In vivo MRI detection of intraplaque macrophages with biocompatible silica-coated iron oxide nanoparticles in murine atherosclerosis

Chan Woo Kim1,2, Byung-Hee Hwang1,2,3, Hyeyoung Moon3, Jongeun Kang3,4, Eun-Hye Park1,2, Sang-Hyun Ihm1,5, Kiyuk Chang1,2 and Kwan Soo Hong3,4

Abstract
Identification of a vulnerable atherosclerotic plaque before rupture is an unmet clinical need. Integrating nanomedicine with multimodal imaging has the potential to precisely detect biological processes in atherosclerosis. We synthesized silica-coated iron oxide nanoparticles (SIONs) coated with rhodamine B isothiocyanate and polyethylene glycol and investigated their feasibility in the detection of macrophages in inflamed atherosclerotic plaques of apolipoprotein E-deficient (ApoE−/−) mice via magnetic resonance (MR) and fluorescence reflectance (FR) imaging. In vitro cellular uptake of SIONs was assessed in macrophages using confocal laser scanning microscopy (CLSM). In vivo MR imaging was performed 24 h after SION injection via the tail vein in 26-week-old ApoE−/− mice fed a high-cholesterol diet (HCD). We also performed FR imaging of the extracted aortas from four different mice: two normal-diet-fed C57BL/6 mice injected with saline or 10 mg/kg SIONs and two HCD-fed ApoE−/− mice injected with 5 or 10 mg/kg SIONs. The harvested aortas were cryosectioned and stained with immunohistochemical staining. The CLSM images at 24 h after incubation showed efficient uptake of SIONs by macrophages, with no evidence of cytotoxicity. The in vivo and ex vivo MR and FR images demonstrated SION deposition in the atheroma. Upon immunohistochemical staining of the aorta, CLSM images revealed colocalization of macrophages and SIONs in the atherosclerotic plaque. These results demonstrate that polyethylene glycosylated SIONs could be a highly effective method to identify macrophage activity in atherosclerotic plaques as a multimodal imaging agent.

Keywords
Nanoparticle, magnetic resonance imaging, macrophage, atherosclerosis

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Introduction

Magnetic nanoparticles (MNPs), which are one of the most widely used nanomaterials, possess great potential for application in clinical diagnostic and therapeutic techniques, which has led to their increasing use in the screening, diagnosis, and treatment of cancer, cardiovascular disease, and neurological disease.1–6 In particular, due to their intrinsic magnetic properties to enhance proton relaxation of specific tissues, the use of MNPs as magnetic resonance (MR) imaging contrast agents is one of the most promising applications of nanomedicine.

Dextran-coated, ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles (NPs) have been utilized to detect inflammatory plaques in preclinical models, including atheroprone rabbits, due to their excellent biocompatibility and ease of synthesis.7,8 As USPIOs circulate and transiently penetrate the vessel wall, plaque macrophages phagocytose these particles, which results in a strong signal loss on T₂-weighted MR imaging. Although USPIOs such as ferumoxtran (Sinerem®, Guerber, Paris, France) have shown promising clinical and preclinical utilities for detecting plaque macrophages, they have several intrinsic weaknesses as contrast agents in molecular imaging, such as a relatively lower relaxivity because of the small iron oxide core. This results in a larger administration dose and difficulty in linking specific ligands to the particles, which would enable the targeting of unique molecules and cells in high-risk plaques.

Silica provides a chemically and mechanically stable scaffold for the iron oxide core, superior biocompatibility with less in vivo toxicity, and an easily functionalized surface for simpler conjugation of various targeting ligands.9–11 In addition, diverse molecules, such as fluorescent dyes or targeting ligands, can simply be integrated into a silica shell.12,13 In this study, we synthesized iron oxide NPs of superparamagnetic particle size and coated the surfaces with organic dye-incorporated silica. This newly synthesized polyethylene glycolylated (PEGylated) silica-coated iron oxide nanoparticle (SION) would serve as a bimodal molecular imaging agent for MR and fluorescence reflectance (FR) imaging.

Since the application of SIONs in the molecular imaging of atherosclerosis is lacking, we investigated the feasibility of SIONs in preclinical macrophages within atherosclerotic plaques of apolipoprotein E (ApoE)−/− mice. To accomplish this, we first investigated the efficiency of SION uptake by macrophages and validated the in vivo ability to detect macrophage-rich plaques in ApoE−/− mice via MR and FR imaging.

Methods

Synthesis of fluorescent silica-coated iron oxide nanoparticles (SIONs)

PEGylated SIONs labeled with rhodamine B isothiocyanate (RITC, Sigma-Aldrich, St. Louis, MO, USA) were synthesized following our previous report.14 Briefly, ferri-rite (Sigma-Aldrich) was added into polyvinylpyrrolidone (PVP, Sigma-Aldrich) solution. The stabilized ferrite NPs were washed with 10% acetone, followed by centrifugation. Then, the supernatant was removed, and the NPs were resuspended in ethanol. Prior to silica coating, 3-amino-propyltriethoxysilane (APS, Gelest, Morrisville, PA, USA) and RITC were reacted under nitrogen, and the resultant solution was mixed with tetraethoxysilane (TEOS, Gelest). This solution was added to the purified NPs and polymerized by adding ammonia. Next, the resulting NPs were centrifuged and resuspended in basic ethanol containing ASP and polyethylene glycol (PEG) (molecular weight of 460–590 Da; Gelest Inc., Tullytown, PA, USA), followed by additional stirring to obtain SIONs.

Characterization of SIONs

The morphology and size of SIONs were confirmed by transmission electron microscopy (TEM, H-7600, Hitachi Ltd., Japan). Fe concentrations of SIONs were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Optima 4300 DV, Perkin-Elmer, Waltham, MA, USA), and T₂ relaxation measurements in aqueous solution containing SIONs were performed on a 4.7-T animal MRI system (Biospec 47/40, Bruker, Germany).

In vitro uptake of SIONs in murine macrophages and viability test

RAW 264.7 cells were maintained in culture dishes with Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% antibiotics. Cells were cultured in a humidified atmosphere of 5% CO₂ at 37°C. To examine the capacity of RAW 264.7 cells to phagocytose SIONs, cells were incubated with SIONs at concentrations lower than 100 µg Fe/ml for 24 h under standard culture conditions. The SION-loaded macrophages were washed three times with Dulbecco’s phosphate-buffered saline (DPBS), and nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). The cellular uptake of SIONs was observed by confocal laser scanning microscopy (CLSM; Zeiss LSM 510, Carl Zeiss, Germany). To assess the potential cytotoxic effect of SIONs, cell viability was assessed in RAW 264.7 cells using an MTT assay.

In vitro MR contrast measurements of RAW 264.7 cells labeled with SIONs

To elucidate the feasibility of SIONs as MR imaging agents, T₂*-weighted MR images were verified at different macrophage concentrations in agarose gel phantoms. RAW 264.7 cells were incubated with SIONs (50 µg Fe/ml) for 24 h, and then the cells were prepared at seven different concentrations (0.2, 0.5, 0.8, 1, 2, 8, and 10 × 10⁶
cells/ml) in 1% agarose phantoms. $T_2^*$-weighted MR images with a gradient-echo (FLASH) pulse sequence were measured with the following parameters: field of view (FOV) $= 3 \times 3$ cm$^2$, matrix $= 128 \times 128$, slice thickness $= 1$ mm, repetition time (TR) $= 130$ ms, number of slices $= 5$, and number of scans $= 4$.

**MR and FR imaging of the ApoE−/− mouse model of atherosclerosis**

All animal experiment procedures were provided in accordance with the Laboratory Animals Welfare Act, the Guide for the Care and Use of Laboratory Animals and the Guidelines and Policies for Rodent Experiments provided by the Institutional Animal Care and Use Committee (IACUC) in the School of Medicine of The Catholic University of Korea. ApoE−/− mice were fed a 1.2% cholesterol diet from 8 to 26 weeks of age. SIONs (10 mg Fe/kg) were administered via tail vein injection in 26-week-old mice. In vivo $T_2^*$-weighted MR imaging was conducted before and 24 h after SION injection. For ex vivo FR imaging, ApoE−/− mice were sacrificed, and their aortas were extracted and fixed in 4% paraformaldehyde solution. C57BL/6 mice fed a normal diet were also injected with SIONs via the tail vein, and their aortas were extracted in a manner similar to that in ApoE−/− mice. We imaged the extracted aortas of C57BL/6 mice with saline injection, C57BL/6 mice with 10 mg/kg SION injection, and two ApoE−/− mice with 5 and 10 mg/kg SION injection using a fluorescence imaging system (Maestro™; CRi, MA, USA).

**Histological evaluation using fluorescence microscopy in the aorta**

To evaluate the distribution of accumulated SIONs within the aorta after undergoing in vivo MR imaging, the aortas were extracted and mounted in optimal cutting temperature (OCT) compound (Sakura Finetek, Torrance, CA, USA), which were then frozen in liquid nitrogen. Each frozen aorta was cut into a 5 μm thickness using a cryostat (Microm HM 525, Thermo Scientific, MA, USA), and immunohistochemical staining for macrophages was performed. The primary antibody was a mouse monoclonal antibody against CD68 (ED-1, Abcam Inc., MA, USA), and the secondary antibody was a goat anti-rat Alexa Fluor 488-conjugated antibody (Abcam Inc., MA, USA). The aortic sections were stained with DAPI (blue color; Sigma-Aldrich Co., MO, USA) to identify cell nuclei and a mouse monoclonal antibody targeting CD68 (green color) to identify atherosclerosis-associated macrophages. These stained aortic sections were imaged by CLSM.

**Results**

**Synthesis and characterization of SIONs**

Monodispersed SIONs incorporating RITC were synthesized via a modified procedure of our previous work, in which this NP coating process with silica and PEG was conducted to provide highly biocompatible properties of the SIONs, resulting in a long blood half-life of approximately 3 h. According to this procedure, approximately 10 nm diameter core iron oxide was first prepared, and sequential modification was performed by a RITC-incorporated silica and PEG coating. The final SIONs were obtained with an average size of 90 ± 7 nm (Figure 1(a)). MR $T_2$ contrast behavior of SIONs was examined using a 4.7-T MRI system, and then the $T_2$ relaxivity ($r_2$) of SIONs revealed approximately 150 s$^{-1}$mM$^{-1}$, indicating a linear correlation with SION concentrations (Figure 1(b)).

![Figure 1. Characterization of SIONs. Representative TEM images exhibited well-dispersed SIONs with an average size of 90 ± 7 nm (scale bar: 100 nm) (a). The relaxation rate $R_2 (=1/T_2)$ was linearly dependent on the SION concentration (b). The $T_2$ relaxivity ($r_2$) calculated from the fitted line was 152.2 ± 6.7 s$^{-1}$mM$^{-1}$.](image)
Cellular uptake and in vitro magnetic properties of SIONs in murine macrophages

RITC-containing SIONs were effectively taken up by macrophages following 24 h of incubation, and there was a concentration-dependent increase in the intracellular accumulation of SIONs (Figure 2(a)). After macrophages were incubated with 50 µg Fe/ml of SIONs for 24 h, nearly all cells were loaded with the SIONs. The intracellular Fe content in the SION-treated macrophages at 50 µg Fe/ml was 28 ± 6 pg Fe/cell, which was comparable to the content reported by others with a similar cell type and particle size. No sign of cytotoxicity was observed up to 100 µg Fe/ml of SIONs within the following 24 h of incubation (Figure 2(b)).

The T2*-weighted MR images of the agarose phantom were measured by treating 100 µg Fe/ml of SIONs at different concentrations of macrophages. Representative MR images of SION-loaded macrophages with cell densities between 2 × 10^5 and 1 × 10^7 cells/ml are shown in Figure 2(c), in which signal intensity (SI) attenuation was observed depending on the cell density. This SI loss in the T2*-weighted MR images of the in vitro phantom demonstrated that SIONs could be used for in vivo imaging of monocyte/macrophage infiltrates in inflammatory sites.

In vivo MR images of the ApoE−/− mouse model

In vivo T2*-weighted MR images were taken before and after SION (10 mg/kg) injections in the ApoE−/− mouse model. In the MR images taken before SION injection (Figure 3(a)), the ascending and descending aortas showed no signal changes in their respective aortic walls. By comparison, the MR images taken after SION injection demonstrated strong focal signal loss (arrowheads) in the aortic wall (Figure 3(b)). This signal loss was a result of SION accumulation mainly in macrophages infiltrating the atherosclerotic plaque, indicating that in vivo MR imaging following biocompatible SION injection provided information on SION-deposited macrophage infiltration in the atherosclerotic plaque.

Ex vivo FR images of the aortas

To clarify SION deposition in the atherosclerotic plaque, ex vivo FR images were obtained from the extracted aortas after MR imaging (Figure 4). C57BL/6 mice injected with saline showed no fluorescence signal, whereas C57BL/6 mice injected with 10 mg/kg SIONs exhibited minimally visible blue-colored fluorescence. ApoE−/− mice treated with 5 mg/kg SIONs presented signal enhancement at the
aortic root; however, a stronger red fluorescence signal was noted in ApoE<sup>−/−</sup> mice injected with 10 mg/kg SIONs. These FR images confirmed the results seen in the in vivo MR images.

**Colocalization of SIONs and macrophages within atherosclerotic plaques**

Atherosclerotic lesions in the aorta were assessed using DAPI (blue color) staining to identify nuclei and mouse monoclonal antibody targeting CD68 (green color) staining to identify macrophages. Based on fluorescence images of the aortic root section, macrophage areas in aortic root plaques (indicated by green fluorescence) overlapped with SION-derived fluorescence signals (red fluorescence). In particular, CD68<sup>+</sup> intimal macrophages within plaque lesions were clearly colocalized with SIONs, presenting yellow signals.

**Discussion**

High-risk atherosclerotic plaques are characterized by their specific cellular and biological compositions. In this setting, macrophages are mainly involved in plaque disruption and thrombus formation, which are responsible for both fatal and nonfatal cardiovascular disease. Approaches to imaging macrophages have been extensively investigated to identify vulnerable atherosclerotic plaques in vivo. In this study, we investigated the cellular targeting and multimodal imaging capabilities of magnetic NPs in an experimental mouse atherosclerosis model and observed clear cellular uptake of SIONs in macrophages in vitro and in vivo. The SIONs allowed imaging of cellular inflammation in a mouse model of atherosclerosis via in vivo MR imaging and ex vivo multimodal imaging systems. The SIONs allowed for the successful imaging of macrophages embedded in atherosclerotic plaques.
Traditional cardiovascular imaging has focused on anatomy, but current molecular imaging is being expanded to interrogate pathological perspectives of initiation and progression of atherosclerotic plaques. These approaches are based on identifying stage-specific molecular markers or inflammatory cells, which can be detected by various contrast agents. USPIO particles, which are representatively used as $T_2$ MR contrast agents, are taken up by macrophages and can induce a significant decrease in in vivo MR imaging signals in inflammatory lesions of atherosclerotic plaques, as has been reported in human studies. A clear correlation of MR imaging signals with macrophage densities was introduced in a murine atherosclerosis model by using Gd$^{3+}$-loaded micelles as a contrast agent. Additionally, increased $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) uptake in human atherosclerotic plaques was detected by positron emission tomography depending on the number of macrophages. Imaging atherosclerosis with SPIO particles, however, is less understood than the other contrast agents mentioned above. We demonstrated the possibilities for this type of particle, such as novel atherosclerosis-targeted imaging NPs and a noninvasive imaging system to assess inflammation in atherosclerosis.

SIONs utilize two mechanisms to improve their use in biomedical applications: a core-shell structure and polymeric coating. The size, shape, and surface modification of nanoparticles can be adjusted by physical and chemical modifications to not only allow for an increase in magnetic properties but also improve its impact on the in vivo behavior of NPs. In its simplest form, magnetic NPs consisting of an inorganic core and biocompatible outer coating layer stabilize the particle under a physiological environment. Numerous strategies have been attempted to enhance coat NPs, forming “core-shell structures”. Some NPs, for example, gold-coated NPs, have proven useful in molecular imaging but have limitations with respect to long-term biocompatibility. Silica coating of NPs theoretically allows for better biocompatibility and prevents the potential degradation of the inner core and encapsulated molecules, including alternative diagnostic agents or drugs. In addition, the polymeric coating is largely used to (i) prevent magnetic NPs from aggregating under physiological conditions; (ii) act as a barrier to protect the magnetic core from the aqueous solvent; and (iii) allow for sites for chemical modification with targeting agents, such as peptides or antibodies. As a representative example, PEG has been widely studied and proposed as an efficient strategy for an NP coating for biomedical applications.

SIONs, a new contrast particle of SPIOs with some of the specialized structures mentioned above, extended the use of MR imaging to cardiovascular diseases and has several advantages over currently available macrophage-targeting USPIOs detecting atherosclerosis. SPIOs approximately 150 nm in diameter are more susceptible to phagocytosis than USPIOs approximately 30 nm in diameter, and earlier reports confirmed that phagocytic uptake of iron oxide increased with increasing particle size. This conception was applied to the currently available SPIO and USPIO, as Metz et al. and Lunov et al. showed that in vitro loading of human monocytes for MR imaging is most effectively obtained with SPIO compared to USPIO. SIONs have an average diameter of 90 nm, which is larger than that of USPIO; therefore, we can assume that SIONs are more efficiently phagocytosed by macrophages based on their size.

SIONs have a higher value of $T_2$ relaxivity than do USPIO-based particles. Ferumoxides, a commercially available SPIO, have $160 \text{s}^{-1}\text{mM}^{-1}$ $T_2$ relaxivity at 0.47 T. The $T_2$ relaxivity of ferumoxtran, an actively investigated USPIO, is $80 \text{s}^{-1}\text{mM}^{-1}$ at 37°C and 0.47 T. Because of its lower $T_2$ relaxivity value, more ferumoxtran is needed to produce the same signal intensity in the $T_2$ MR image. Given the same amount of iron oxide NPs, ferumoxides elicit a higher signal intensity than ferumoxtran. The $T_2$ relaxivity of the SIONs in our current study is $150 \text{s}^{-1}\text{mM}^{-1}$, which is approximately twice that of currently available USPIO particles. This implies that 50% fewer SIONs can create the same signal intensity as that by USPIO particles.

Coating the SIONs with PEG gives these particles several benefits, as mentioned above. PEG is nontoxic, nonimmunogenic and resistant to protein aggregation of magnetic NPs and conveys an extended circulation time. Magnetic NPs coated with charged polymers are prone to opsonization due to strong electrostatic interactions between their surface and plasma proteins. By contrast, neutral polymers contain an abundant number of neutral and hydrophilic groups that offer excellent resistance against opsonization. SPIO was originally investigated as a contrast agent for liver and spleen imaging because of its selective uptake by Kupffer cells and reticuloendothelial systems. On the other hand, USPIO is small enough to evade the reticuloendothelial systems. The half-life of ferumoxide in rats, which is 0.1 h, is shorter than that of ferumoxtran, 1.4–3 h. Coating the SIONs with PEG rendered them able to evade uptake by the reticuloendothelial systems, resulting in a longer half-life in the blood of rats (approximately 2 h). Therefore, SIONs are more likely to be phagocytosed by macrophages in the blood and other inflamed tissues, such as atherosclerotic lesions.

Coating NPs with silica and PEG provides multifunctional capacities. Ma et al. described core-shell magnetic NPs composed of iron oxide cores approximately 10 nm in diameter surrounded by a SiO$_2$ shell 10–15 nm thick. In this study, an organic dye, tris (2,2’-bipyridine) ruthenium, was incorporated with a second silica shell to provide a luminescence signal and prevent quenching by interacting with the magnetic core. In recent years, a wide variety of in vitro and in vivo applications have been demonstrated, suggesting that silica-coated NPs allow peripheral labeling
of cancer cells and mesenchymal stem cells. In addition, antibody-conjugated silica-coated NPs allow for multitarget monitoring of bacterial species. Surface modification of magnetic NPs with PEG and folic acid has been used to facilitate their uptake to specific cancer cells for diagnosis and treatment purposes.

Our main study limitation is that the colocalization of SION and macrophages bound by CD68 antibodies in the CLSM images does not necessarily mean that the same macrophages were directly inside pre-established atherosclerotic lesions. We cannot be sure that SIONs truly accumulated at the site of atherosclerotic lesions based on the CLSM images. In addition, the results obtained in the murine atherosclerosis model may not fully reflect the pathogenesis in the human atherogenesis, thereby limiting direct translation into clinical setting. Accordingly, many aspects of clinical applications concerning e.g. clinical dose, iron oxide content, and signal properties should be further refined and examined in order to extend this study toward clinical trials, including multiple comparisons with currently used imaging agents.

Conclusion

Our findings indicate that silica-coated magnetic fluorescent NPs are a superior contrast agent owing to their unique silica shells and polymeric coatings. Our study may provide the foundation for the noninvasive assessment of cellular components of vulnerable plaques in concert with structural atherosclerosis in MR imaging. Assessment of atherogenesis in the early stage of development using this noninvasive imaging modality would allow for early treatment before the disease processes. Further studies are required to monitor the safety and application of SIONs in atherosclerosis in humans.

Declaration of conflicting interests

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ORCID iDs

Chan Woo Kim https://orcid.org/0000-0001-8430-3903
Byung-Hee Hwang https://orcid.org/0000-0001-7770-0791

References

1. Corot C, Petry KG, Trivedi R, et al. Macrophage imaging in central nervous system and in carotid atherosclerotic plaque using ultrasmall superparamagnetic iron oxide in magnetic resonance imaging. Invest Radiol 2004; 39: 619–625.
2. Ferrari M. Cancer nanotechnology: opportunities and challenges. Nat Rev Cancer 2005; 5: 161–171.
3. Wickline SA, Neubauer AM, Winter PM, Caruthers SD and Lanza GM. Molecular imaging and therapy of atherosclerosis with targeted nanoparticles. J Magn Reson Imaging 2007; 25: 667–680.
4. Cardoso VF, Francesco A, Ribeiro C, Bañobre-López M, Martins P and Lancereros-Mendez S. Advances in magnetic nanoparticles for biomedical applications. Adv Healthc Mater 2018; 7: 1700845.
5. Ansari SAMK, Ficiara E, Ruffinatti FA, et al. Magnetic iron oxide nanoparticles: synthesis, characterization and functionalization for biomedical applications in the central nervous system. Materials 2019; 12: 465.
6. Dadfar SM, Roemhild K, Drude NI, et al. Iron oxide nanoparticles: diagnostic, therapeutic and theranostic applications. Adv Drug Deliv Rev 2019; 138: 302–325.
7. Reehm SG, Corot C, Vogt P, Kolb S and Debatin JF. Magnetic resonance imaging of atherosclerotic plaque with ultrasmall superparamagnetic particles of iron oxide in hyperlipidemic rabbits. Circulation 2001; 103: 415–422.
8. Morishige K, Kacher DF, Libby P, et al. High-resolution magnetic resonance imaging enhanced with superparamagnetic nanoparticles measures macrophage burden in atherosclerosis. Circulation 2010; 122: 1707–1715.
9. Vivero-Escoto JL, Huxford-Phillips RC and Lin WB. Silica-based nanoprobes for biomedical imaging and theranostic applications. Chem Soc Rev 2012; 41: 2673–2685.
10. Zhang Y, Hsu BYW, Ren CL, Li X and Wang J. Silica-based nanocapsules: synthesis, structure control and biomedical applications. Chem Soc Rev 2015; 44: 315–335.
11. Fathy MM, Fahmy HM, Saad OA and Elshemey WM. Silica-coated iron oxide nanoparticles as a novel nanoradiosensitizer for electron therapy. Life Sci 2019; 234: 116756.
12. Saint-Cricq P, Deshayes S, Zink JI and Kasko AM. Magnetic field activated drug delivery using thermodegradable azo-functionalised PEG-coated core-shell mesoporous silica nanoparticles. Nanoscale 2015; 7: 13168–13172.
13. Li J, Liu K, Chen HY, et al. Functional built-in template directed siliceous fluorescent supramolecular vesicles as diagnostics. ACS Appl Mater Interfaces 2017; 9: 21706–21714.
14. Lee K, Park C, Moon HY, et al. Magnetic resonance tracking of multifunctional nanoparticle-labeled mouse mesenchymal stem cells in a mouse model of myocardial infarction. Curr Appl Phys 2009; 9: S12–S14.
15. Moon H, Park HE, Kang J, et al. Noninvasive assessment of myocardial inflammation by cardiovascular magnetic resonance in a rat model of experimental autoimmune myocarditis. Circulation 2012; 125: 2603–2612.
16. Trivedi RA, Mallavarachi C, U-King-Im JM, et al. Identifying inflamed carotid plaques using in vivo USPIO-enhanced MR imaging to label plaque macrophages. Arterioscler Thromb Vasc Biol 2006; 26: 1601–1606.
17. Amirbekian V, Lipinski MJ, Briley-Saebo KC, et al. Detecting and assessing macrophages in vivo to evaluate atherosclerosis noninvasively using molecular MRI. Proc Natl Acad Sci U S A 2007; 104: 961–966.

18. Tawakol A, Migrino RQ, Bashian GG, et al. In vivo 18F-fluorodeoxyglucose positron emission tomography imaging provides a noninvasive measure of carotid plaque inflammation in patients. J Am Coll Cardiol 2006; 48: 1818–1824.

19. Gupta AK and Gupta M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials 2005; 26: 3995–4021.

20. Aisida SO, Akpa PA, Ahmad I, Zhao T-K, Maaza M and Ezema Fl. Bio-inspired encapsulation and functionalization of iron oxide nanoparticles for biomedical applications. Eur Polym J 2020; 122: 109371.

21. Zhi DF, Yang T, Yang J, Fu S and Zhang S. Targeting strategies for superparamagnetic iron oxide nanoparticles in cancer therapy. Acta Biomater 2020; 102: 13–34.

22. Fang C and Zhang M. Multifunctional magnetic nanoparticles for medical imaging applications. J Mater Chem 2009; 19: 6258–6266.

23. Peng E, Wang FH and Xue JM. Nanostructured magnetic nanocomposites as MRI contrast agents. J Mater Chem B 2015; 3: 2241–2276.

24. Shi YM, Gao YP, Zou X, Chen L and Li Y. Imaging of carotid artery inflammatory plaques with superparamagnetic nanoparticles and an external magnet collar. J Mater Chem B 2017; 5: 797–806.

25. Daldrup-Link HE, Rudelius M, Oostendorp RA, et al. Targeting of hematopoietic progenitor cells with MR contrast agents. Radiology 2003; 228: 760–767.

26. Matuszewski L, Persigehl T, Wall A, et al. Cell tagging with clinically approved iron oxides: feasibility and efficiency of lipofection, particle size, and surface coating on labeling efficiency. Radiology 2005; 235: 155–161.

27. Metz S, Bonaterra G, Rudelius M, Settles M, Rummeny EJ and Daldrup-Link HE. Capacity of human monocytes to phagocyte approved iron oxide MR contrast agents in vitro. Eur Radiol 2004; 14: 1851–1858.

28. Lunov O, Zablotskii V, Syrovets T, et al. Modeling receptor-mediated endocytosis of polymer-functionalized iron oxide nanoparticles by human macrophages. Biomaterials 2011; 32: 547–555.

29. Kohler N, Sun C, Fichtenholtz A, Gunn J, Fang C and Zhang M. Methotrexate-immobilized poly(ethylene glycol) magnetic nanoparticles for MR imaging and drug delivery. Small 2006; 2: 785–792.

30. Ma DL, Guan JW, Normandin F, et al. Multifunctional nano-architecture for biomedical applications. Chem Mater 2006; 18: 1920–1927.

31. O’Connell CL, Nooney R and McDonagh C. Cyanine5-doped silica nanoparticles as ultra-bright immunospecific labels for model circulating tumour cells in flow cytometry and microscopy. Biosens Bioelectron 2017; 91: 190–198.

32. Keteb AA, Shin TH, Jun M, Lee G and Park S. Effect of silica-coated magnetic nanoparticles on rigidity sensing of human embryonic kidney cells. J Nanobiotechnol 2020; 18: 170.

33. Sung CK, Hong KA, Lin S, et al. Dual-modal nanoparticles for imaging of mesenchymal stem cell transplant by MRI and fluorescence imaging. Korean J Radiol 2009; 10: 613–622.

34. Jang Y, M, Yoon YI, Kwon YS, et al. Trastuzumab-conjugated liposome-coated fluorescent magnetic nanoparticles to target breast cancer. Korean J Radiol 2014; 15: 411-422.

35. Wang L, Zhao W, O’Donoghue MB and Tan W. Fluorescent nanoparticles for multiplexed bacteria monitoring. Bioconjug Chem 2007; 18: 297–301.

36. Wen CY, Jiang YZ, Li XY, et al. Efficient enrichment and analyses of bacteria at ultralow concentration with quick-response magnetic nanospheres. ACS Appl Mater Interfaces 2017; 9: 9416–9425.

37. Zhang Y, Kohler N and Zhang MQ. Surface modification of superparamagnetic magnetite nanoparticles and their intracellular uptake. Biomaterials 2002; 23: 1553–1561.

38. Yin T, Huang P, Gao G, et al. Superparamagnetic Fe3O4-PEG2K-FA@Ce6 nanoparticles for in vivo dual-mode imaging and targeted photodynamic therapy. Sci Rep 2016; 6: 36187.

39. Ai PH, Wang H, Liu K, et al. The relative length of dual-target conjugated on iron oxide nanoparticles plays a role in brain glioma targeting. RSC Adv 2017; 7: 19954–19959.