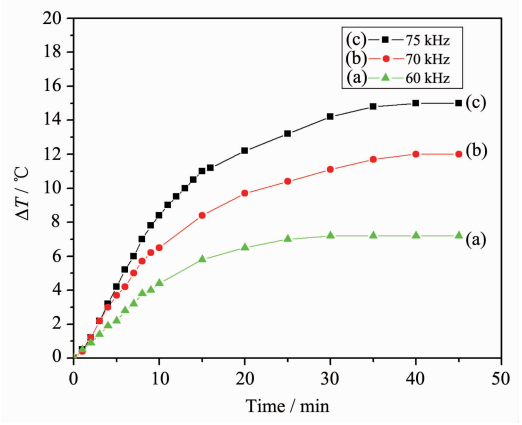

Fig.6 Deionized water temperature as a function of time for samples of F10 in AC magnetic field with various frequencies

frequency of magnetic field, with the greatest temperature rise in samples exposed to the highest frequency of the magnetic field.

The heat generation of nanoparticles is mainly due to Brownian and Neel relaxation mechanisms. The heat generation ability, therefore, can be determined by magnetic moment, frequency, and particle amount. The heating power can be deduced by equation **2**[16]:

$$P = -\mu_0 f \oint M\mathrm{d}H = \mu_0 \pi \chi'' f H_0^2 \qquad (2)$$

where P is the measured heating power, μ_0 is the permeability of free space; f is frequency of applied alternation magnetic field and H is the amplitude of the magnetic field intensity. Obviously, our data can be explained by equation **2**. For hyperthermia application, the temperature is needed to increase to 42~47 ℃ from physiological temperature (37 ℃), i.e., the temperature rise is 5~10 ℃. The thermosensitive magnetic particles satisfy a requirement for in vivo hyperthermia, because the temperature rise is suitable for hyperthermia and can be adjusted by changing the concentration of particles or the applied alternation magnetic field. When the composite particles are loaded with drugs and dispersed in simulate body fluid, they can be specific to tumor place and heated by an external magnetic field.

The heat generated by the composite particles can kill tumor cells and trigged the release of drugs at the same time. Thus, the composite particles have a potential application on hyperthermia and chemotherapy treatment of cancer.

3 Conclusions

Fe_3O_4/P(NIPAAm-co-Am) composite particles were prepared by emulsion free polymerization of NIPAAm and Am in the presence of oleic acid modified Fe_3O_4 nanoparticles. DLS measurement shows that the composite particles exhibit a LCST about 40 ℃, which is the desired temperature for hyperthermia and chemotherapy treatment of cancer. The obtained composite particles show a superparamagnetic property which is important for biomedical application. Additionally, the magnetic property measurements show that composite particles exhibit relatively high magnetic property and heat generation ability, though it is lower than that of pure Fe_3O_4 nanoparticles. The heat generated by the composite particles is dependent on both the amount of magnetic particles and alternation magnetic field. The desired heating to achieve the temperature of hyperthermia and trigger drug release can be achieved by adjusting the concentration of magnetic particles and/or mag netic field.

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