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Effect of magnetic nanoparticles size on rheumatoid arthritis targeting and photothermal therapy

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ABSTRACT

Nanoparticles based multifunctional system exhibits great potential in diagnosis and therapy of rheumatoid arthritis (RA). The size of nanoparticles plays an essential role in biodistribution and cellular uptake, in turn affects the drug delivery efficiency and therapeutic effect. To investigate the optimal size for RA targeting, Fe_3O_4 nanoparticles with well-defined particle sizes (70–350 nm) and identical surface properties were developed as model nanoparticles. The synthesized Fe_3O_4 nanoparticles exhibited excellent biocompatibility and showed higher temperature response under irradiation of near infrared light. Size-dependent internalization was observed when incubated with inflammatory cells. Compared with large ones, small nanoparticles were more readily be phagocytized, leading to higher cytotoxicity *in vitro*. However, the *in vivo* experiment in CIA mice demonstrated a quite different result that nanoparticles with size of 220 nm exerted better accessibility to inflamed joint and resulted in higher temperature and better therapeutic effect under laser irradiation. This study not only offered a novel method for RA therapy but also a guideline for RA targeted drug carrier design.

1. Introduction

As a chronic autoimmune disease, rheumatoid arthritis (RA) is characterized by inflammatory cell infiltration, synovial hyperplasia, pannus formation and the continuous destruction of the cartilage and bone [1,2]. Recently, various therapeutic agents such as methotrexate, glucocorticoids and monoclonal antibody have been applied for the therapy of RA [3–5]. However, the severe adverse effects of these agents significantly limit their application in clinic, especially for long-term medication [6–8]. To address this issue, targeted drug delivery strategy has been developed to improve the accumulation of therapeutic agents in the target tissue [9,10]. Notably, the size of nanocarrier plays a key role in targeted drug delivery. Optimal gold nanoparticles, magnetic nanoparticles and micelles can significantly enhance the drug delivery efficiency [11–14]. Guo et al. indicated that small nanoparticles could realize deeper penetration, while large ones displayed greater tumor accumulation [15]. However, systematic assessment of nanoparticle size on RA targeting efficiency has not been reported and need to be further studied.

Here, we try to explore the effect of nanoparticles size on RA targeted drug delivery. Nevertheless, the properties of nanoparticles, including zeta potential, roughness and elemental composition, display significant influence on the biodistribution. As a result, nanoparticles with adjustable size and identical surface properties shall be synthesized for the biodistribution study. Magnetic iron oxide (Fe_3O_4), which has been extensively applied in biomedicine, are easily fabricated within the size range of 50–400 nm [16–18]. Besides, iron oxide has been reported to be used as photothermal agent for tumor hyperthermia therapy [19,20], which also indicates a great potential in ablating activated or hyperplastic cells in RA therapy. As such, iron oxide provides an ideal candidate for investigating the effect of nanoparticles size on distribution and photothermal treatment of RA.

In this study, we synthesized citrate group coated Fe_3O_4 nanoparticles with diameters of 70, 110, 220, and 350 nm *via* a facile solvothermal method. All the nanoparticles exhibited similar surface properties. We compared the photothermal performances of the iron oxide nanoparticles by exposing them to near infrared (NIR) light. To elucidate the effect of nanoparticles size on cellular endocytosis, four kinds of nanoparticles were incubated with macrophage cells after labeling with rhodamine B. The biodistribution of the nanoparticles was performed on collagen-induced arthritic (CIA) mice models using indocyanine green (ICG) as a probe. We further carried out *in vivo*

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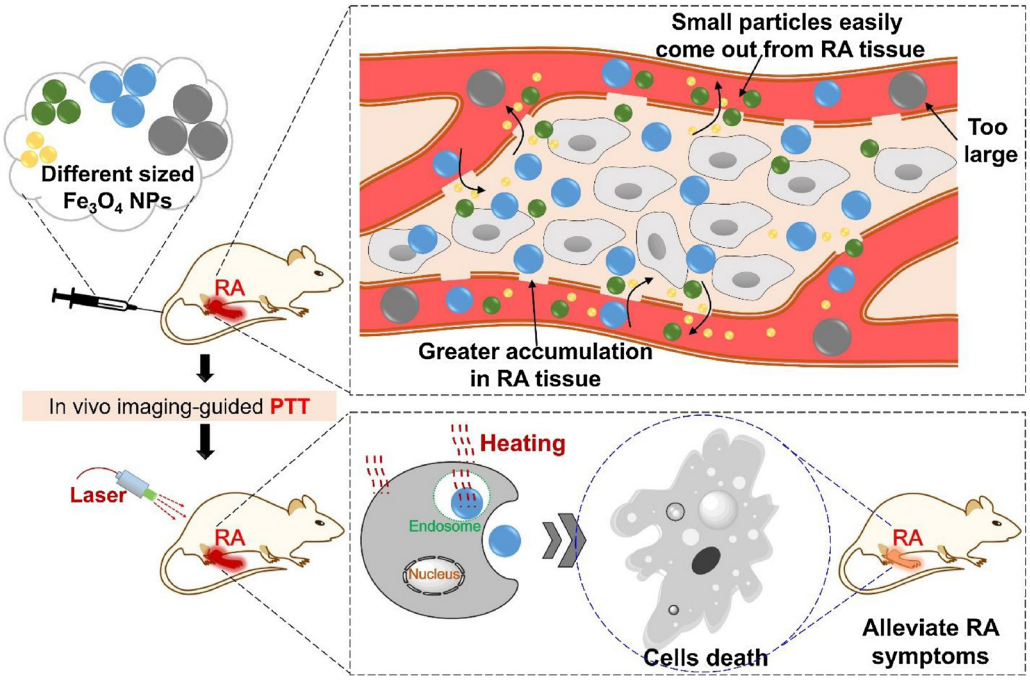

Scheme 1. Schematic illustration of the optimal size of Fe_3O_4 nanoparticles (Fe_3O_4 NPs) as photothermal agent in rheumatoid arthritis photothermal therapy. The leaky blood vessel in the RA tissue has an increased pore size for selective penetration of nanoparticles. After intravenous injection, if the nanoparticles are larger than the cutoff size of the pathological vessel, they will lack permeability to exhibit a reduced level of RA lesions accumulation. When the nanoparticles are too small, they will easily enter the RA tissue through the leak but prefer to readily extravasate, resulting in little tissue retention. While the optimal sized nanoparticles exhibit enhanced permeability and retention that realize the excellent photothermal therapy (PTT).

imaging guided photothermal therapy and assessed the relation RA therapeutic effect with biodistribution. This work is aimed to provide the optimal nanoparticles size for RA targeting and evaluate the potential of using iron oxide as agent for RA photothermal therapy (Scheme 1).

2. Experiment and methods

2.1. Materials

Iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), sodium acetate trihydrate ($NaAc{\cdot}3H_2O$), trisodium citrate dehydrate (Na_3Cit), polyethylene glycol (PEG, Mw = 4000), ethylene glycol (EG) and rhodamine B (RhB) were purchased from Sino-pharm Chemical Reagents Company. Collagen Type II, complete Freund's adjuvant (CFA), Hoechst 33342 and 3-[4,5-dimethylthialzol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich. Indocyanine green (ICG) was purchased from Tokyo Chemical Industry Co., Ltd. (Japan). RPMI-1640 medium (RPMI), fetal bovine serum (FBS), and penicillin/streptomycin (100 $U{\cdot}mL^{-1}$) were from Ji No Biotechnology Co., Ltd. (Zhejiang, China). All chemicals were used without further purification. Deionized water (18.2 MΩ cm) was prepared by Milli-Q system (Millipore, USA) and was used in all experiments.

2.2. Cell culture

RAW 264.7 cells (a type of mouse macrophage cell line) were used to simulate the macrophages in RA, which were purchased from Cell Resource Centre of Shanghai Institute for Biological Sciences (Chinese Academy of Sciences, Shanghai, China). The cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum and maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

2.3. Preparation and characterization of Fe_3O_4 nanoparticles

The citrate groups stabilized Fe_3O_4 nanoparticles were prepared by a solvothermal method [21,22]. We chose 110 nm nanoparticles as model to explain the synthesis process. Typically, 0.333 g PEG 4000 was first dissolved in ethylene glycol (47 mL). Then, 1.270 g $FeCl_3{\cdot}6H_2O$ (4 mM) and 0.466 g trisodium citrate (1.6 mM) were added followed by stirring at room temperature (RT) to form a clear solution. After that, 4.640 g NaOAc (32 mM) was added and the mixture was stirred vigorously for 1 h at RT. The mixture was transferred into a Teflon-lined stainless-steel autoclave (70 mL capacity) and reacted at 200 °C for 10 h. The black products were successively washed with ethanol and distilled water for several times and then stored in distilled water for further use. Fe_3O_4 nanoparticles with other sizes were synthesized by varying the reaction times and the concentrations of $FeCl_3$ (as shown in Table S1).

2.4. Photothermal conversion in vitro

The photothermal effect of 110-nm Fe_3O_4 nanoparticles was evaluated using an 808 nm laser at a power density of 2 $W{\cdot}cm^{-2}$. Fe_3O_4 nanoparticles suspensions with serious concentrations of 0, 20, 50, 100, 200, 400 $\mu g{\cdot}mL^{-1}$ were irradiated to explore the change of temperature along with the concentration. The temperature of the suspensions was monitored in 30 s intervals with a thermocouple immersed in the solution for a total of 5 min. The thermal images were captured by Infrared Thermal Camera in 60 s intervals companied with heating curve. Besides, the photothermal effects of 70-nm, 220-nm, and 350-nm Fe_3O_4 nanoparticles at the concentration of 200 $\mu g{\cdot}mL^{-1}$ were also investigated.

2.5. Cytotoxicity and photothermal toxicity assay

MTT assay was used to evaluate the cytotoxicity of Fe_3O_4 nanoparticles. RAW 264.7 cells were seeded into 96-well plates at a density of 5×10^3 cells/well. Different sized nanoparticles with serious concentrations were added into the medium and incubated for 24 h. Then the cultural medium was replaced with 100 μL of fresh medium containing 1 $mg{\cdot}mL^{-1}$ MTT. 4 h later, the medium was discarded and 200 μL of dimethyl sulfoxide (DMSO) was added to dissolve crystal. The absorbance at 570 nm was measured by using an ELISA plate reader.

Photothermal toxicity of Fe_3O_4 nanoparticles was also evaluated by MTT assay. Briefly, the different sized nanoparticles suspensions at the concentration of 100 $\mu g{\cdot}mL^{-1}$ (the final concentration of nanoparticles in medium) were incubated with RAW 264.7 cells for 4 h. After replacing the nanoparticles suspension with fresh medium, the cells were exposed to NIR laser irradiation (2 $W{\cdot}cm^{-2}$) for 3 min. After incubation