Construction of dual nanomedicines for the imaging and alleviation of atherosclerosis

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ABSTRACT

Magnetic resonance imaging (MRI) is an essential tool for the diagnosis of atherosclerosis, a chronic cardiovascular disease. MRI primarily uses superparamagnetic iron oxide (SPIO) as a contrast agent. However, SPIO integrated with therapeutic drugs has rarely been studied. In this study, we explored biocompatible paramagnetic iron-oxide nanoparticles (NPs) in a complex with low pH-sensitive cyclodextrin for the diagnostic imaging and treatment of atherosclerosis. The NPs were conjugated with profilin-1 antibody (PFN1) to specifically target vascular smooth muscle cells (VSMCs) in the atherosclerotic plaque and integrated with the anti-inflammatory drug, rapamycin. The PFN1-CD-MNPs were easily bound to the VSMCs, indicating their good biocompatibility and low renal toxicity over the long term. \textit{Ex vivo} near-infrared fluorescence (NIRF) imaging and \textit{in vivo} MRI indicated the accumulation of PFN1-CD-MNPs in the atherosclerotic plaque. The RAP@PFN1-CD-MNPs alleviated the progression of arteriosclerosis. Thus, PFN1-CD-MNPs served not only as multifunctional imaging probes but also as nanovehicles for the treatment of atherosclerosis.

INTRODUCTION

Atherosclerosis is a chronic progressive inflammatory disease [1–4]. Atherosclerotic lesions are characterized by the accumulation of vascular smooth muscle cells (VSMCs) and extracellular matrix proteins, especially proteoglycan, collagen and elastin. The proliferation and migration of VSMCs in the plaque area constitute fibrous caps, which are highly related to plaque stability [5]. Profilin-1 is abundant in the VSMCs of atherosclerosis and participates in the recombination of cytoskeleton polymerization [6,7]. Recent studies have indicated that profilin-1 overexpression triggers cardiovascular diseases, such as atherosclerosis, by regulating the migration and proliferation of VSMCs [8]. Hence, profilin-1 is considered a molecular marker of atherosclerosis, as it reflects the pathological conditions of VSMCs.

Molecular imaging technology enables the investigation of lesions at the cellular and molecular levels and accurate diagnosis of the atherosclerotic plaque. In the past few decades, magnetic iron-oxide nanoparticles (NPs) for magnetic resonance imaging (MRI), such as magnetite (Fe\textsubscript{3}O\textsubscript{4}), have attracted considerable attention, owing to their high biocompatibility [9–12]. The targeted therapies explore functional NPs by attaching antibodies, peptides or other ligands to their surface [13,14]. Therefore, the molecular pathways of NPs targeted to atherosclerotic plaque can be monitored. However, NPs with both imaging and therapeutic abilities (theranostics) have rarely been reported for atherosclerosis [15,16]. Winter et al. developed an antiangiogenic atherosclerosis theranostic strategy by incorporating fumagillin into the surface layer of integrin \(\alpha_v\beta_3\)-targeted NPs, thus forming a platform for molecular MRI of plaque angiogenesis [17]. The usage of dual diagnostic and therapeutic tools in one NP formulation allows synchronized and site-specific diagnosis and therapy of the disease [1,18].

Currently, several clinic drugs are available that slow the progression of atherosclerosis by lowering plasma lipid levels [19,20]. However, the equally important contribution of inflammation to the development of cardiovascular disease is overlooked by current therapies [21]. For example, rapamycin is a promising and effective drug for the atherosclerotic plaque with anti-inflammatory properties [22]. However, most drugs including anti-inflammatory medicines are orally administered and exhibit poor bioavailability and high toxicity [23–25]. Hence, rapamycin can serve as a model drug for targeting delivery without the severe side effects [26]. In addition, inflammatory tissue has an acidic...
microenvironment, nanomedicine that releases drug molecules in response to low pH indicates great potential for high efficacy [27]. Recently, cyclodextrin has been investigated as a reliable nanomaterial and can be modified for pH-sensitive nanocarriers [26,28,29]. Cyclodextrin has been widely used as an excipient to improve the stability, solubility and bioavailability of hydrophobic drugs [30,31]. Most importantly, the byproducts of cyclodextrin are neutral and induce negligible inflammation on plaque [26].

In this study, we aimed to construct a dual diagnostic and therapeutic probe for imaging the atherosclerotic plaque of ApoE/−/− mice using MRI and near-infrared fluorescence (NIRF) imaging to dynamically monitor plaque changes and evaluate treatment efficacy. We obtained composite NPs comprising non-proinflammatory cyclodextrin, the profilin-1 antibody and rapamycin. These composite NPs exhibited a promising therapeutic potential for the treatment of atherosclerosis.

**Materials and methods**

**Ethical approval**

All animal studies were approved by the Animal Care and Use Committee of South China Normal University, Guangzhou, China, and were in accordance with the revised Animals (Scientific Procedures) Act, 1986 and the Directive 2010/63/EU.

**Fabrication of RAP@Fe₃O₄-PDA-PEG-PEI-profilin-1 (RAP@PFN1-CD-MNPs) targeted drug delivery system**

The steps for the synthesis of PFN1-CD-MNPs NPs are described in the Supplementary materials. To prepare RAP-loaded NPs, rapamycin was dissolved in DMSO to a final concentration of 10 mg/mL. Then, 10 mL of the PFN1-CD-MNP dispersion (1 mg/mL) was ultrasonicated for 30 min, followed by the addition of 0.1 mL of RAP solution. The mixture was then stirred at 4 °C for 12 h, centrifuged at 12,875 × g for 30 min, and then washed with deionized water several times. Finally, 1 mg/mL of RAP-loaded NPs (RAP@PFN1-CD-MNPs) were dissolved in deionized water, and samples were stored at −20 °C for further use. The conception and synthesis of the composite probe are illustrated in Figure 1.

**Characterization of NPs**

The morphology of NPs was examined using high-resolution transmission electron microscopy (TEM; Hitachi HT7700, Tokyo, Japan) at 80 kV. Particle distribution and zeta potential were measured by dynamic light scattering (DLS) using Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). The magnetic properties of samples were characterized on a vibrating sample magnetometer (VSM, Lakeshore, Westerville, OH). UV–vis absorption spectroscopy (Shimadzu, Kyoto, Japan) was used to confirm the conjugation of the profilin-1 antibody with Fe₃O₄-PDA-CD-PEG-PEI NPs. XRD and FTIR were also used to characterize the NPs.

**In vitro MR imaging of nanocarriers**

The PFN1-CD-MNPs were diluted in 500 μL of a 2% high-grade agarose gel at various concentrations (0, 2.5, 5, 10, 12.5, 25 and 50 μg/mL). The T2 mapping-TSE scans were acquired using a knee coil on a 3.0T MRI system (PHILIPS, Best, the Netherlands). The parameters of imaging were as follows: matrix = 332 × 271, field of view (FOV) = 140 mm, slice thickness = 4.5 mm, slice gap = 0.6 mm, repetition time (TR) = 2000 ms and multiple echo times (TE) = n × 13 ms. The relaxivity at various iron concentrations was determined using a linear fit.

**Quantification of RAP in RAP@PFN1-CD-MNPs**

To quantify drug content, 10.0 mg freeze-dried RAP-loaded NPs were thoroughly dissolved in DMSO by ultrasonication for 30 min. The RAP concentration was determined by UV absorption at 278 nm. The drug-loading content and entrapment efficiency were calculated according to the following equations:

Drug-loading content (%) = \( \frac{\text{weight of RAP in NPs}}{\text{weight of RAP–loaded NPs}} \times 100 \)

and

Entrapment efficiency (%) = \( \frac{\text{RAP content in NPs}}{\text{theoretical RAP content}} \times 100 \)

**Figure 1.** Schematic diagram of RAP@Fe₃O₄-PDA-CD-PEG-PEI-Profilin1-Cy5.5 nanoparticles.
In vitro release study

In vitro release of RAP-loaded MNPs was tested under various pH conditions. Briefly, 10 mg RAP@PFN1-CD-MNPs was dispersed in 10 mL PBS (pH 5.5 or 7.4) and incubated at 37 °C. At predetermined time points, the samples were centrifuged, and 10 mL of the supernatant of each sample was freeze-dried. Subsequently, 1 mL of DMSO was added to each supernatant, and the absorbance was measured at 278 nm using an ultraviolet spectrophotometer.

Smooth muscle cell culture

MOVAS cells were grown in Dulbecco’s modified Eagle medium (DMEM) containing 10% foetal bovine serum and 1% penicillin–streptomycin at 37 °C in a humidified incubator under 5% CO2 and counted with a haemocytometer.

Cytotoxicity assay of NPs

To assess the cytotoxicity of NPs, the cell counting kit-8 (CCK-8, Beijing, China) was used to count viable MOVAS cells after treatment with NPs. A fresh medium without cells served as a blank control.

Haemolytic assay of PFN1-CD-MNPs

Haemolytic properties are important for in vivo applications of NPs, especially if the NPs interact with blood. Haemolytic effect of the nanocarrier was measured by the absorbance of the supernatant at 542 nm after exposing the NPs to mouse red blood cell (MRBC) suspension for 6 h, followed by centrifugation. The absorbance at 542 nm represents the haemoglobin released from the ruptured MRBCs.

In vitro Prussian blue staining and laser confocal imaging

To detect the cellular uptake of PFN1-CD-MNPs, MOVAS cells were incubated with NPs and stained with a Prussian blue stain to observe the cells fixed in 4% paraformaldehyde. Cells were also stained with DAPI, and images of intracellular targeting were captured using a laser scanning confocal microscope (LSCM; Carl Zeiss, Jena, Germany) after treatment with PFN1-CD-MNPs-Cy5.5.

Animals

Twelve-week-old male ApoE−/− mice were fed with a high-fat diet containing 1% cholesterol for 16 weeks to establish atherosclerosis model.

C57BL/6 male mice (Guangzhou, China) were used as a normal group to detect the potential toxicity of NPs.

Western blot analysis

The level of profilin-1 protein in the MOVAS cell and tissues harvested from the aorta of mice was determined by western blot analysis, as described previously [6].

In vivo MRI with PFN1-CD-MNPs

The carotid arteries of ApoE−/− mice were imaged in a 7.0 T small animal MRI system (PharmaScan70/16, Bruker, Ettlingen, Germany) with body coil. T2-weighted images (T2WIs) and T2 mapping were captured at 0 and 24 h post-administration of PFN1-CD-MNPs with 2 mg/kg Fe. T2WIs were captured using the following parameters: repetition time (TR)/echo time (TE) = 2500/35 ms, FOV = 23 mm × 23 mm, matrix = 256 × 256 and slice thickness = 0.7 mm. T2 mapping employed the following parameters: TR/TE = 2200/9.5 ms, FOV = 23 mm × 23 mm, matrix = 256 × 256 and slice thickness = 0.8 mm.

Ex vivo fluorescence imaging with PFN1-CD-MNPs-Cy5.5

After MRI, animals were euthanized. The aorta of mice was carefully dissected and thoroughly cleaned. Additionally, major organs including the heart, the liver, the spleen, the lung and the kidney were excised carefully. All organs were subjected to NIRF at a 700 nm excitation wavelength (Odyssey system, Omaha, NE).

In vivo assessment of the therapy effect

After a week of acclimation in the laboratory, 30 ApoE−/− mice were fed with a high-fat diet. Two months later, the mice were randomized into three groups and given two additional months of different treatments; one group received normal saline treatment (control), while the other two groups received intraperitoneal injection of nanomedicine (3.0 mg/kg) every three days. Intraperitoneal injections are advised, as the tail vein is prone to occlusion during long-term injection. At the end of the treatment, the whole aortic plaque was assessed by gross Oil red O (ORO) staining.

Blood biochemistry analysis

The blood samples were centrifuged after coagulation. The serum was quantitated for the following molecules: triglycerides (TGs), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (Cr) and blood urea nitrogen (BUN). This analysis was performed by the blood test centre of the First Affiliated Hospital of Jinan University.

Morphological histology and immunohistochemical analysis

Pathological section examination included haematoxylin and eosin (H&E) staining, Prussian blue staining, Masson’s trichrome and immunohistochemistry. Immunohistological
examination indicators included α-smooth muscle actin (α-SMA; quantification of smooth muscle cells), as described previously [26]. Additionally, the main organs including heart, liver, spleen, lung and kidney were harvested for pathological examination.

**Statistical analysis**

All experiments were carried out at least in triplicate. Statistical analysis was conducted using the GraphPad Prism 5 software (GraphPad Software, San Diego, CA). Data were presented as mean ± standard deviation (SD). Differences between the control and test groups were analysed using the two-tailed Student’s t-test. One-way analysis of variance test was used for experiments comprising multiple groups. Significant differences were indicated at \( p < 0.05 \) (*), \( p < 0.01 \) (**), or \( p < 0.001 \) (***)

**Results and discussion**

**Characterization of the basal Fe\(_3\)O\(_4\) NPs**

The Fe\(_3\)O\(_4\) NPs were the basal material of the targeted molecular imaging probe. The data in the Figure S1 suggest that Fe\(_3\)O\(_4\) NPs could be used as superparamagnetic iron oxide (SPIO).

**Characterization of PFN1-CD-MNPs**

Although the Fe\(_3\)O\(_4\) NPs are generally well tolerated in vivo, proper surface modification, particle size and core ligand composition play an important role in physiological reactions [9,10]. To improve the colloid stability in physiological conditions, the lifetime in blood circulation should be increased, and toxicity in vivo should be reduced; magnetite NPs are often coated or modified with biocompatible polymer molecules such as dextran and polyvinyl alcohol [9,11,12]. In this study, we used PEG-PEI to modify SPIO NPs, as it provides good solubility in aqueous solvents and prolongs circulation time in the bloodstream [20,32]. The TEM image indicated that profilin-1-labeled NPs displayed mono-dispersion, uniform size (average size = 15–20 nm in the dry state) and spherical morphology (Figure 2(a)). The average hydrodynamic size of nanocomposites was 15–30 nm, with a PDI of 0.3207 ± 0.01936 (Figure 2(b)). The hysteresis curves of Fe\(_3\)O\(_4\) NPs (Figure 2(c)) indicated an \( M_s \) value of 69.85 emu g\(^{-1}\), which was smaller than that of the unmodified Fe\(_3\)O\(_4\) NPs, despite meeting the imaging requirement of >60 emu g\(^{-1}\), and larger than that of PFN1 magnetic NPs reported previously [6]. The XRD pattern indicated that the peak intensity of PFN1-CD-MNPs was weaker than that of unmodified Fe\(_3\)O\(_4\) (Figure S2). FTIR confirmed the successful synthesis of PFN1-CD-MNP nanocomposites (Figure 2(d)). UV–vis absorption spectroscopy indicated that profilin-1-labeled NPs exhibited characteristic absorption at 280 nm, suggesting that the profilin-1 antibody was successfully conjugated onto CD-MNPs (Figure 2(e)). The NPs carried a positive zeta potential of 18.37 mV (Figure S3) with good storage ability (Figure S4) and were subsequently bound to the negatively charged cell membrane, leading to cellular internalization.

**MRI of PFN1-CD-MNPs in vitro**

To observe the different iron concentrations of PFN1-CD-MNPs in vitro, 3.0 T MRI was performed. The signal intensity of NPs decreased gradually with the increase in iron concentration (Figure 2(f)). When the concentration of PFN1-CD-MNPs was >10 µg/mL, the T2WI indicated no significant change in signal intensity that could not be distinguished by the naked eye, unlike the T2 mapping colour image. Thus, the T2WI may not reveal the real T2 values, although most
studies use this technique [6,33]. In this study, we used T2 mapping to study MNPs, as this technique should be closer to the true T2 relaxation time. In addition, the calculated relativity ($r^2$) of 90.3 mM$^{-1}$ s$^{-1}$ for NPs (Figure 2(g)) suggests that the synthesized PFN1-CD-MNPs are suitable as an MRI contrast agent for atherosclerosis.

**RAP content in RAP@PFN1-CD-MNPs**

Drug-loading and encapsulation efficiency (EE) is critical to drug delivery systems. The EE and loading capacity (LC) of

| Rapamycin | 1 | 2 | 3 | 4 | 5 | Mean ± SD |
|-----------|---|---|---|---|---|-----------|
| EE (%)    | 7.89 | 7.93 | 8.01 | 7.86 | 7.90 | 7.89 ± 0.06 |
| LC (%)    | 86.88 | 87.33 | 88.10 | 86.41 | 85.93 | 86.93 ± 0.75 |

RAP-loaded PFN1-CD-MNPs were validated by their UV spectra at 278 nm. The EE and LC of RAP-loaded nanocomposites are listed in Table 1. The LC of up to 87% should meet therapeutic requirements of nanomedicine.

**In vitro biocompatibility of PFN1-CD-MNPs nanocomposites**

The biocompatibility of nanocomposites is an important parameter that determines their utility in human clinical trials [22]. To administer an intravenous injection, a haemolysis test was performed on the haemocompatibility of NPs. No obvious haemolysis was detected after 6 h incubation of PFN1-CD-MNPs to MRBCs (Figure 3(a,b)). To investigate the cellular toxicity of NPs, the nanocarriers were cultured with MOVAS for 1, 3 and 7 days at 50, 10 and 1 µg/mL, respectively. The CCK8 assay indicated that the cytotoxicity of PFN1-CD-MNPs and CD-MNPs increased slightly with the increase in the
concentration of NPs (Figure 3(c)). Despite the slight increase in cytotoxicity after prolonged incubation, the cell viability of > 80% after three-day incubation demonstrates that the multifunctional nanocarriers are not toxic to the VSMCs, even at concentrations as high as 50 \( \mu \text{g/mL} \). Therefore, PFN1-CD-MNPs with favourable properties such as haemocompatibility, and low cytotoxicity are suitable for MRI in vivo.

**In vitro drug release studies**

Since the microenvironment of the atherosclerotic plaque is mildly acidic [26], drug delivery systems sensitive to changes in pH in lesions are designed to improve the targeted delivery of drugs, thereby enhancing drug efficacy [34]. To investigate the pH sensitivity of RAP@PFN1-CD-MNPs, hydrolysis of these NPs was studied under various pH conditions. The hydrolysis of RAP@PFN1-CD-MNPs was correlated with the pH of buffer solutions, as expected (Figure 3(d)). The hydrolysis rate of NPs was faster at lower pH. The nanomedicine within RAP@PFN1-CD-MNPs was released rapidly, reaching a plateau within the first 24 h. At pH 5.5, rapamycin in the target vector was almost completely released at 60 h.

**In vitro targeting ability and fluorescence imaging of multifunctional nanocomposites**

*In vitro* fluorescence images of LCSM indicated high binding of PFN1-CD-MNPs to MOVAS cells. The rate of binding decreased when MOVAS cells were treated with CD-MNPs (Figure 3(e,f)). Similarly, Prussian blue staining also revealed high cell binding of PNF1-CD-MNPs to MOVAS cells (Figure S5); this is consistent with the finding of a previous study [6]. Previously, it has been demonstrated that transfection of short interfering RNA (siRNA) of profilin-1 in MOVAS cells attenuated the binding of the nanoprobe to VSMCs, mainly because of diminished proliferation and profilin-1 expression in VSMCs. This implies that specific binding of profilin-1 binds to MOVAS cells.

**Serum lipid profile of the atherosclerosis model**

The atherosclerosis model ApoE/−/− mice were successfully constructed by feeding the mice with a high-fat diet for 4 months. An arterial plaque was confirmed by general ORO staining (Figure 4(a)). Additionally, the serum lipid level of the HDL-C, LDL-C and TC levels was significantly higher in ApoE/−/− mice than in normal mice (Figure 4(b)). The serum level of TC and lipoproteins was higher in mice fed the high-fat diet for 7 months than in those fed the same diet for 4 months. Altogether, these findings indicated that the high-fat diet increases TC and lipoprotein levels.

**Profilin-1 is widely expressed in the plaque of atherosclerosis model mice**

Profilin-1, a small actin-binding protein, regulates the dynamics of actin polymerization, which is key for cell motility in vivo [35]. Profilin-1 expression and dysfunction play an
important role in the development of cardiovascular diseases [36,37]. Western blot analysis revealed that the expression of profilin-1 in the arterial wall of ApoE<sup>−/−</sup> mice was significantly higher than that in the normal group (Figure 4(c,d)). This result was consistent with the upregulated expression of profilin-1 in MOVAS cells treated with oxidized low-density lipoprotein (Figure S6). These results suggest that profilin-1 can be used as a targeted molecule for atherosclerosis; this is consistent with previous studies [6,8]. Thus, profilin-1 may be a potential new target for the diagnosis and treatment of cardiovascular diseases.

**MRI and fluorescence imaging of atherosclerotic plaque with PFN1-CD-MNPs-Cy5.5 in vivo or ex vivo**

There are several ways for NPs (see Scheme 1) to reach the lesion in vivo, including enlarged endothelial gaps, new microvessels that arise from neovascularization extended into the base of the plaque, and PFN1-mediated active targeting [6,22,38]. To trace NPs in the atherosclerotic plaque, in vivo 7.0 T MRI was performed before and 24 h after the injection of PFN1-CD-MNPs. The results indicated that the wall thickness of the carotid artery increased after the injection of NPs, and plaque formation was prominent compared with that in the control (Figure 5(a)). Twenty-four hours post injection, a significant T2-weighted MRI signal attenuation was observed in ApoE<sup>−/−</sup> mice (Figure 5(c)). However, after the administration of CD-MNPs, no changes in signal intensity were observed in the carotid artery wall in T2WI. Since quantitative T2 mapping can provide absolute physical measurements of plaque components [39,40], it characterizes the atherosclerotic plaque precisely and provides in vivo T2 values of plaque components. The Fe<sub>3</sub>O<sub>4</sub> NPs deposit on the plaque area and shorten the T2 relaxation time. The T2 values of mice treated with PFN1-CD-MNPs decreased more drastically than those of mice treated with CD-MNPs. This MRI result is consistent with
the result of Cy5.5 NIR fluorescence imaging. The ex vivo images of NIRF captured 24 h after the intravenous injection of PFN1-CD-MNPs-Cy5.5 or CD-MNPs-Cy5.5 revealed that the fluorescence signal in the aorta in the PFN1-CD-MNPs-Cy5.5 injection group was significantly higher than that in the CD-MNPs-Cy5.5 injection group (Figure 5(b,d)). These results were supported by the histological Prussian blue staining, where iron deposition in the PFN1-CD-MNP group was more than that in the CD-MNP group (Figure 5(e)). Thus, NPs conjugated with the profilin-1 antibody better target the atherosclerotic plaque. High expression of profilin-1 in the atherosclerotic plaque was verified indirectly.

**Therapeutic effect of RAP nanomedicine on atherosclerosis**

To determine the potential therapeutic effect of RAP on atherosclerosis, ex vivo fluorescence imaging was conducted 2 months after the various treatment of ApoE−/− mice. Ex vivo fluorescence imaging revealed significant lower fluorescence intensity in mice treated with RAP-loaded NPs than that in the control group (Figure 6(a)). In the representative stereomicrographs of stained aortas, lesions were reduced in the groups treated with RAP-loaded NPs (Figure 6(b)); this result was consistent with the results of ex vivo fluorescence imaging. Intraperitoneal administration of RAP@PFN1-CD-MNPs notably delayed plaque development. Moreover, RAP@PFN1-CD-MNPs indicated better therapeutic potential than the RAP@CD-MNPs. The plaque area in the aorta was remarkably reduced after treatment with RAP@PFN1-CD-MNPs. Similarly, cross-sections of the aortic arch stained with ORO also revealed remarkably reduced atherosclerotic plaque (Figure S7). These results suggest that RAP nanomedicine conjugated with the profilin-1 antibody effectively slows the progression of atherosclerosis compared with RAP nanomedicine without active target molecules.

To detect whether the nanomedicine affects the vulnerability of the plaque, specific components such as collagen, and α-SMA were evaluated by Masson’s trichrome and immunohistochemical staining. Thick collagen was observed after Masson’s trichrome staining after intervention with RAP@CD-MNPs; RAP@PFN1-CD-MNPs even produced stronger effect (Figure 6(c)). In addition, plaque stability is related to the content of SMCs. Immunohistochemical staining suggested that α-SMA (for SMC staining) was increased (Figure 6(d)) significantly in the RAP-loaded group. Since severe inflammation will speed up the death of VSMCs until the plaques take shape [41]. In this study, we found that RAP-containing NPs significantly increased SMCs in the fibrous cap of plaque, indicating that rapamycin exerts its stabilization effects by increasing SMCs in the plaque; this is consistent with previous studies [28,42]. Moreover, profilin-1-conjugated drug delivery systems exhibited better efficacy than CD-MNPs.

**In vivo toxicity evaluation**

To assess potential in vivo toxicity of RAP@PFN1-CD-MNP nanosystem, the major organs H&E staining of the mice indicated no major injury in the major organs of ApoE−/− mice (Figure 7(a)). However, levels of creatinine and uric acid...
Kidney function indicators and creatine kinase (cardiac function indicator) were significantly higher in the RAP@CD-MNP group than in the control and RAP@PFN1-CD-MNP groups (Figure 7(b)). These results suggested that RAP@CD-MNPs might be potentially toxic to kidney and myocardial functions of the atherosclerosis model mice in long-term therapy. The RAP@PFN1-CD-MNP nanosystem significantly alleviated the damage to kidney and myocardium functions of mice and the development of atherosclerosis. Serum lipid indicators (CHOL, HDL, LDL-C and TG) indicated no significant differences among the control, RAP@CD-MNP and RAP@PFN1-CD-MNP groups. This result is consistent with a previous study [42], where rapamycin effectively attenuated inflammation and inhibited the progression of the atherosclerotic plaque, without altering serum lipid levels. Other biochemical indices for liver function (ALT, AST and kidney function (urea nitrogen)) exhibited no significant differences among these groups. However, the level of the cardiac function indicator, lactate dehydrogenase (LDH), was higher in the ApoE−/− mice, the nanoprobe was capable of locating the atherosclerotic plaque. In addition to passive targeting via the enlarged endothelial space, the PFN1-mediated targeted VSMCs may be another pathway for the diagnosis of atherosclerosis. The CD-MNPs conjugated with the profilin-1 antibody could non-invasively visualize atherosclerotic lesions in live mice. Thus, the nanomedicine RAP@PFN1-CD-MNP is not only a promising biocompatible contrast agent for imaging atherosclerotic lesions but can also rapidly release therapeutic molecules at low pH for atherosclerosis treatment. This study provides key insights into the therapy of atherosclerosis and a new direction for the theranostics and personalized treatment of atherosclerosis.

Conclusions

Overall, the PFN1-CD-MNPs used in this study indicated low cytotoxicity, high targeting efficiency and high hemocompatibility in murine MOVAS and were useful for the treatment of atherosclerosis in vivo. After its administration to ApoE−/− mice, the nanprobe was capable of locating the atherosclerotic plaque. In addition to passive targeting via the enlarged endothelial space, the PFN1-mediated targeted VSMCs may be another pathway for the diagnosis of atherosclerosis. The CD-MNPs conjugated with the profilin-1 antibody could non-invasively visualize atherosclerotic lesions in live mice. Thus, the nanomedicine RAP@PFN1-CD-MNP is not only a promising biocompatible contrast agent for imaging atherosclerotic lesions but can also rapidly release therapeutic molecules at low pH for atherosclerosis treatment. This study provides key insights into the therapy of atherosclerosis and a new direction for the theranostics and personalized treatment of atherosclerosis.
Disclosure statement
The authors declare that there is no conflict of interest.

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Figure 7. Toxicity assessment of RAP\{PFN1-CD-MNPs. (a) Histological analysis of the major organs in ApoE\(-/-\) mice treated with the nanomedicine for 2 months. (b) Haematological analysis of the control and treatment groups of ApoE\(-/-\) mice (*p < .05, **p < .001).
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