Light-Addressable Nanoclusters of Ultrasmall Iron Oxide Nanoparticles for Enhanced and Dynamic Magnetic Resonance Imaging of Arthritis

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Design of novel nanoplatforms with single imaging elements for dynamic and enhanced $T_1/T_2$-weighted magnetic resonance (MR) imaging of diseases still remains significantly challenging. Here, a facile strategy to synthesize light-addressable ultrasmall $\text{Fe}_3\text{O}_4$ nanoparticles (NPs) that can form nanoclusters (NCs) under laser irradiation for enhanced and dynamic $T_1/T_2$-weighted MR imaging of inflammatory arthritis is reported. Citric acid-stabilized ultrasmall $\text{Fe}_3\text{O}_4$ NPs synthesized via a solvothermal approach are linked with both the arthritis targeting ligand folic acid (FA) and light-addressable unit diazirine (DA) via polyethylene glycol (PEG) spacer. The formed ultrasmall $\text{Fe}_3\text{O}_4$-PEG-(DA)-FA NPs are cytocompatible, display FA-mediated targeting specificity to arthritis-associated macrophage cells, and can form NCs upon laser irradiation to have tunable $r_1$ and $r_2$ relaxivities by varying the laser irradiation duration. With these properties owned, the designed $\text{Fe}_3\text{O}_4$-PEG-(DA)-FA NPs can be used for $T_1$-weighted MR imaging of arthritis without lasers and enhanced dual-mode $T_1/T_2$-weighted MR imaging of arthritis under laser irradiation due to the formation of NCs that have extended accumulation within the arthritis region and limited intravasation back to the blood circulation. The designed light-addressable $\text{Fe}_3\text{O}_4$-PEG-(DA)-FA NPs may be used as a promising platform for dynamic and precision $T_1/T_2$-weighted MR imaging of other diseases.

Inflammatory arthritis is one of the most common diseases affecting human health because of its high incidence and disability rate of up to 50% in the patients with an age of over 50.\cite{1} Although many techniques have been developed for the diagnosis of arthritis such as biomarkers, computed tomography, and ultrasonic imaging, their efficacy for patients at an advanced stage is not satisfactory.\cite{2} As such, development of multifunctional nanoplatforms for early diagnosis of arthritis still remains an urgent challenge. Until now, noninvasive magnetic resonance (MR) imaging with high spatial resolution has been considered to be a powerful technique for early detection of arthritis.\cite{3}

In general, to improve the sensitivity and accuracy of diagnosis, multifunctional contrast agents with dual-mode or multimode imaging elements integrated have been designed.\cite{4} In particular, for dual-mode $T_1/T_2$-weighted MR imaging, the reported contrast agents are usually composed of two types of imaging agents, such as gadolinium- or manganese-based NPs ($T_1$ positive agents) combined with superparamagnetic iron oxide ($\text{Fe}_3\text{O}_4$) NPs ($T_2$ negative agents) within or onto a single nanosystem.\cite{5} However, the strategy used for simple combination of two types of functional elements often leads to unsatisfactory results,\cite{6} due to the existing issues of colloidal instability, the composition-dependent $r_1/r_2$ ratio, and the difficulty for further surface functionalization. Furthermore, it is difficult to achieve

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the tunable conversion or coexistence of $T_1$-weighted and $T_2$-weighted MR imaging capability based on one kind of NPs for the clinical applications.

Ultrasmall Fe$_3$O$_4$ NPs with a size smaller than 5 nm have been reported to have $T_1$ enhancing effect and $T_2$ decreasing effect, and can be used as an excellent contrast agent for $T_1$-weighted MR imaging.\(^{[8]}\) Through assembly of ultrasmall Fe$_3$O$_4$ NPs onto generation 5 (G5) poly(amidoamine) dendrimers, the generated nanoclusters can be readily developed as a contrast agent for $T_2$-weighted MR imaging of tumors.\(^{[7]}\) Recent reports have also shown that ultrasmall Fe$_3$O$_4$ NPs can be assembled to form larger clusters in a suitable environment (e.g., specific pH or enzyme) and realize the conversion from $T_1$-weighted to $T_2$-weighted MR imaging.\(^{[8]}\) However, these approaches using pH- or enzyme-responsive assembly may generate unwanted particle aggregation due to the sophisticated biological environment that is susceptible to nucleases in blood and may further induce immune response after particle exposure.\(^{[9]}\) Additionally, the pH- or enzyme-based responsive assembly of particles mostly occurs in a few types of tumor microenvironment,\(^{[8b,10]}\) and may not be suitable for the design of responsive assembly of ultrasmall Fe$_3$O$_4$ NPs for precision imaging of other diseases, in particular inflammatory arthritis. Moreover, a part of NPs with a small size are capable of escaping the rapid renal clearance, easily extravasate from vessels around lesion, and subsequently penetrate into the lesion region. However, these NPs also readily intravasate back into circulation, leading to decreased accumulation in the lesion.\(^{[11]}\) Therefore, it is vital to realize the enhanced retention of particles and obtain precise dynamic dual-mode $T_1/T_2$-weighted MR imaging of arthritis in vivo based on the ultrasmall Fe$_3$O$_4$ NPs. In particular, light-triggered assembly and hierarchical targeting\(^{[12]}\) may be used as an efficient strategy to bypass some hurdles because of most of the diseases (e.g., inflammation, tumors, etc.) can be spatiotemporally addressed by light.\(^{[13]}\)

In our previous work, we synthesized G5 dendrimer-stabilized gold (Au) nanoflowers embedded with ultrasmall Fe$_3$O$_4$ NPs.\(^{[14]}\) The designed hybrid nanoplatform possessed a higher $r_1$ relaxivity of 3.22 mm s$^{-1}$ by embedding ultrasmall Fe$_3$O$_4$ NPs within Au nanoflowers with a well distribution and limited aggregation. In another work, Gao and co-workers designed light-triggered Au NP-based nanoclusters that enable photothermal therapy and photacoustic imaging of tumors in vivo,\(^{[15]}\) where the Au NPs are able to be clustered via a polyethylene glycol (PEG)-linked diazirine (DA) terminal group under laser exposure. These prior works stimulate us to hypothesize that by reasonable adjustment of the aggregation degree of the ultrasmall Fe$_3$O$_4$ NPs through spatiotemporal manipulation, the NPs may be used as an excellent nanoprobe for dynamic dual-mode $T_1/T_2$-weighted MR imaging of arthritis.

In this present work, we designed light-addressable assemblies of ultrasmall Fe$_3$O$_4$ NPs for enhanced retention and dynamic $T_1/T_2$-weighted MR imaging of inflammatory arthritis in vivo. First, ultrasmall citric acid-stabilized Fe$_3$O$_4$ NPs were synthesized via a solvothermal method, and modified with arthritis-targeting ligand folic acid (FA)\(^{[15]}\) and molecular switch DA via a PEG spacer through 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) coupling reaction. The linked molecular switch DA on the surface of Fe$_3$O$_4$-PEG-(DA)-FA NPs was transformed to carbene under 405 nm laser excitation, which was further reacted with adjacent ligands onto the Fe$_3$O$_4$ NPs to form covalent bonds of C-C, C=H, O-H, and X=H (X represents heteroatom), leading to the formation of nanoclusters (NCs) that were used for dynamic light-addressable $T_1/T_2$-weighted MR imaging of arthritis (Figure 1). The main idea to be tested was as follows: After intravenous delivery, the Fe$_3$O$_4$-PEG-(DA)-FA NPs can easily extravasate through the vasculature around arthritis and subsequently penetrate inside the inflammation region, allowing for $T_1$-weighted MR imaging of the arthritis. After 405 nm laser irradiation to induce the formation of NCs in the inflammation region, the formed NCs are not able to intravasate back into circulation and remain in the inflammation region of arthritis, thus allowing for enhanced $T_1/T_2$-weighted dual-mode MR imaging. We systematically

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**Figure 1.** Schematic illustration of the synthesis of Fe$_3$O$_4$-PEG-(DA)-FA NPs for enhanced retention and tunable $T_1/T_2$-weighted MR imaging of inflammatory arthritis.
characterized the materials, tested their cytocompatibility, FA-mediated targeting specificity, and dynamic light-addressable MR imaging performance of arthritis in vivo. To our knowledge, this is the very first example to design an FA-targeted, light-addressable nanoplatform that can be used for dynamic dual-mode $T_1/T_2$-weighted precision MR imaging of arthritis.

To prepare light-addressable NCs composed of ultrasmall Fe$_3$O$_4$ NPs, we first synthesized citric acid-stabilized ultrasmall Fe$_3$O$_4$ NPs with an average diameter of 2.8 nm via a solvothermal method. The size and uniform morphology of the particles were characterized and confirmed by transmission electron microscopy (TEM, Figure S1, Supporting Information). We then synthesized NHS-DA, which was characterized by $^1$H NMR (Figure S3, Supporting Information). Next, NH$_2$-PEG-NHS was simultaneously modified with NHS-DA and NHS-FA, and deprotected by hydrochloric acid. The formed mixture of NH$_2$-PEG-(DA)-FA was characterized by $^1$H NMR (Figure S3, Supporting Information). Through NMR integration, the numbers of DA and FA conjugated to each PEG were estimated to be 0.68 and 0.26, respectively.

After that, NH$_2$-PEG-(DA)-FA was immobilized onto the surface of citric acid-stabilized ultrasmall Fe$_3$O$_4$ NPs via EDC chemistry, leading to the formation of Fe$_3$O$_4$-PEG-(DA)-FA NPs. The amount of NH$_2$-PEG-(DA)-FA modified onto the surface of the Fe$_3$O$_4$ NPs was quantified by thermogravimetric analysis (TGA, Figure S4, Supporting Information). Based on the weight loss data, the percentage of PEG-(DA)-FA modified onto the particle surface can be calculated to be 14.3%. Inductively coupled plasma-optical emission spectroscopy (ICP-OES) measurements were used to quantify the Fe element within the Fe$_3$O$_4$-PEG-(DA)-FA NPs and the Fe content was quantified to be 220 $\mu$g mg$^{-1}$.

The light-responsive aggregation of the Fe$_3$O$_4$-PEG-(DA)-FA NPs was investigated via TEM imaging (Figure 2). Before laser irradiation, the Fe$_3$O$_4$-PEG-(DA)-FA NPs display a certain degree of aggregation, which is likely due to the sample drying process of the PEGylated ultrasmall Fe$_3$O$_4$ NPs. After irradiated by a 405 nm laser (1.0 W cm$^{-2}$) for different time periods, the aggregation of the NPs is apparent (Figure 2a). The aggregation degree of the NPs is dependent on the laser irradiation duration. The formed Fe$_3$O$_4$-PEG-(DA)-FA NCs comprise of many single Fe$_3$O$_4$-PEG-(DA)-FA NPs with anisotropic crystalline orientations and the crystal structure and composition of the ultrasmall Fe$_3$O$_4$ NPs have not changed upon laser irradiation for different time periods (inset of Figure 2a, b). The laser irradiation time-dependent aggregation of the Fe$_3$O$_4$-PEG-(DA)-FA NPs was further confirmed by dynamic light scattering measurements (Figure 2c), where the hydrodynamic size of the Fe$_3$O$_4$-PEG-(DA)-FA NPs increases as a function of laser irradiation time. The Fe$_3$O$_4$-PEG-(DA)-FA NPs exhibit an initial hydrodynamic size of around 45.7 nm, and after laser irradiation for 12 min eventually reach a size up to 798.4 nm. Further, the clear aggregation of Fe$_3$O$_4$-PEG-(DA)-FA NCs can be observed in aqueous solution after laser irradiation for 12 min (Figure S3, Supporting Information), and the aggregates can be redispersed to form a stable solution after shaking. This indicates that the light-addressable DA terminal groups enable the self-crosslinking of the particles under laser irradiation, likely via the formation of covalent bonds of C–C, C–H, O–H, and X–H (X represents heteroatom) between the formed DA carbine and adjacent ligands onto the Fe$_3$O$_4$ NPs, in agreement with the literature.[13d] Moreover, the agglomeration degree of Fe$_3$O$_4$-PEG-(DA)-FA NCs could be precisely controlled through variation of the laser irradiation time period.

The MR relaxometry of the Fe$_3$O$_4$-PEG-(DA)-FA NPs and NCs formed after laser irradiation was next explored. The $T_1$ and $T_2$ relaxation rate of the particles as a function of laser irradiation time period was determined (Figure 3a,b). The $r_1$ and $r_2$ relaxivities of Fe$_3$O$_4$-PEG-(DA)-FA NCs are size-dependent and can be precisely tuned by different laser irradiation durations. The initial Fe$_3$O$_4$-PEG-(DA)-FA NPs possess a high $r_1$ relaxivity (3.83 ms$^{-1}$ s$^{-1}$) and low $r_2$ relaxivity (9.04 ms$^{-1}$ s$^{-1}$). After laser irradiation for 12 min, their $r_1$ and $r_2$ relaxivities change to 1.61 and 31.6 ms$^{-1}$ s$^{-1}$, respectively. It is worth mentioning that the developed Fe$_3$O$_4$-PEG-(DA)-FA NPs possess a higher $r_1$ relaxivity than that of ultrasmall Fe$_3$O$_4$ NPs (about 1.2–1.4 ms$^{-1}$ s$^{-1}$) in previous reports.[7] This may be due to the fact that the PEGylation modification along with the DA and FA functional groups may enable them to form a certain degree of aggregation with extended interparticle distance, thereby enhancing their $r_1$ relaxivity, in consistence with our previous work.[14]

Furthermore, the change of the $r_1$ and $r_2$ relaxivities can be reflected by the switch between $T_1$ effect (hyperintensity contrast) and $T_2$ effect (hypointensity contrast) of the Fe$_3$O$_4$-PEG-(DA)-FA NCs (Figure 3c,d and Figure S6, Supporting Information). The Fe$_3$O$_4$-PEG-(DA)-FA NPs only possess hyperintensity of $T_1$ effect due to their high $r_1$ relaxivity and low $r_2$ relaxivity. Interestingly, the intensity of $T_1$ effect of Fe$_3$O$_4$-PEG-(DA)-FA NCs gradually decreases with the time of laser irradiation (0–12 min), while the intensity of $T_2$ effect of Fe$_3$O$_4$-PEG-(DA)-FA NCs gradually increases with the time of laser irradiation at the same Fe concentrations. The Fe$_3$O$_4$-PEG-(DA)-FA NPs and Fe$_3$O$_4$-PEG-(DA)-FA NCs after laser irradiation for 2 min exhibit the excellent $T_1$ effect, while the Fe$_3$O$_4$-PEG-(DA)-FA NCs after laser irradiation for 8 and 12 min turn to have the stronger $T_2$ effect. This means that the Fe$_3$O$_4$-PEG-(DA)-FA NPs after laser irradiation exhibit a clear switch from $T_1$ to $T_2$ effect and the switch process is precisely tuned through variation of laser irradiation time. The reasons for the gradual decrease of $r_1$ relaxivity and increase of $r_2$ relaxivity of the Fe$_3$O$_4$-PEG-(DA)-FA NPs after laser irradiation may stem from the reduced interparticle distance after the crosslinking reaction and simultaneous increase of the mass magnetization values of the Fe$_3$O$_4$ NPs.[16] In a recent work, Dullens and co-workers synthesized superparamagnetic nickel colloidal NCs displaying the property of tunable size and mass magnetization by a spontaneous self-organization procedure, and their results also supported our conclusion.[17] In addition, the merits of Fe$_3$O$_4$-PEG-(DA)-FA NCs are remarkable since the agglomeration degree of NCs can be precisely tuned through the external laser irradiation, hence the retention of the particles in the disease site may be controlled by changing the size of the aggregated NCs.

In order to further understand the $T_1$--$T_2$ switching property of the Fe$_3$O$_4$-PEG-(DA)-FA NPs, we calculated the $r_1/r_2$ ratios of the NPs after laser irradiation (Figure S7, Supporting Information) to explain the competition of $T_1$ and $T_2$ effect according to theory[14] and the literature,[18] since the $r_1/r_2$ ratio is an important parameter widely used for evaluating the $T_1$ or $T_2$ effect.
Figure 2. a) TEM images, b) energy-dispersive X-ray spectroscopy, and c) hydrodynamic size of Fe$_3$O$_4$-PEG-(DA)-FA NPs under 405 nm laser irradiation (1.0 W cm$^{-2}$) for different periods of time.
of Fe3O4 NPs. It is quite obvious that the laser irradiation time has strongly impacted the \( r_1 \), \( r_2 \), and \( r_2/r_1 \) ratio. In general, the small Fe3O4 NPs with a high \( r_1 \) relaxivity (>3.0 \text{ m}\text{s}^{-1}\text{s}^{-1}) and low \( r_2/r_1 \) ratio (<5) show strong \( T_1 \) effect and do not show obvious \( T_2 \) effect. In addition, the large Fe3O4 NPs with a high \( r_2 \) relaxivity and \( r_2/r_1 \) ratio (more than 25) result in a dominant \( T_2 \) effect overwhelming the \( T_1 \) effect. Interestingly, the relatively high \( r_1 \) (1.61 \text{ m}\text{s}^{-1}\text{s}^{-1}) and \( r_2 \) (31.60 \text{ m}\text{s}^{-1}\text{s}^{-1}) relaxivities of the Fe3O4-PEG-(DA)-FA NCs formed after laser irradiation for 12 min are suitable to be used for potential dual-mode \( T_1/T_2 \)-weighted MR imaging applications. The Fe3O4-PEG-(DA)-FA NPs having a low \( r_2 \) relaxivity (9.04 \text{ m}\text{s}^{-1}\text{s}^{-1}) and \( r_2/r_1 \) ratio (2.36) can realize a sharp switch from \( T_1 \) to \( T_2 \) effect after formation of NCs with a larger size under laser irradiation. Meanwhile, the agglomeration degree and \( r_2/r_1 \) ratio of Fe3O4-PEG-(DA)-FA NCs can be precisely controlled by varying the laser irradiation time period, providing a powerful platform for accurate dynamic dual-mode \( T_1/T_2 \)-weighted MR imaging applications.

Before in vivo applications, we first tested the cytotoxicity of the Fe3O4-PEG-(DA)-FA NPs and evaluated the FA-mediated targeting specificity of the particles to arthritis-associated macrophage cells. CCK8 cell proliferation assay data (Figure S8, Supporting Information) reveal that the viability of Raw264.7 cells treated with the Fe3O4-PEG-(DA)-FA NPs only has slight changes and can maintain above 86.1% in the studied concentration range ([Fe] = 0.2–3.0 \times 10^{-3} \text{ M}) when compared to control cells treated with phosphate buffered saline (PBS). This implies that the Fe3O4-PEG-(DA)-FA NPs display excellent cytocompatibility. In order to validate the FA-mediated specific targeting of Fe3O4-PEG-(DA)-FA NPs to arthritis-associated macrophage cells, we quantitatively analyzed the Fe uptake by Raw264.7 via ICP-OES, and the free FA-blocked cells were also tested under the same conditions (Figure S9, Supporting Information). It can be seen that with the increase of Fe concentration, the Fe uptake by either Raw264.7 cells or free FA-blocked Raw264.7 cells gradually increases. The Fe uptake by the Raw264.7 cells is about 1.66–1.81 times higher than that by the free FA-blocked Raw264.7 cells at the Fe concentration range of 0.2–3.0 \times 10^{-3} \text{ M} (p < 0.01), suggesting that the attached FA ligand onto the particle surface can actually mediate the particle targeting to macrophage cells expressing FA receptors. The targeting specificity and light-addressable assembly of the Fe3O4-PEG-(DA)-FA NPs was further validated by TEM imaging in vitro (Figure S10, Supporting Information). It is apparent that the Fe3O4-PEG-(DA)-FA NPs are more significantly taken up by Raw264.7 cells than by free FA-blocked Raw264.7 cells after incubation for 12 h. Meanwhile, the Fe3O4-PEG-(DA)-FA NCs are formed in the cytoplasm of Raw264.7 cells after exposure to 405 nm laser (1.0 \text{ W cm}^{-2}) for 3 min, while the Fe3O4-PEG-(DA)-FA NPs without laser irradiation do not seem to have the NCs composed of the aggregated Fe3O4-PEG-(DA)-FA NPs. These results demonstrate that the modification of FA renders the Fe3O4-PEG-(DA)-FA NPs with targeting specificity to FA receptor-expressing macrophage cells and the light-triggered assembly of Fe3O4-PEG-(DA)-FA NCs can be realized in vitro.

We next explored the potential to use the Fe3O4-PEG-(DA)-FA NPs for targeted \( T_1 \)-weighted and dual-mode \( T_1/T_2 \)-weighted MR imaging of inflammatory arthritis in vivo, respectively. Animal experiments were carried out following the protocols approved by the institutional committee for animal care and the policy of the National Ministry of Health. For the
targeted $T_1$-weighted MR imaging, the $T_1$-weighted MR images (Figure 4a,b) and corresponding $T_1$ MR signal-to-noise ratio (SNR) (Figure 4c,d) were collected from arthritis and free FA-blocked arthritis model at different time points postinjection of the Fe$_3$O$_4$-PEG-(DA)-FA NPs, respectively. It can be seen that for the free FA-blocked arthritis model, the MR signal intensity of the arthritis region is the highest at 30 min postinjection and decreases with the time postinjection. Excitingly, for the regular arthritis model, the strong MR signal intensity can be lasted from 30 to 60 min postinjection and the MR signal can be still detectable even at 120 min postinjection (Figure 4a and Figure S11, Supporting Information). Quantitative MR SNR data show that the MR SNR of the regular arthritis model is about 1.79–2.66 times higher than that of the free FA-blocked arthritis model at 15–60 min postinjection (Figure 4c,d). These results indicated that with the FA-mediated targeting, the designed Fe$_3$O$_4$-PEG-(DA)-FA NPs enable enhanced accumulation and prolonged residence in the arthritis region, thereby allowing for enhanced $T_1$-weighted MR imaging of arthritis.

In order to avoid the individual differences of mice that influence the targeted $T_1$/$T_2$-weighted MR imaging, the arthritis models in both left and right hind legs for each mouse were established. Then, the $T_1$/$T_2$-weighted MR images (Figure 5a,b) and corresponding MR SNR (Figure 5c,d) of the arthritis model were collected and calculated. The laser irradiation of arthritis region in the left hind leg (red arrow) was carried out, while the arthritis region of right hind leg (white arrow) received no laser irradiation. Clearly, for the $T_1$-weighted MR imaging, the arthritis region of both hind legs shows a significant MR contrast enhancement at 30 min postinjection of the Fe$_3$O$_4$-PEG-(DA)-FA NPs before laser irradiation, which is much higher than that before injection ($p < 0.001$, Figure 5a,c). As expected, the signal intensity and MR SNR of arthritis region in left hind leg after laser irradiation slightly decrease, while the $T_1$ MR signal intensity of arthritis region in right hind leg without laser irradiation does not have any prominent changes ($p > 0.05$, ns). The MR signal intensity and SNR of left hind leg after laser irradiation are always higher than that of the control ($p < 0.01$). These results demonstrate that the formed Fe$_3$O$_4$-PEG-(DA)-FA NCs after laser irradiation retain their $T_1$ contrast capability for $T_1$-weighted MR imaging of arthritis.

For $T_2$-weighted MR imaging, the arthritis region of both hind legs display similar MR signal intensity for control mouse before injection and at 30 min postinjection of the Fe$_3$O$_4$-PEG-(DA)-FA NPs before laser irradiation ($p > 0.05$, ns, Figure 5b,d), implying that the Fe$_3$O$_4$-PEG-(DA)-FA NPs have no $T_2$-weighted MR imaging effect. In contrast, the MR signal intensity and SNR value of arthritis region in left hind leg after laser irradiation significantly decrease ($p < 0.01$), which is opposed to the arthritis region in right hind leg without laser irradiation.
Figure 5. In vivo a) $T_1$-weighted, b) $T_2$-weighted MR imaging, and c,d) the corresponding MR SNR of arthritis model before (control) and after intravenous injection of Fe$_3$O$_4$-PEG-(DA)-FA NPs (before and after laser irradiation). The white and red arrows indicate the arthritis region without and with laser irradiation, respectively. e,f) Safranin O and Prussian blue-stained tissue section of inflammatory arthritis after different treatments. Inset in each panel of (e) shows the magnified region of arthritis. In (f), the red circle indicates the blue staining area.
(p > 0.05, ns). It is apparent that the aggregated Fe3O4-PEG-(DA)-FA NCs after laser irradiation can exert their T2 contrast effect in the arthritis. Overall, the aggregated Fe3O4-PEG-(DA)-FA NCs after laser irradiation for 12 min can be used as a precision diagnosis nanoprobe for targeted dual-mode T1/T2-weighted MR imaging of arthritis.

We have distinctly validated that the modification of FA renders the Fe3O4-PEG-(DA)-FA NPs with specific accumulation in vitro and prolonged residence time of arthritis region in vivo through MR imaging. Further, the enhanced retention and efficient accumulation of aggregated Fe3O4-PEG-(DA)-FA NCs after laser irradiation in the arthritis region in vivo were investigated by Prussian blue staining (Figure 5f). According to the results of Safranin O and hematoxylin and eosin (H&E) staining (Figure 5e and Figure S12, Supporting Information), the knee joint region of the mice with arthritis model shows some large area of inflammation (dashed red circles or ellipses) when compared to normal mice. For Prussian blue staining, the mice with arthritis model and normal mice in saline groups do not display any blue staining. The normal mice in NPs group and the mice with NPs injected to free FA-blocked arthritis model group display slight blue staining. In contrast, the arthritis model mice injected with NPs (NPs group) and the further laser irradiation to generate NCs (Laser-NCs group) exhibit much larger blue areas (red circles) than the above groups, and the blue staining areas of the inflammation region in NCs groups are much larger than those of NPs groups. This indicates that the NPs with ultrasmall size and FA-mediated targeting specificity could effectively penetrate into the inflammation region, and upon laser irradiation, the NCs are able to better retain in the inflammatory region. After feeding the mice for additional 2 h, the blue color of the stained inflammation region disappears for the NPs + 2 h group because of the fast metabolism of NPs in the mice, whereas the Laser-NCs + 2 h group still maintains a relatively large blue staining area. These results are in consistence with the results of T1/weighted and T1/T2-weighted MR imaging data, suggesting that laser irradiation greatly enhances the retention of NCs with a large size in the arthritis regions. Our study can readily prove our design concept: the small Fe3O4-PEG-(DA)-FA NPs can not only extravasate from the blood vessels to interstitial space of lesion region but also readily intravasate back into circulation system; upon laser irradiation to generate larger NCs, the NCs sufficiently retained in the lesion region hardly intravasate back to the blood vessels.\[8b,13a\] thereby enabled enhanced dual-mode T1/T2-weighted MR imaging. Overall, the efficient penetration and accumulation in the arthritis are attributed to the ultrasmall size and FA-mediated specific targeting of the Fe3O4-PEG-(DA)-FA NPs, and the aggregated Fe3O4-PEG-(DA)-FA NCs after laser irradiation display enhanced retention in arthritis due to the increased size, in agreement with our hypothesis (K0 >> K10) described in Figure 1.

In summary, we present a facile method to generate light-addressable Fe3O4-PEG-(DA)-FA NPs that can be used as a novel nanoplatform for dynamic and enhanced T1/T2-weighted MR imaging of inflammatory arthritis presumably due to the fact that the easily extravasated individual Fe3O4-PEG-(DA)-FA NPs are able to be located in the arthritis region, and form NCs upon laser irradiation that are difficult to intravasate back to the blood circulation. The designed light-addressable Fe3O4-PEG-(DA)-FA NPs may be used as a promising nanoplatform for dynamic and enhanced T1/T2-weighted dual-mode MR imaging of other diseases (e.g., cancer) with high precision.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

folic acid-mediated targeting, inflammatory arthritis, light-addressable nanoclusters, T1/T2-weighted MR imaging, ultrasmall Fe3O4 NPs

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