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Review

Treatment of atherosclerotic plaque: perspectives on theranostics

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Abstract

Objectives Atherosclerosis, a progressive condition characterised by the build-up of plaque due to the accumulation of low-density lipoprotein and fibrous substances in the damaged arteries, is the major underlying pathology of most cardiovascular diseases. Despite the evidence of the efficacy of the present treatments for atherosclerosis, the complex and poorly understood underlying mechanisms of atherosclerosis development and progression have prevented them from reaching their full potential. Novel alternative treatments like usage of nanomedicines and theranostics are gaining attention of the researchers worldwide. This review will briefly discuss the current medications for the disease and explore potential future developments based on theranostics nanomaterials that may help resolve atherosclerotic cardiovascular disease.

Key findings Various drugs can slow the effects of atherosclerosis. They include hyperlipidaemia medications, anti-platelet drugs, hypertension and hyperglycaemia medications. Most of the theranostic agents developed for atherosclerosis have shown the feasibility of rapid and noninvasive diagnosis, as well as effective and specific treatment in animal models. However, there are still some limitation exist in their structure design, stability, targeting efficacy, toxicity and production, which should be optimized in order to develop clinically acceptable nanoparticle based theronostics for atherosclerosis.

Summary Current medications for atherosclerosis and potential theranostic nanomaterials developed for the disease are discussed in the current review. Further investigations remain to be carried out to achieve clinical translation of theranostic agents for atherosclerosis.

Introduction

Cardiovascular diseases (CVDs), a set of disorders of the heart and blood vessels, are a major cause of mortality, representing 31% of global deaths (reported in 2015).[1] The prevalence of CVD increases with age so with an ageing population worldwide the prevalence of CVD is increasing. Genetic factors, race and sex of the individual are determining factors in the occurrence of cardiovascular disease. The risk factors of more modern relevance include environmental and behavioural risk factors such as lack of physical exercise, sedentary lifestyle, cigarette smoking and alcohol consumption and intake of high-fat diet.[1] In some developed countries, reduced cigarette smoking and better treatment of hypertension is leading to a reduced rate of CVD but even then it still remains the largest cause of death. This favourable trend is opposed by the global epidemic of obesity and its sequelae where obesity is also a risk factor for CVD.

The majority of this global disease burden is associated with atherosclerotic cardiovascular disease (ACD). Atherosclerosis occurs in medium to large vessels. It is the underlying cause of ischaemic heart pain, cardiac and cerebrovascular ischaemia and fatal incidences due to myocardial infarction and stroke. It is also the cause of peripheral artery disease that can lead to lower limb amputations. ACD is characterised by the progressive build-up of atherosclerotic

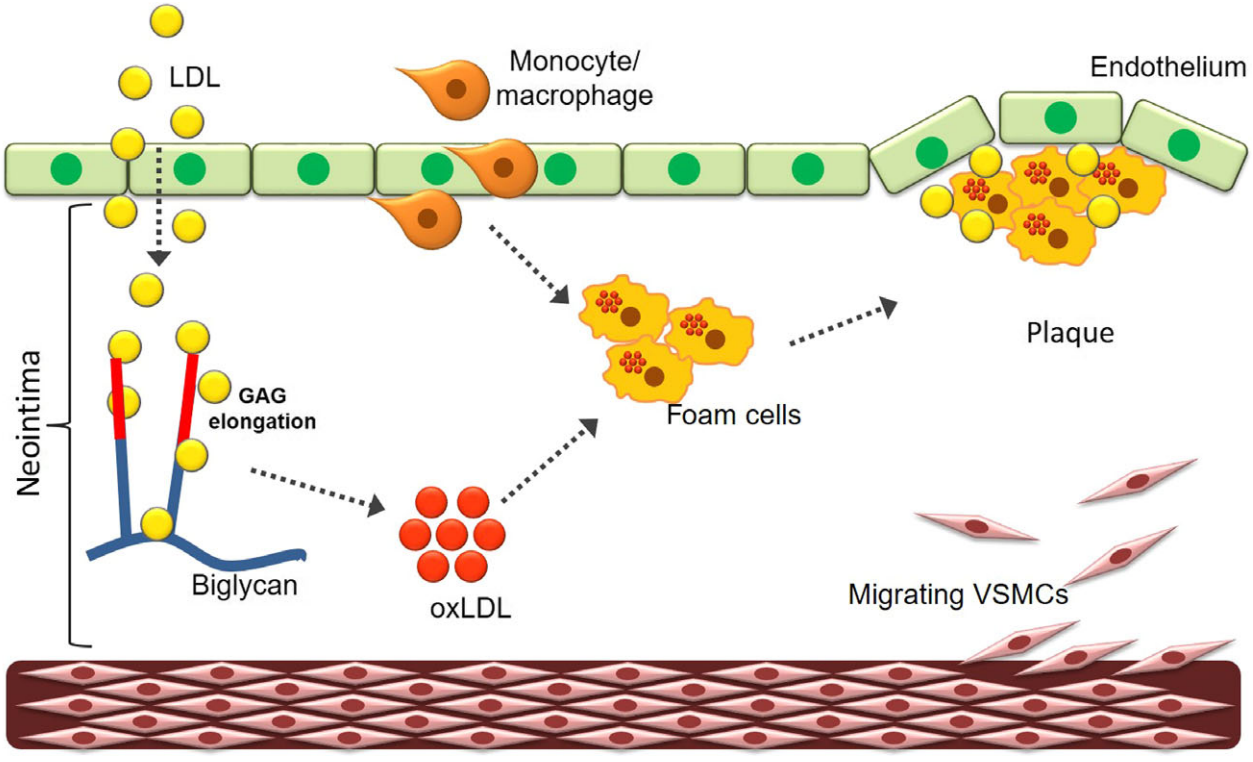

Figure 1 Schematic representation of the initiation and progression of atherosclerosis. The figure depicts the development of atherosclerosis inside the neointima of the arterial wall. Vascular smooth muscle cells secrete a range of proteoglycans, such as biglycan. When growth factors are released, the glycosaminoglycan chains on proteoglycans become elongated. Low-density lipoproteins diffuse into the vessel wall where it is bound by the modified proteoglycans and subsequently retained. The retained low-density lipoproteins are oxidised leading to the invasion of monocytes. Monocytes differentiate into macrophages and engulf the oxidised low-density lipoproteins to form foam cells. Foams cells and lipids accumulate to form atherosclerotic plaque.

plaque on the vessel walls due to the initial accumulation and retention of lipids, and subsequent thrombosis formation on plaque lesions and plaque rupture leading to partial or complete occlusion of the vessel.[2] An outline of the mechanisms underlying the early and critical events in atherosclerosis within the vessel wall is shown in Figure 1. With a complicated aetiology, the disease also induces complex and rather unique immune responses. The occlusion leads to life-threatening clinical outcomes. This article will briefly discuss the current medications and then review the potential future treatments for atherosclerosis with the focus on theranostic nanomedicine.

Clinically available pharmaceutical treatments

The treatment of the atherosclerosis underlying CVD is mostly aimed at the risk factors. These are the modifiable or treatable risk factors of low physical activity, sedentary lifestyle such as cigarette smoking, mental health (depression), atherogenic diets leading to obesity and type 2 diabetes and the non-modifiable factors such as genes, age and gender.[3] Lifestyle factors are the first line of medical guidance for the prevention of atherosclerosis but despite their efficacy, in modern societies, lifestyle modifications have proven to be extremely intractable to interventions in spite of the evidence. Cigarette smoking is a powerful cardiovascular risk factor and smoking cessation is the single most effective lifestyle measure for the prevention of a large number of CVDs.[4] Common medicines for atherosclerosis include cholesterol medications, antiplatelet and antihypertension medications. Antiplatelet drugs such as aspirin are prescribed to prevent platelets to clump in the narrow arteries, form a blood clot and cause further blockage.[5] Antihyperglycaemia medications are also suggested to control specific risk factors for atherosclerosis, such as diabetes.[6]

Hyperlipidaemia (high LDL cholesterol and triglyceride), one of the major drivers of the process of atherosclerosis, has a very strong epidemiological association with the occurrence of cardiovascular events.[3] Fibrates, fibric acid derivatives such as ethylchlorphenoxyisobutyrate,

clofibrate, bezafibrate, fenofibrate, are drugs used to reduce plasma triglycerides.[7] They may also reduce LDL cholesterol, but the extent of its effect is variable. HMG-CoA reductase inhibitors, widely known as statins, are LDL lowering drugs.[8] The mechanism of action of statins involves competitive inhibition of the rate-limiting enzyme HMG-CoA reductase in the cholesterol biosynthesis pathway, leading to the lack of the cholesterol synthesis in the liver. This leads to the synthesis and activation of LDL receptors in the liver, thus stimulating the facilitated removal of excess LDL from the body via the liver. This removal in turn causes the rise in the levels of the HDL. Statins have been reported to reduce the morbidity and mortality caused due to ACD.[8] Some studies have also highlighted the anti-inflammatory effects of statins and proposed that they also contribute to plaque stabilisation.[9]

A range of statins such as atorvastatin, fluvastatin, lovastatin, pravastatin, cerivastatin, pitavastatin, rosuvastatin and simvastatin are currently on the market. There have been some rare adverse events associated with the usage of statins, such as rhabdomyolysis, liver damage and neurological events whereby some reports suggest that the drastic decrease in the cholesterol level by statins could lead to death due to causes other than ACD.[10,11] These adverse events have been rare and have been outweighed by the benefits of statin therapy. However, a meta-analysis using data from eight randomised statin trials shows that more than 40% of patients treated with high-dose statin therapy did not reach an LDL target of less than 70 mg/dl.[12]

Hypertension contributes to atherosclerosis at different levels and stages, including the development of endothelial dysfunction, fatty streaks, early atherosclerotic plaque, plaque progression and plaque rapture.[13] It was found that 77% of those who have a first stroke have had a blood pressure (BP) above 140/190 mmHg. Major classes of antihypertensive drugs include angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), beta blockers, calcium channel blockers, diuretics or combination therapy.[14] Current evidence suggests that treatment and gradual control of hypertension to levels below 120 mmHg systolic and below 65 mmHg diastolic reduce cardiovascular events and improve survival.[13]

The role of (hyper)glycaemia and its treatment in ameliorating atherosclerosis and cardiovascular disease is controversial. Elevated HbA1c (type 2 diabetes) is strongly associated with the initiation and/or progression of atherosclerosis[6] but the role of treating hyperglycaemia in reducing ischaemic atherosclerotic cardiovascular events in people with diabetes (called macrovascular disease) is unclear. This lack of treatment efficacy may possibly be due to a mismatch between the mechanism causing the hyperglycaemia and atherosclerosis, for example insulin resistance, and the mode of action of drugs to reduce HbA1c.

Despite the evidence of the efficacy of the present treatments for atherosclerosis, the complex and poorly understood underlying mechanisms of atherosclerosis development and progression have prevented them from reaching their full potential. Extensive investigation carried out over the years has provided novel insights into the potential treatments and possible prevention of atherosclerosis. In addition to targeting the derangements in lipid metabolism, therapeutic modulation to regulate chronic inflammation and the immune system response may prove to be very promising strategies in the management of atherosclerosis. Evolving targets for the treatment of atherosclerosis include PCSK9 (proprotein convertase subtilisin/kexin type 9), Tregs (regulation T cells), TREM-1 (Triggering Receptor Expressed on Myeloid cells-1), CD47 (cluster of differentiation-47), proatherogenic TMAO (trimethylamine N-oxide), ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) and Notch signalling.[15] Most of these are still under research and development stage. Various approaches to inhibit PCSK9 are currently under clinical trials, including siRNA (phase II), adnectins (phase I), vaccines (phase I) and different antibodies (phase I and II).[15] It has been reported that clinically available statins can upregulate Tregs and, therefore, suppress atherosclerotic lesion formation and inflammation.[15] Recently, CANTOS trial showed that anti-inflammatory therapy with canakinumab, a monoclonal antibody that delectively neutralises IL-1β, reduced the rate of major adverse cardiovascular events in patients with stable coronary disease.[16] CIRT trial, however, showed that low-dose methotrexate, a drug used to treat inflammatory conditions such as rheumatoid arthritis, did not reduce adverse cardiovascular effects in patients with stable atherosclerosis who were at high cardiovascular risk.[17]

Nanomaterials have been employed as vehicles for drug delivery in atherosclerosis, although most of them are still under development stage. The majority of the approaches used currently for the synthesis of therapeutic nanoparticles have employed FDA-approved nanoparticles like PLGA, hyaluronic acid (HA) and liposomes. The most promising application of therapeutic nanoparticles, however, comes in the form of HDL-imitating nanoparticles[18–26] and gene silencing approaches,[26–29] the numerous studies performed for which, have shown promising results that have the potential of clinical success. HDL is considered to have anti-atherogenic properties and believed to reduce the plaque volume by reverse cholesterol efflux methods. Engineering nanoparticles that mimic or imitate the properties of HDL have been trialled lately (CER-001, phase II clinical trial).[20] Gene silencing approaches target miR-712, a proatherogenic miRNA that is upregulated during initiation of atherosclerosis[26]; chemokine receptor (CCR2) involved with the generation of inflammatory signals and

immune cell recruitment[27]; and apoB present in LDL.[28] Phase I clinical trials have been done on the use of siRNA liposome-based gene silencing to lower the LDL levels, with 60% reduction in the LDL plasma concentration with one dose of the formulation, effects of which last for about 3 weeks.[29] The therapy uses siRNA to silence the production of PCSK9 that regulates low-density lipoprotein receptor (LDLR) protein levels and function. Loss of PCSK9 increases LDLR levels in liver and reduces plasma LDL cholesterol (LDLc).

Theranostic nanomedicine for atherosclerosis

Recently, advances in nanoparticulate therapeutics have promoted the emergence of theranostics, a multifunctional agent that can provide simultaneous diagnosis and treatment for diseases.[30] Nanoparticle delivery systems not only can increase the circulation time of therapeutic or diagnostic agents, but also can lower the required dose and reduce off-target toxicity of the drugs.[31] Apart from inorganic nanoparticles, such as gold,[32] silver,[33] silica,[34] silicon[35] and metal oxides,[36] there are several other alternative nanocarriers, including lipid-based nanoparticles, gel-like nanoparticles, micelles, polymeric nanoparticles and dendrimers have been studied.[31,37] These nanocarriers can be targeted to and accumulated in a specific site and simultaneously deliver both imaging agents and therapeutic agents. In theory, theranostic agents shall provide efficient, image-guided and personalised treatment for cardiovascular patients. These compounds can be simply in the form of cardiovascular drug labelled with fluorophores or radionuclide, which can provide the information about disease location, drug release and therapeutic efficacy. Compared with the bulk of theranostic agents for cancer, the development of theranostic agents for CVD is underrepresented. Herein, we discuss some of the impressive development of theranostic agents for diagnosis and treatment of atherosclerosis. We will also critically evaluate factors affecting clinical translation of these nanoparticles for atherosclerosis. Table 1 summarises theranostic nanomaterials developed for atherosclerosis. These approaches are still under development stage, and none has been proceeded to clinical trials yet.

As shown in Figure 2, there are a number of potential cell and receptors involved in atherosclerosis can be used for theranostic agents targeting, such as macrophage, integrin $\alpha_v\beta_3$ and VCAM-1. Among these targets, macrophages are the most commonly studied and used target because of its pathogenic roles, phagocytic property and abundance in the atherosclerosis lesion. The nanomaterials can be labelled with binding ligands such as antibodies (CD11b, anti-VCAM1) and peptides to target macrophages. Macrophages can also phagocytose the theranostic nanoparticles into the atherosclerosis lesion site when the nanomaterials are modified with molecules such as dextran[38] or mannose.[39] There are two strategies for the treatments of atherosclerosis targeting macrophages, including macrophage ablation and macrophage repair. During the progression of atherosclerosis, macrophages play a critical role in pro-inflammatory regulation. Therefore, the reduction in macrophages may slow the inflammatory development.[40] Presently, there are several therapeutic strategies are utilised for macrophage depletion, including photodynamic therapy (PDT), photothermal therapy (PTT) and cytotoxic chemotherapy.[41]

Photodynamic therapy of atherosclerosis

Photodynamic therapy is a treatment modality that uses light to activate photosensitisers to produce cytotoxic singlet oxygen.[42] The activated photosensitisers can react with oxygen to produce reactive oxygen species (ROS), singlet oxygen and radicals, which can cause cytotoxicity and damage to macrophages.[43] Additionally, the activated photosensitisers also have fluorescence properties and thus photosensitisers can be used as theranostic agents. Previously, photosensitiser-based theranostic agents have been used as an imaging-guided therapy for some diseases, including cancer and skin diseases.[42] Photosensitiser-based theranostic agents specifically accumulate in the disease area and then image and treat the disease upon illumination.

Generally, the common clinically used photosensitisers are porphyrin and the derivant of porphyrin, which only are cytotoxic in the presence of light.[44] Non-selective small molecule photosensitisers, such as 5-aminolevulinic acid (ALA), were employed for the treatment of atherosclerosis in an animal model.[45] However, due to their low molecule weight and non-selective characteristics, the accumulation of ALA occurred not only in macrophages but also in nearby region, and they also showed a short half-life *in vivo*. To overcome these limitations, photosensitisers are combined with nanoparticles to increase specific accumulation, half-life and loaded dose. In the previous studies, McCarthy *et al.*[46] developed a cross-linked dextran-coated iron oxide (CLIO) nanoparticles for the macrophages ablation in atherosclerosis. Iron oxide-based nanoparticles have been developed widely for molecular imaging of CVDs.[47–59] The CLIO nanoparticles in McCarthy study were coated with dextran to specifically target to macrophages and labelled with the near-infrared (NIR) fluorescent dye, Alexa Fluor 750 (AF750) and a chlorin-based photosensitiser, 5-(4-car-boxyphenyl)-10,15,20-triphenyl-2,3-dihydroxychlorin (TPC), which make CLIO can be detected by MRI and fluorescence imaging (Figure 3a).

Table 1 Theranostic agent of cardiovascular diseases

Target	Name of agent	Imaging mode	Drug/therapeutic factor	Binding ligand	Disease
Macrophage	Cross-linked dextran-coated iron oxide (CLIO) nanoparticles	Fluorescence imaging/MRI	Chlorin-based photosensitiser, 5-(4-car-boxyphenyl)-10,15, 20-triphenyl-2,3-dihydroxychlorin (TPC)	Dextran	Atherosclerosis[46]
Macrophage	Cross-linked dextran-coated iron oxide (CLIO) nanoparticles	Fluorescence imaging/MRI	Chlorin-based photosensitiser, *meso*-tetra(*m*-hydroxyphenyl)chlorin (THPC)	Dextran	Atherosclerosis[36]
Macrophage	Protease-mediated theranostic agent	NIRF	L-SR15 cathepsin-B (CatB) activatable photosensitisers	–	Atherosclerosis[60]
Macrophage	Small multifunctional nanoclusters (nanoroses)	MRI/NIRF	NIR (700-850 nm)	Dextran	Atherosclerosis[65]
Macrophage	Bioconjugated gold nanorods	Fluorescent imaging	NIR irradiation (650 nm)	Monoclonal antibody (CD11b)	Atherosclerosis[68]
Macrophage	Gold nanorods	CT	NIR irradiation (808 nm)	/	Atherosclerosis[66]
Macrophage	Silica-coated gold nanorod	IVUS/IVPA imaging	Continuous wave laser	Poly-L-Lysine	Atherosclerosis[71]
Macrophage	Single-Walled Carbon Nanotubes (SWNTs)	NIRF	NIR irradiation (808 nm)	/	Atherosclerosis[72]
Macrophage	^{18}F-labelled liposome	PET/MRI	Glucocorticoid	/	Atherosclerosis[74]
Macrophage	Drug-loaded hyaluronic acid– polypyrrole nanoparticles (HA–PPyNPs)	Fluorescence imaging	Doxorubicin (DOX)	/	Atherosclerosis[78]
Macrophage	High-density lipoprotein-like magnetic nanostructures (HDL-MNS)	MRI	HDL-mimics	/	Atherosclerosis[79]
Macrophage	Hybrid lipid–latex (LiLa) n anoparticles	MRI	Rosiglitazone	Phosphatidylserine (PtdSer), cholesterol-9-carboxynonanoate (9-CCN)	Atherosclerosis[80]
Macrophage	Phosphatidylserine-presenting liposomes	MRI/Fluorescence imaging	Phosphatidylserine	Phosphatidylserine	Atherosclerosis[82]
Macrophage	Monocyte-targeting siRNA nanomaterials	Fluorescence imaging	CCR2-silencing short interfering RNA	Chemokine receptor CCR2	Atherosclerosis[83]
$\alpha_v\beta_3$-integrin	$\alpha_v\beta_3$-targeted paramagnetic nanoparticles	MRI	Fumagillin	Peptidomimetic vitronectin antagonist	Atherosclerosis[84]
$\alpha_v\beta_3$-integrin	$\alpha_v\beta_3$-targeted paramagnetic nanoparticles	CMR molecular imaging	Fumagillin	Peptidomimetic $\alpha_v\beta_3$-integrin antagonist	Atherosclerosis[85]
VCAM-1	Magnetic microbubbles	MRI	Doxorubicin (DOX)	Anti-VCAM-1 antibody	Atherosclerosis[86]
Platelet	Solid lipid nanoparticles	MRI	α-tocopherol or prostacyclin (PGI2)	/	Atherosclerosis[87]
Collagen IV	USPIO + paclitaxel-loaded polymer-lipid hybrid theranostic nanoparticles conjugated with C11 (UP-NP-C11)	MRI	Paclitaxel	Polypeptide C11	Atherosclerosis[88]

CMR, cardiac magnetic resonance; CT, computed tomography; IVUS/IVPS, intravascular ultrasound and photoacoustic; MRI, magnetic resonance imaging; NIRF, Near-infrared fluorescent; PET, positron emission tomography; VCAM-1, vascular cell adhesion molecule 1.

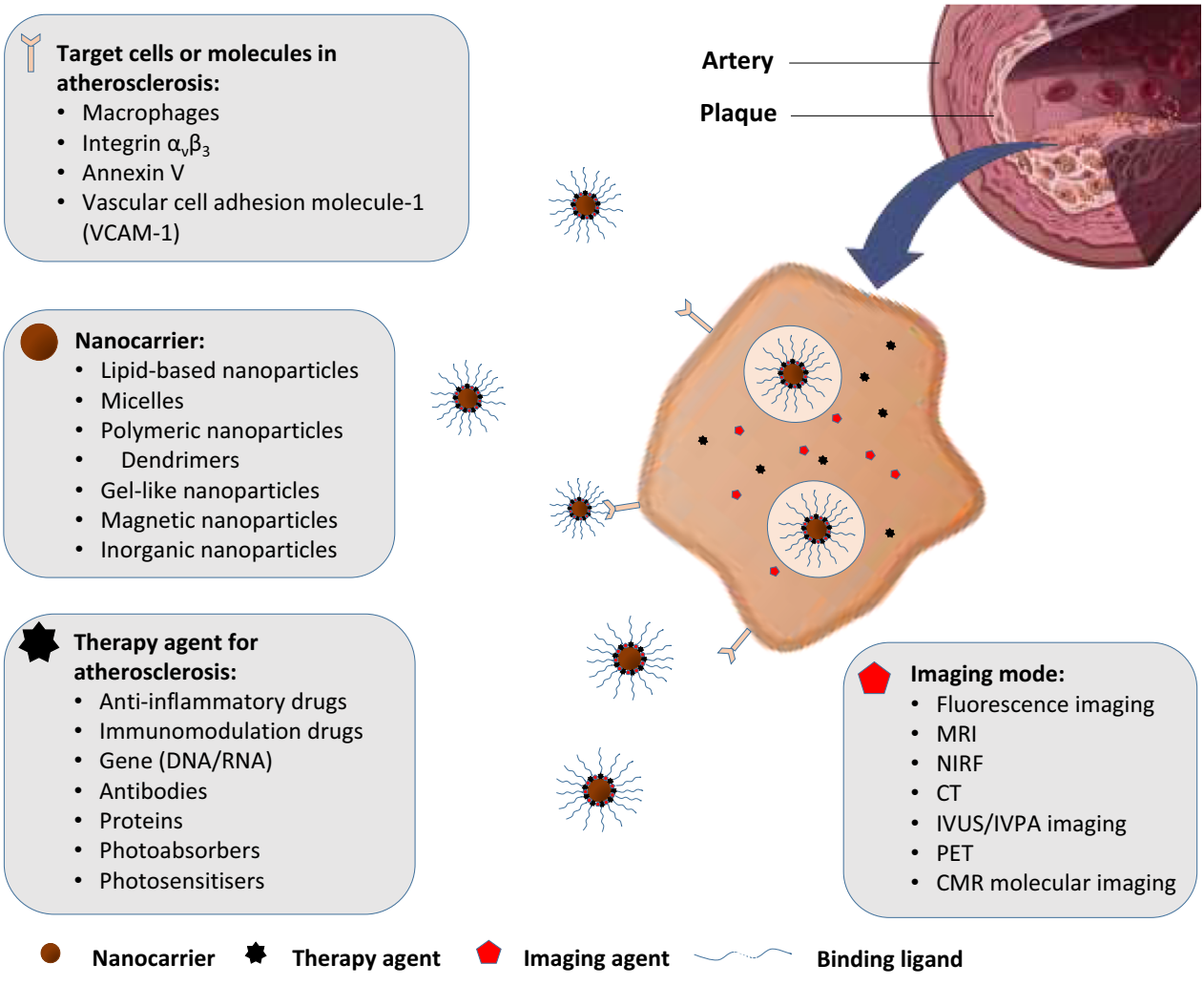

Figure 2 Schematic of theranostic agents used in atherosclerosis.

The *in vitro* study with RAW 264.7 mouse cell line showed that the theranostic nanoparticle (TNP) had cytotoxicity only in the presence of light (Figure 3b). However, according to the *in vitro* study result, the first generation CLIO nanoparticles are not stable. Therefore, the second generation CLIO nanoparticles were conjugated with a new chlorin-based photosensitiser, *meso*-tetra (*m*-hydroxyphenyl) chlorin (THPC), which is loaded three times more than TPC on the CLIO nanoparticles.[36] The *in vivo* and *in vitro* studies were also taken place in the murine model of atherosclerosis and RAW 264.7 cells. As expected, CLIO-THPC caused macrophage death upon a 650 nm laser irradiation and did not display any toxicity in the absence of light. In the *in vivo* study, mice were injected with CLIO-THPC or CLIO-AF750 as the control nanoparticles. Fluorescence imaging shows an accumulation of CLIO-THPC in the atherosclerotic plaque (Figure 3c). CLIO-THPC exhibited a lower skin phototoxic than chlorin e6 (Figure 3d). In this study, an exposed carotid artery murine model was employed for ease of imaging and therapy. In further development, photosensitiser with longer wavelength should be utilised to achieve deep tissue imaging and therapy.

In another study, a protease-mediated theranostic agent (L-SR15) was developed to increase the selectivity of theranostic agents.[60] L-SR15 was modified with a cathepsin-B (Cat B) activatable photosensitiser to selectively deplete Cat B, which is a protease generated by macrophages.[61] L-SR15 cathepsin-B (Cat B) activatable photosensitisers are non-toxic at low dose and can be used to target and localise the nanoparticles to the disease region before the application of antimacrophage irradiation. L-SR15 is activated via proteolytic cleavage by macrophage Cat B, producing the fluorescent photosensitiser, chlorin e6.[62] The *in vivo* and *in vitro* studies were conducted with RAW 264.7 cell line and murine model of atherosclerosis. A Cy7-Cat B sensing fluorescent

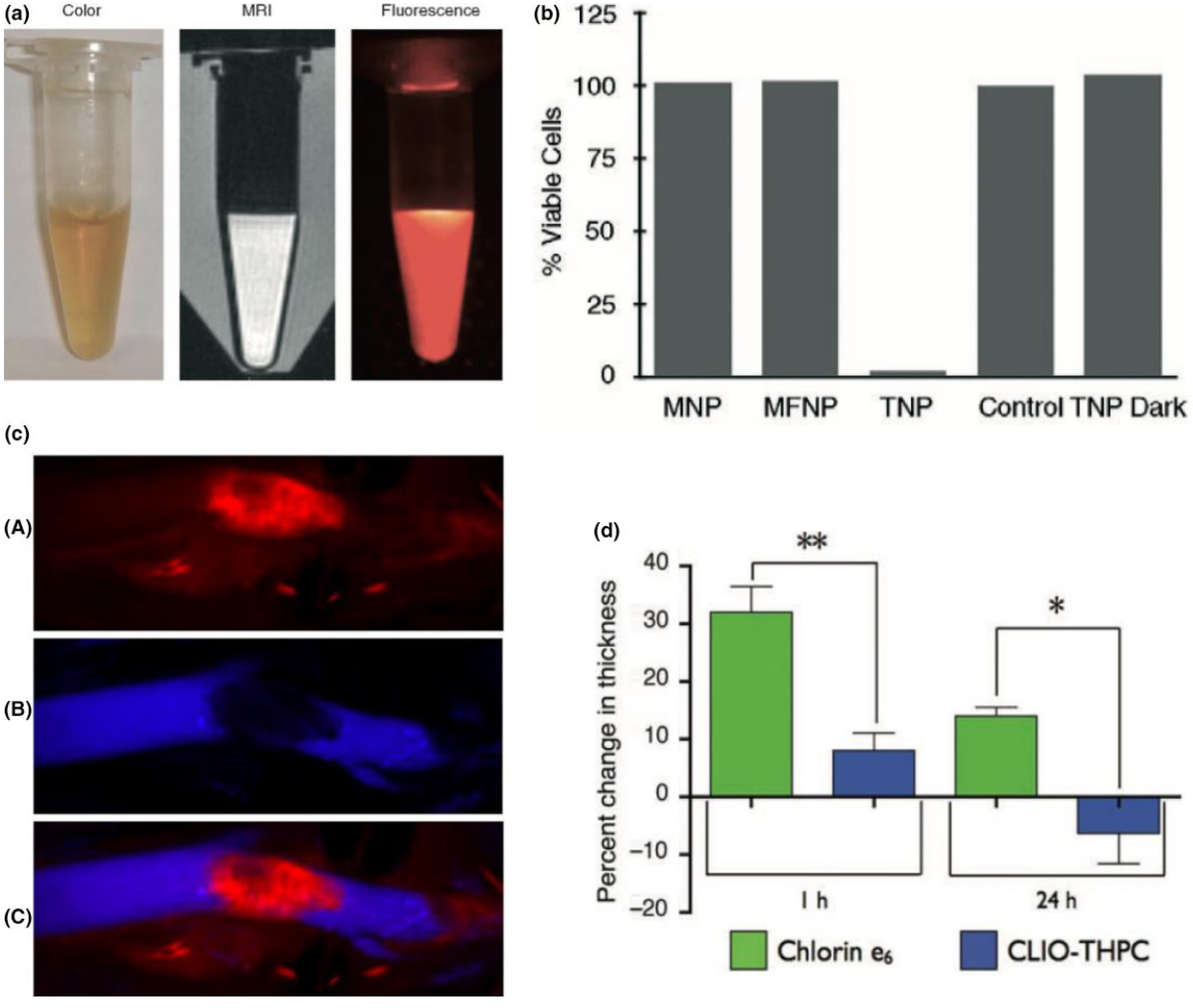

Figure 3 (a) Colour, MR, and fluorescence images of CLIO. (b) Cell viability of macrophages after incubation (1 h) with the respective nanoparticles (0.2 mg Fe/ml) and light treatment (42 mW/cm2, 7.5 J). The theranostic nanoparticle dark experiment consisted of the cells exposed on theranostic nanoparticle but without photodynamic therapy treatment. Control cells were incubated with PBS. (c) Intravital fluorescence microscopy image of CLIO-THPC localised to carotid atheroma *in vivo*. (A) Fluorescence image determined in the AF750 channel proving particle uptake. (B) Fluorescence angiogram utilising fluorescein-labelled dextran to outline the vasculature. (C) Merged image of the two fluorescence channels. (d) Skin photosensitivity of chlorin e6 vs CLIO-THPC based upon the change in thickness in the treated paw 24 h after laser irradiation. (**P = 0.009, *P = 0.02). Reproduced with permission from Refs [36, 46].

probe was used to detect the Cat B activity. Compared with D-SR16 that is a Cat B-inactivated control group, L-SR15 effectively enhanced fluorescent signal and reduced Cat B activity. In addition, subsequent PDT could cause macrophage death and further reduced Cat B activity in atherosclerosis lesion. In the skin phototoxicity study, the damage caused by L-SR15 was much smaller than chlorin e6. Although L-SR15 offers a stimuli-sensitive strategy for the development of high selectivity theranostic agents, the therapeutic efficacy of L-SR15 is relatively weak. Overall, PDT provides a novel therapy strategy which can simultaneously detect the location of atherosclerotic lesion and effect the macrophage ablation in atherosclerosis. In addition, more stable, efficacy and longer wavelength photosensitisers should be used in further development, which enable non-invasive and effective treatment for atherosclerosis.

Photothermal therapy of atherosclerosis

Photothermal therapy employs photoabsorbers such as gold to generate hyperthermia in the disease region by the irradiation of light such as NIR light.[63,64] Hyperthermia can cause protein denaturation and destroy cellular structure followed by cell death.[32] Similar to photosensitisers,

photoabsorbers also have optical properties for imaging, while photoabsorbers can be used for deep tissue treatment. Gold nanoparticles are promising theranostic agents used in PTT because of their non-photobleaching and rapid heat generation.[63] Ma *et al.*[65] have developed iron oxide nanoparticles coated with thin gold film for the PTT macrophages ablation. These theranostic nanoparticles are also coated with dextran for macrophage targeting. Dextran coating also reduced the conglomeration of nanoparticles and improves colloidal stability. Both *in vitro* and *in vivo* studies in rabbit atherosclerosis model showed selective uptake of the theranostic nanoparticles by macrophages in atherosclerosis plaque, and the subsequent NIR irradiation effectively killed macrophages. As the core of this theranostic agent is iron oxide nanoparticles, MRI could be utilised to detect the location of atherosclerosis plaque after macrophage-specific uptake. Although, this study provided a one-step self-assembly for the synthesis of the theranostic nanoparticle, its therapeutic efficacy has not been explicitly studied. In a more recent study, GNPs were synthesised for the use of PTT in inflammatory macrophages ablation.[66] GNPs showed good biocompatibility and were non-cytotoxic in the absence of light in an *in vitro* study with macrophages. Upon 808 nm NIR irradiation, GNPs exhibited excellent PTT efficacy on macrophages ablation *in vitro* (Figure 4a) and also *in vivo* in an apolipoprotein E (Apo E) knockout mouse model.[66]

Similar to gold nanoparticles (GNPs), gold nanorods (GNRs) are also used as theranostic agents in PTT, but GNRs exhibit intense and tunable optical absorption due to different aspect ratios.[67] In the previous study of Pissuwan *et al.*,[68] the gold nanorods conjugated to a macrophage targeting monoclonal antibody (CD11b) have been developed to kill macrophages upon NIR irradiation (650 nm). The fluorescent imaging showed a selective present of GNPs-CD11b in macrophages, and around 80% macrophages were killed by NIR irradiation. However, there is only little increase in therapeutic efficacy compared with naked GNPs. When the NIR irradiation increases to more than 800 nm, there is a significant rise in therapeutic efficacy.[32] This strategy is feasible in the development of theranostic agents for cancer; similar studies have been reported.[69,70] In another study, silica-coated gold nanorods (SiO_2AuNR) were detected and monitored for temperature change during the PTT process via the combined intravascular ultrasound and photoacoustic (IVUS/IVPA) imaging.[71] This method provides a feasible strategy to specifically monitor the induced thermal damage and the delivered thermal dose, which can promote the development of GNRs-based PTT theranostic agents in clinical use.

Single-walled carbon nanotubes (SWNTs) also have been developed for PTT in macrophages ablation. In a study, the feasibility of the fluorescent dye Cy5.5-conjugated SWNTs as PTT theranostic agents was demonstrated.[72] *In vitro* results showed that SWNTs had excellent uptake (94%) in mouse macrophages and caused macrophage death upon exposure to 808 nm NIR light, while both SWNTs and NIR light showed no toxicity (Figure 4b). In addition, the fluorescence imaging obtained from *ex vivo* and *in vivo* studies showed that Cy5.5-conjugated SWNTs had excellent selective accumulation in ligated carotids compared with the sham-operated group (Figure 4c). As a result, subsequent PTT with 808 nm NIR light caused macrophage apoptosis in the ligated carotids, instead of operated sham. Although this research demonstrated that SWNTs are capable of simultaneous fluorescence imaging and PTT macrophages ablation, further studies should be conducted to increase the selectivity of SWNTs by surface modification and to investigate the biocompatibility of SWNTs. Overall, even though the feasibility of PTT theranostic agents in macrophage ablation has been demonstrated, there are still a number of challenges that need to be overcome before clinical use, such as specificity, colloidal stability and the pharmacokinetics.

Cytotoxic chemotherapy of atherosclerosis

Although the non-invasive and non-toxic PDT and PTT are promising theranostic approaches for macrophage ablation in atherosclerosis, they may not be effective to treat the atherosclerosis in deeper tissue due to the limitation of light penetration in tissue. In the deeper tissue condition, targeted cytotoxic chemotherapy can be a more effective treatment of atherosclerosis. There are several cytotoxic drugs such as glucocorticoid, doxorubicin and paclitaxel that can be used for chemotherapy. However, the utilisation of these non-specific drugs can result in an overall decrease in the number of macrophages.[73] In a previous study, an effective anti-inflammatory drug prednisolone phosphate (PLP) was encapsulated in a nanomedicinal liposome (L-PLP) to reduce its adverse effect.[74]

The L-PLP theranostic agents have been successfully studied in several cancer models[75,76] and rheumatoid arthritis.[77] In the study of Lobatto *et al.*,[74] several imaging modalities, including ^{18}F-Fluoro-deoxy-glucose positron emission tomography (^{18}F-FDG-PET) and Dynamic contrast-enhanced MRI (DCE-MRI) were employed to monitor and evaluate the delivery and therapeutic efficacy of L-PLP. An intensive signal increase was observed from MRI images (Figure 5a) at 2 days after the administration of L-PLP, indicating a considerable accumulation of L-PLP in atherosclerotic lesions. In addition, ^{18}F-FDG-PET result (Figure 5b) showed that anti-inflammatory effects of L-PLP were rapid and lasted up to 7 days, while there was no effect from free PLP. The histological analysis for macrophage density also corroborated the above findings (Figure 5c).

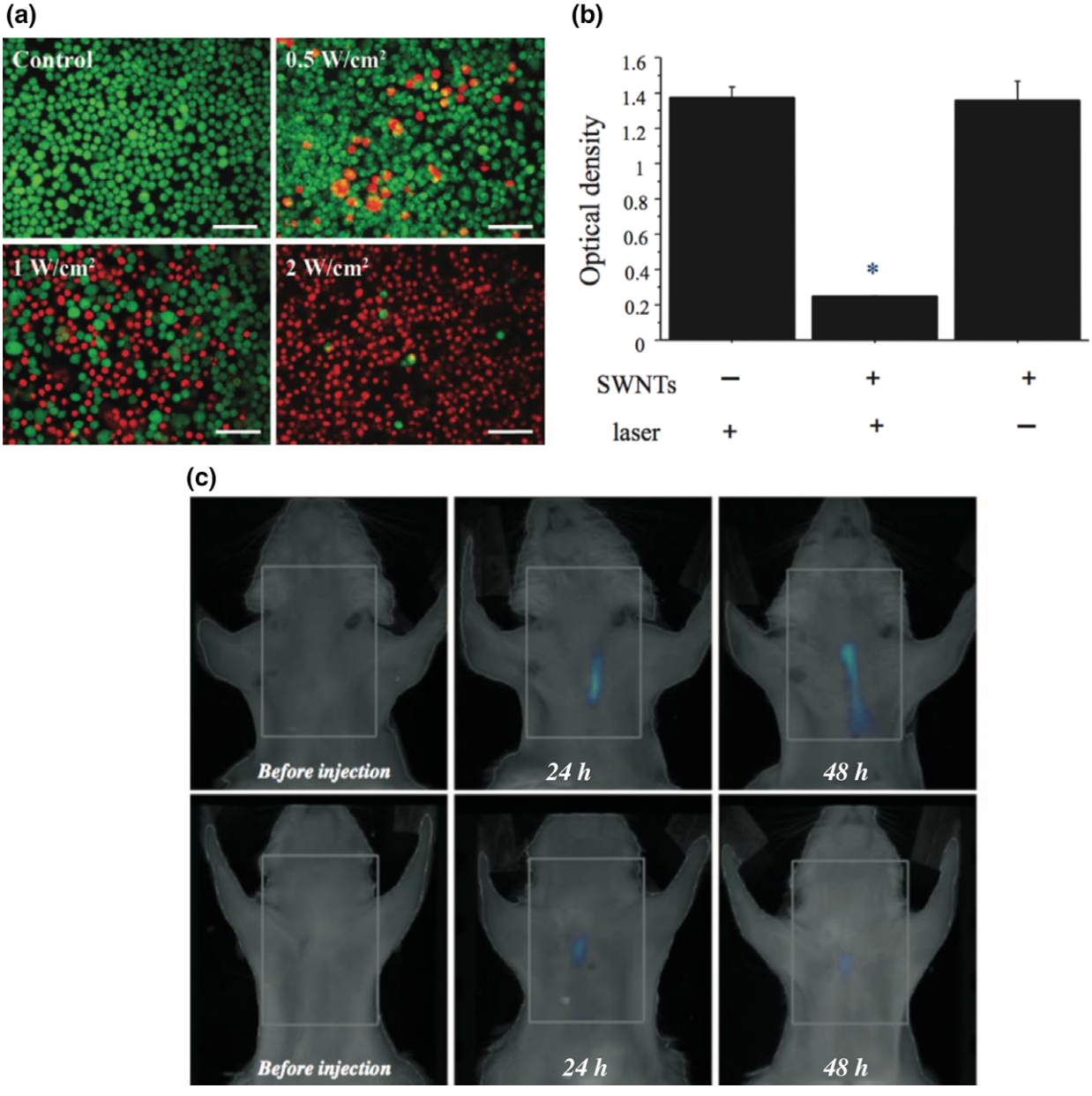

Figure 4 (a) Live cells and dead cells were stained with calcein AM (green) and PI (red), after incubation of Ana-1 cells with or without the Au NRs (20 μg/ml) and being exposed to an 808 nm laser at different power densities (0.5, 1 and 2 W/cm^2). (b) Cell viability by MTT assay 24 h after thermal ablation was significantly decreased in macrophages with Single-walled carbon nanotubes. (c) Representative serial *in vivo* FMT images of a carotid-ligated mouse (top) and a sham-operated mouse (bottom) before and after injection of Cy5.5-conjugated SWNTs. Reproduced with permission from Refs [66, 72].

In addition to L-PLP, there are a number of promising theranostic agents for cytotoxic chemotherapy have been development. For example, doxorubicin-loaded hyaluronic acid-polypyrrole nanoparticles (DOX@HA–PPyNPs) is a promising pH-responsive theranostic agent for proliferating macrophages in atherosclerosis lesions.[78] High-density lipoprotein-like magnetic nanostructures (HDL-MNS) have been studied for their imaging (via MRI) and therapeutic effects (reverse cholesterol transport) on macrophage-rich and cholesterol-rich atherosclerotic lesions. Non-invasive synergistic therapeutic effects may be achieved with this kind of nanomaterial upon loading with other anti-inflammatory drugs.[79] Bagalkot *et al.*[80] reported lipid–latex (LiLa) hybrid nanoparticles using 'eat me' signals (by coating the particles with phosphatidylserine and oxidised cholesterol ester derivative cholesterol-9-carboxynonanoate) which can result in

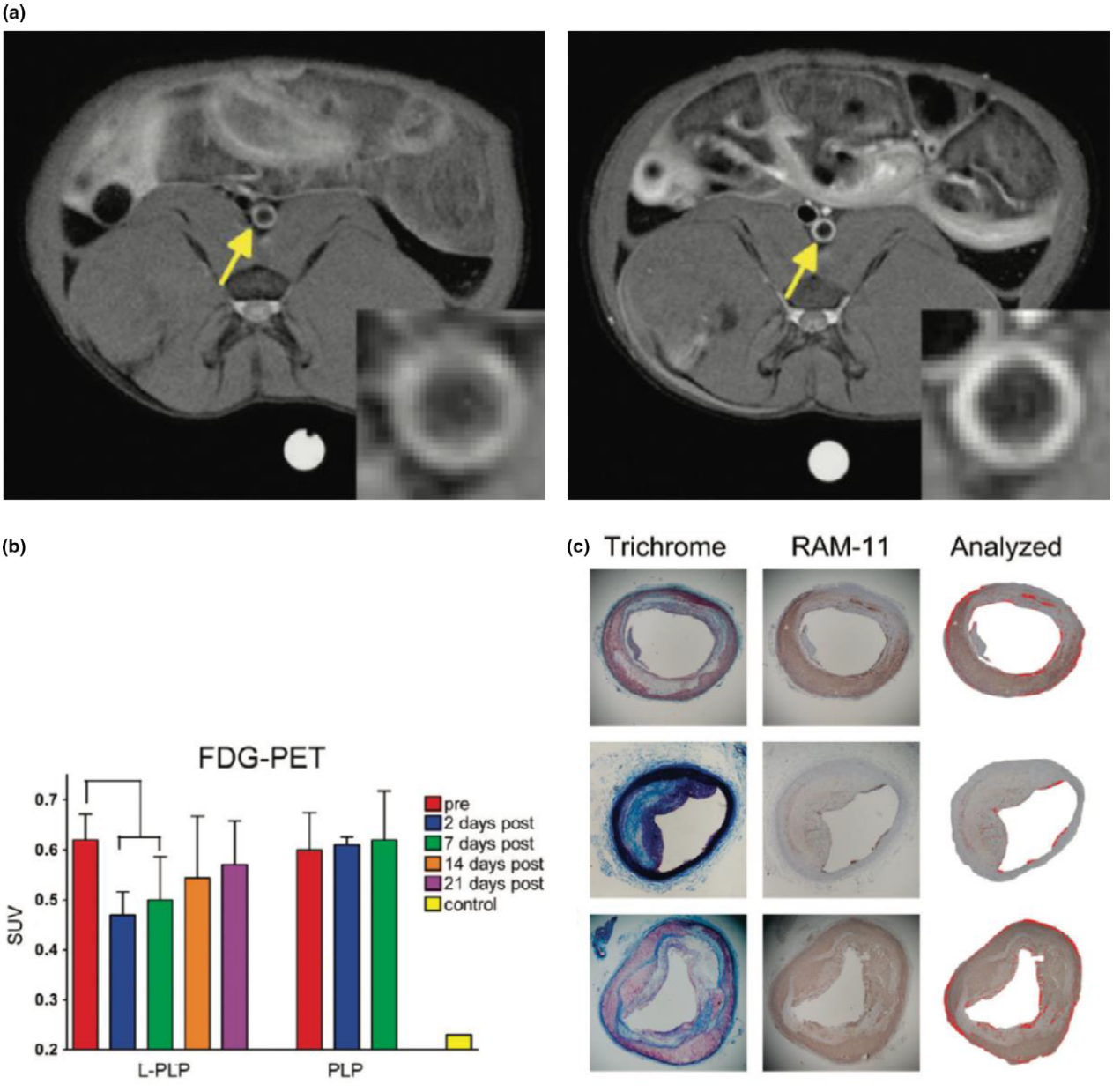

Figure 5 (a) *In vivo* magnetic resonance imaging of the abdominal aorta before (left) and 2 days after (right) the administration of liposomes. A marked signal intensity increase was observed throughout the atherosclerotic lesion. (b) The mean SUV for the different time points pre- and postinjection of liposomal prednisolone phosphate and free prednisolone phosphate are given. (c) Representative histological slices of aortic sections stained with Masson's trichrome and stained for macrophages with RAM-11. (top) Section from a free prednisolone phosphate-treated animal, 7 days after intravenous administration. (middle) Macrophage density is significantly reduced 7 days post-treatment with liposomal prednisolone phosphate and was (bottom) back to baseline after 21 days. Reproduced with permission from Ref. [74].

efficient and specific phagocytosis of the nanomaterials by macrophages. LiLa nanoparticles are coated with gadolinium DTPAbis (stearylamide) in order to enable MRI detection and anti-inflammatory drug loading (rosiglitazone, tamoxifen and paclitaxel). Although LiLa is a promising multifunctional theranostic agent for atherosclerosis, it is unable to enter clinical trial due to its poor biodegradability.[80]

Other theranostic approaches for atherosclerosis

In certain cases, macrophage ablation therapy can affect homeostasis and lead to infections.[81] Non-ablation methods have been developed to only reduce the pathogenic activity of macrophages, which can reduce some side effects. For

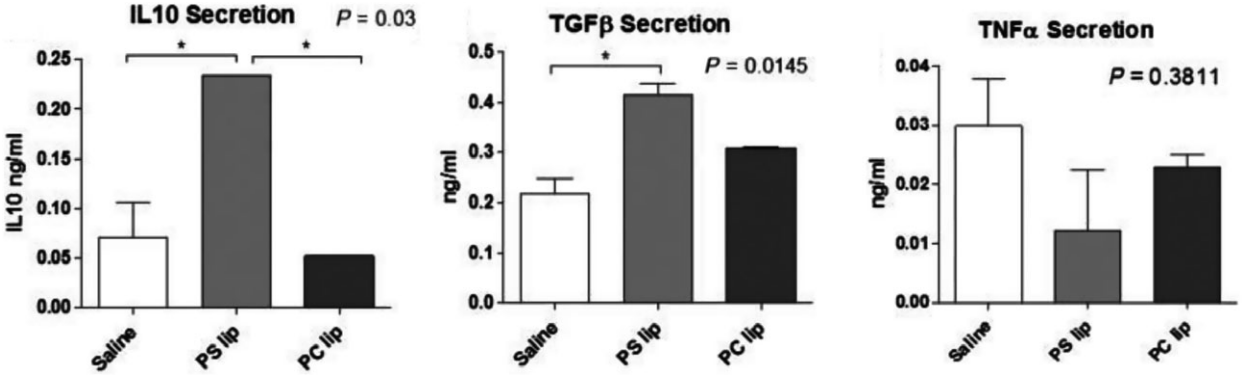

Figure 6 Cytokine analysis of supernatants from macrophage cultures exposed to phosphatidylserine liposomes. Phosphatidylserine liposomes show an increase in IL-10 and TGF-β and a reduction in TNF-α compared with saline and phosphatidyl choline liposomes. Reproduced with permission from Ref. [82].

example, phosphatidylserine (PS)-presenting liposomes were developed for the modulation of cardiac macrophages in post-MI.[82] The strategy mimicked the anti-inflammatory effects of apoptotic cells, which are known to actively suppress inflammation by inhibiting the release of pro-inflammatory cytokines from macrophages while augmenting the secretion of anti-inflammatory cytokines. PS is expressed on the surface of apoptotic cells, which can be recognised by macrophages and following by 'silent' clearance. Thus, apoptotic cells can produce anti-inflammatory effects through inhibiting the pro-inflammatory activity of macrophages. In this study, MRI agent iron oxide was encapsulated in the PS-presenting liposomes, which is mimicking apoptotic cells.[82] These PS liposomes were injected into the rat model of acute MI. As shown in Figure 6, there was an increase in the secretion of anti-inflammatory cytokines (TGF-β and IL-10) and the expression of the mannose receptor (CD206), while a decrease in the secretion of pro-inflammatory TNF-α and the expression of surface marker CD86, which verified the anti-inflammatory effects of PS liposomes on the modulation of cardiac macrophages. In the development of atherosclerosis, the recruitment of macrophages to lesions site can aggravate pro-inflammatory activity and destabilise atheromata. Therefore, fluorescent-tagged siRNA modified lipid nanoparticles were developed to target monocyte and silence the activity of C-C chemokine receptor type 2 (CCR2) on the monocyte, which effectively prevent the recruitment of macrophages in the plaque.[83]

Additionally, there are several other potential targets for the theranostic agents of atherosclerosis. For example, integrin-targeted paramagnetic nanoparticles loaded with the anti-angiogenic drug fumagillin was used to specifically target $\alpha_v\beta_3$ integrin in angiogenesis.[84] Angiogenic expansion of the vasa vasorum is a well-known feature of progressive atherosclerosis, suggesting that anti-angiogenic therapies may stabilise or regress plaques. This study illustrated the potential of anti-angiogenic drug-loaded theranostic agents for non-invasive therapy of atherosclerosis and also quantified the response to the theranostic agents. In their further study,[85] the anti-angiogenic pharmacodynamics of $\alpha_v\beta_3$-targeted paramagnetic nanoparticles dosage and the sustained anti-angiogenic effects of $\alpha_v\beta_3$-targeted paramagnetic nanoparticles have been investigated. The anti-angiogenic effects of single low dose $\alpha_v\beta_3$-targeted paramagnetic nanoparticles was sustained for 3 weeks. Moreover, magnetic microbubbles conjugated with anti-VCAM-1 antibodies were designed as theranostic agents to effectively attach to inner wall, which offered a method for detection and early treatment of atherosclerosis in high shear stress.[86] In more recent studies, novel lipid magnetic nanoparticles have been designed for atherosclerosis theranostic agents that can directly deliver cytotoxic drugs to platelet and collagen IV, respectively.[87,88] Therefore, theranostic agents can be used for specific delivery of cytotoxic drugs combined with imaging modality for the imaging-guided therapy of atherosclerosis. Recently, iron oxide–cerium oxide core–shell nanoparticles have been developed as a potential theranostic nanomaterial for reactive oxygen species (ROS)-related inflammatory diseases such as atherosclerosis.[89] The study showed that this nanoparticle was effective for scavenging ROS and provided excellent MRI performance *in vitro*.

Perspectives

Atherosclerosis is the primary cause of CVDs, affecting millions of people worldwide. Many risk factors play a role in the occurrence of atherosclerosis; however, the exact and defined mechanism of action of these risk factors in atherosclerosis still remains elusive. With lack of early observable symptoms, atherosclerosis usually progresses to a very later stage before it is diagnosed, leading to high mortality. Various drugs can slow the effects of atherosclerosis.

They include hyperlipidaemia medications, antiplatelet drugs, hypertension and hyperglycaemia medications. Despite the evidence of the efficacy of the present treatments for atherosclerosis, the complex and poorly understood underlying mechanisms of atherosclerosis development and progression have prevented them from reaching their full potential.

With the advance of nanotechnology and the enormous demands for effective therapy of CVDs, a large amount of theranostic agents have been developed for CVDs. We have reviewed novel theranostic agents for atherosclerosis. Most of them have shown the feasibility of rapid and non-invasive diagnosis, as well as effective and specific treatment in animal models. However, there are still some limitation exist in their structure design, stability, targeting efficacy, toxicity and production, which should be optimised in order to develop clinically acceptable nanoparticle-based theranostics for atherosclerosis. Using porous materials such as metal-organic frameworks (MOFs),[90] silica,[34] silicon,[35] metal oxides[36] combined with imaging agent is certainly an exciting prospect and future research should focus on improving its stability, and effective *in-vitro* and *in-vivo* correlation.

Although nanomedicine promises effective results, cementing clinical evidence of their efficiency remains to be published. Another obstacle is the manufacturing process of nanomedicines, which is far more complicated than the current biopharmaceutical manufacturing. It is therefore necessary to increase the interaction between small laboratories and the pharmaceutical industry. An additional area of concern is that atherosclerosis is quite different in animal models and humans. In animal models, the inflammatory response predominates whereas in humans the initial interaction between the extracellular matrix components and blood-derived cholesterol predominates. This mismatch of human disease and its animal models provides the reason why many successful animal studies have not been successfully translated to the clinic. New animal models that better mimic human atherosclerosis conditions are highly in need.

Research imperatives include the need for better assessment and analysis of stable and labile atherosclerotic plaques, preferably by techniques of low or minimal intervention, and new treatments which most desirably address the disease process(es) in the vessel wall. The development of theranostic strategies for atherosclerosis has attracted much attention. The complexity of theranostic nanosystems, however, presents a significant challenge to meet FDA standards and achieve clinical translation, despite its benefits and potential. Continued innovations in nanomaterial and atherosclerosis drug research will help the early achievement of clinical translation of theranostic agents for atherosclerosis.

Declarations

Conflict of interest

Nothing to declare.

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References

1. World-Health-Organisation. Cardiovascular diseases (CVDs). 2017. Available from: http://www.who.int/mediacentre/factsheets/fs317/en/. Accessed 29 September 2017.
2. Tabas I *et al.* Recent insights into the cellular biology of atherosclerosis. *J Cell Biol* 2015; 209: 13–22.
3. Rafieian-Kopaei M *et al.* Atherosclerosis: process, indicators, risk factors and new hopes. *Int J Prev Med* 2014; 5: 927–946.
4. Virdis A *et al.* Cigarette smoking and hypertension. *Curr Pharm Des* 2010; 16: 2518–2525.
5. Janardan J, Gibbs H. Combining anticoagulation and antiplatelet drugs in coronary artery disease. *Aust Prescr* 2018; 41: 111–115.
6. Goto A *et al.* Hemoglobin A1c levels and the risk of cardiovascular disease in people without known diabetes: a population-based cohort study in Japan. *Medicine (Baltimore)* 2015; 94: e785.
7. Steiner G. Fibrates and coronary risk reduction. *Atherosclerosis* 2005; 182: 199–207.
8. Chou R *et al.* Statins for prevention of cardiovascular disease in adults: evidence Report and systematic review for the US preventive services task force. *JAMA* 2016; 316: 2008–2024.
9. Goff DC *et al.* 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2014; 63: 2935–2959.
10. Satoh M *et al.* Cellular and molecular mechanisms of statins: an update on pleiotropic effects. *Clin Sci* 2015; 129: 93.
11. Bruikman CS *et al.* New Drugs for Atherosclerosis. *Can J Cardiol* 2017; 33: 350–357.
12. Boekholdt SM *et al.* Very low levels of atherogenic lipoproteins and the risk for cardiovascular events: a meta-analysis of statin trials. *J Am Coll Cardiol* 2014; 64: 485–494.
13. Zaheer M *et al.* Hypertension and atherosclerosis: pathophysiology, mechanisms and benefits of BP

control. In: Andreadis E, ed. *Hypertension and Cardiovascular Disease*. Cham: Springer, 2016: 201–216.
14. Thompson AM *et al.* Antihypertensive treatment and secondary prevention of cardiovascular disease events among persons without hypertension: a meta-analysis. *JAMA* 2011; 305: 913–922.
15. Solanki A *et al.* Evolving targets for the treatment of atherosclerosis. *Pharmacol Ther* 2018; 187: 1–12.
16. Ridker PM *et al.* Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017; 377: 1119–1131.
17. Ridker PM *et al.* Low-dose methotrexate for the prevention of atherosclerotic events. *N Engl J Med* 2019; 380: 752–762.
18. Keyserling CH *et al.* Abstract 15525: CER-001, a synthetic HDL-mimetic, safely mobilizes cholesterol in healthy dyslipidemic volunteers. *Circulation* 2011; 124(Suppl 21): A15525.
19. Sean M, Shanta D. Biodegradable synthetic high- density lipoprotein nanoparticles for atherosclerosis. *Proc Natl Acad Sci USA* 2013; 110: 9445.
20. Tardif J-C *et al.* Effects of the high-density lipoprotein mimetic agent CER-001 on coronary atherosclerosis in patients with acute coronary syndromes: a randomized trial†. *Eur Heart J* 2014; 35: 3277–3286.
21. Guo Y *et al.* Synthetic high-density lipoprotein-mediated targeted delivery of liver X receptors agonist promotes atherosclerosis regression. *EBioMedicine* 2018; 28: 225–233.
22. Zhang M *et al.* Plaque-hyaluronidase-responsive high-density-lipoprotein-mimetic nanoparticles for multistage intimal-macrophage-targeted drug delivery and enhanced anti-atherosclerotic therapy. *Int J Nanomed* 2017; 12: 533–558.
23. Zhao Y *et al.* Multifunctional dextran sulfate-coated reconstituted high density lipoproteins target macrophages and promote beneficial antiatherosclerotic mechanisms. *Bioconjug Chem* 2017; 28: 438–448.
24. Sanchez-Gaytan B *et al.* HDL-mimetic PLGA nanoparticle to target atherosclerosis plaque macrophages. *Bioconjug Chem* 2015; 26: 443–451.
25. Raphaël D *et al.* A statin- loaded reconstituted high- density lipoprotein nanoparticle inhibits atherosclerotic plaque inflammation. *Nat Commun* 2014; 5: 3065.
26. Kheirolomoom A *et al.* Multifunctional nanoparticles facilitate molecular targeting and miRNA delivery to inhibit atherosclerosis in ApoE(−/−) mice. *ACS Nano* 2015; 9: 8885–8897.
27. Majmudar DM *et al.* Monocyte-directed RNAi targeting CCR2 improves infarct healing in atherosclerosis-prone mice. *Circulation* 2013; 127: 2038–2046.
28. Denis GM *et al.* Gene inactivation of proprotein convertase subtilisin/kexin type 9 reduces atherosclerosis in mice. *Circulation* 2012; 125: 894–901.
29. Fitzgerald K *et al.* Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled, phase 1 trial. *Lancet* 2014; 383: 60–68.
30. Gundogdu E *et al.* Nanomedicine for the diagnosis and treatment of cardiovascular disease: current status and future perspective. In: iConcept Press, ed. *Cardiovascular Disease*. Hong Kong: Concept Press Ltd., 2014: 187–201.
31. Lewis DR *et al.* Polymer-based therapeutics: nanoassemblies and nanoparticles for management of atherosclerosis. *Wiley Interdiscip Rev* 2011; 3: 400–420.
32. Iancu C. Photothermal therapy of human cancers (PTT) using gold nanoparticles. *Biotechnol Mol Biol Nanomed* 2013; 1: 53–60.
33. Mukherjee S *et al.* Potential theranostics application of bio-synthesized silver nanoparticles (4-in-1 system). *Theranostics* 2014; 4: 316.
34. Popat A *et al.* Enzyme-responsive controlled release of covalently bound prodrug from functional mesoporous silica nanospheres. *Angew Chem* 2012; 124: 12654–12657.
35. Wang C-F *et al.* Multifunctional porous silicon nanoparticles for cancer theranostics. *Biomaterials* 2015; 48: 108–118.
36. McCarthy JR *et al.* A light-activated theranostic nanoagent for targeted macrophage ablation in inflammatory atherosclerosis. *Small* 2010; 6: 2041–2049.
37. Naahidi S *et al.* Biocompatibility of engineered nanoparticles for drug delivery. *J Controlled Release* 2013; 166: 182–194.
38. Choi R *et al.* Thiolated dextran-coated gold nanorods for photothermal ablation of inflammatory macrophages. *Langmuir* 2010; 26: 17520–17527.
39. Kelly C *et al.* Targeted liposomal drug delivery to monocytes and macrophages. *J Drug Deliv* 2011; 2011: 727241.
40. Swindell WR *et al.* Heterogeneity of inflammatory and cytokine networks in chronic plaque psoriasis. *PLoS One* 2012; 7: e34594.
41. Patel SK, Janjic JM. Macrophage targeted theranostics as personalized nanomedicine strategies for inflammatory diseases. *Theranostics* 2015; 5: 150.
42. Dolmans DE *et al.* Photodynamic therapy for cancer. *Nat Rev Cancer* 2003; 3: 380.
43. Celli JP *et al.* Imaging and photodynamic therapy: mechanisms, monitoring, and optimization. *Chem Rev* 2010; 110: 2795–2838.
44. Detty MR *et al.* Current clinical and preclinical photosensitizers for use in photodynamic therapy. *J Med Chem* 2004; 47: 3897–3915.
45. Peng C *et al.* Detection and photodynamic therapy of inflamed atherosclerotic plaques in the carotid artery of rabbits. *J Photochem Photobiol B* 2011; 102: 26–31.
46. McCarthy JR *et al.* A macrophage-targeted theranostic nanoparticle for biomedical applications. *Small* 2006; 2: 983–987.
47. Ta H *et al.* Activatable magnetic resonance nanosensor as a potential imaging agent for detecting and discriminating thrombosis. *Nanoscale* 2018; 10: 15103–15115.
48. Ta HT. Activatable magnetic resonance nanosensor as a potential

imaging agent for detecting and discriminating thrombosis. *Atherosclerosis* 2018; 32: 159.
49. Gaston E *et al.* Nanomaterials in the treatment of internal bleeding and uncontrolled haemorrhage. *Nanomedicine* 2018; 14: 507–519.
50. Ta HT *et al.* Effects of magnetic field strength and particle aggregation on relaxivity of ultra-small dual contrast iron oxide nanoparticles. *Mater Res Express* 2017; 4: 116105.
51. Ta HT *et al.* Self-confirming molecular imaging of activated platelets via iron oxide nanoparticles displaying unique dual MRI contrast. *Atherosclerosis* 2017; 263: e146.
52. Ta HT *et al.* Molecular imaging of activated platelets via antibody-targeted ultra-small iron oxide nanoparticles displaying unique dual MRI contrast. *Biomaterials* 2017; 134: 31–42.
53. Ta HT *et al.* Targeted molecular imaging and cell homing in cardiovascular disease via antibody-sortagging. *Atherosclerosis* 2015; 241: E26.
54. Hagemeyer CE *et al.* Particle generation, functionalization and sortase a-mediated modification with targeting antibodies for diagnostic and therapeutic use. *Nat Protoc* 2015; 10: 90–105.
55. Ta HT *et al.* Enzymatic antibody tagging: towards a universal biocompatible targeting tool. *Trends Cardiovasc Med* 2012; 22: 105–111.
56. Ta HT *et al.* Enzymatic single-chain antibody tagging: a universal approach to targeted molecular imaging and cell homing in cardiovascular disease. *Circ Res* 2011; 109: 365–373.
57. Ta HT *et al.* Antibody-sortagging: a universal approach towards targeted molecular imaging and cell homing in cardiovascular disease. *Circ Res* 2010; 107: e32–e40.
58. Ta HT *et al.* A novel biotechnological approach for targeted regenerative cell therapy and molecular imaging of atherothrombosis. *Heart Lung Circ* 2010; 19: S10.
59. Ta HT *et al.* Novel bionanotechnological solutions based on metal oxide and metal to preserve and assess organs for transplantation. *Cryobiology* 2018; 81: 233.
60. Shon S-M *et al.* Photodynamic therapy using a protease-mediated theranostic agent reduces cathepsin-B activity in mouse atheromata in vivo significance. *Arterioscler Thromb Vasc Biol* 2013; 33: 1360–1365.
61. Deguchi J-O *et al.* Inflammation in atherosclerosis: visualizing matrix metalloproteinase action in macrophages in vivo. *Circulation* 2006; 114: 55–62.
62. Choi Y *et al.* Selective antitumor effect of novel protease-mediated photodynamic agent. *Can Res* 2006; 66: 7225–7229.
63. Rai P *et al.* Development and applications of photo-triggered theranostic agents. *Adv Drug Deliv Rev* 2010; 62: 1094–1124.
64. Lim YT *et al.* Diagnosis and therapy of macrophage cells using dextran-coated near-infrared responsive hollow-type gold nanoparticles. *Nanotechnology* 2008; 19: 375105.
65. Ma LL *et al.* Small multifunctional nanoclusters (nanoroses) for targeted cellular imaging and therapy. *ACS Nano* 2009; 3: 2686–2696.
66. Qin J *et al.* Gold nanorods as a theranostic platform for in vitro and in vivo imaging and photothermal therapy of inflammatory macrophages. *Nanoscale* 2015; 7: 13991–14001.
67. Tong L *et al.* Gold nanorods as contrast agents for biological imaging: optical properties, surface conjugation and photothermal effects. *Photochem Photobiol* 2009; 85: 21–32.
68. Pissuwan D *et al.* Targeted destruction of murine macrophage cells with bioconjugated gold nanorods. *J Nanopart Res* 2007; 9: 1109–1124.
69. Loo C *et al.* Nanoshell-enabled photonics-based imaging and therapy of cancer. *Technol Cancer Res Treat* 2004; 3: 33–40.
70. El-Sayed IH *et al.* Selective laser photo-thermal therapy of epithelial carcinoma using anti-EGFR antibody conjugated gold nanoparticles. *Cancer Lett* 2006; 239: 129–135.
71. Yeager D *et al.* Intravascular photoacoustics for image-guidance and temperature monitoring during plasmonic photothermal therapy of atherosclerotic plaques: a feasibility study. *Theranostics* 2014; 4: 36.
72. Kosuge H *et al.* Near infrared imaging and photothermal ablation of vascular inflammation using single-walled carbon nanotubes. *J Am Heart Assoc* 2012; 1: e002568.
73. Burnett SH *et al.* Conditional macrophage ablation in transgenic mice expressing a Fas-based suicide gene. *J Leukoc Biol* 2004; 75: 612–623.
74. Lobatto ME *et al.* Multimodal clinical imaging to longitudinally assess a nanomedical anti-inflammatory treatment in experimental atherosclerosis. *Mol Pharm* 2010; 7: 2020–2029.
75. Banciu M *et al.* Anti-angiogenic effects of liposomal prednisolone phosphate on B16 melanoma in mice. *J Controlled Release* 2006; 113: 1–8.
76. Banciu M *et al.* Antitumor activity of liposomal prednisolone phosphate depends on the presence of functional tumor-associated macrophages in tumor tissue. *Neoplasia* 2008; 10: 108–117.
77. Metselaar JM *et al.* Complete remission of experimental arthritis by joint targeting of glucocorticoids with long-circulating liposomes. *Arthritis Rheum* 2003; 48: 2059–2066.
78. Park D *et al.* Hyaluronic acid–polypyrrole nanoparticles as pH-responsive theranostics. *Chem Commun* 2014; 50: 15014–15017.
79. Nandwana V *et al.* High-density lipoprotein-like magnetic nanostructures (HDL-MNS): theranostic agents for cardiovascular disease. *Chem Mater* 2017; 29: 2276–2282.
80. Bagalkot V *et al.* Hybrid nanoparticles improve targeting to inflammatory macrophages through phagocytic signals. *J Controlled Release* 2015; 217: 243–255.
81. Cailhier JF *et al.* Conditional macrophage ablation demonstrates that resident macrophages initiate acute peritoneal inflammation. *J Immunol* 2005; 174: 2336–2342.
82. Harel-Adar T *et al.* Modulation of cardiac macrophages by phosphatidylserine-presenting liposomes improves infarct repair. *Proc Natl Acad Sci USA* 2011; 108: 1827–1832.

83. Leuschner F *et al.* Therapeutic siRNA silencing in inflammatory monocytes in mice. *Nat Biotechnol* 2011; 29: 1005.
84. Winter PM *et al.* Endothelial $\alpha_v\beta_3$ integrin–targeted fumagillin nanoparticles inhibit angiogenesis in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2006; 26: 2103–2109.
85. Winter PM *et al.* Antiangiogenic synergism of integrin-targeted fumagillin nanoparticles and atorvastatin in atherosclerosis. *JACC Cardiovasc Imaging* 2008; 1: 624–634.
86. Wu W *et al.* Comparison of magnetic microbubbles and dual-modified microbubbles targeted to P-selectin for imaging of acute endothelial inflammation in the abdominal aorta. *Mol Imag Biol* 2017; 19: 183–193.
87. Oumzil K *et al.* Solid lipid nanoparticles for image-guided therapy of atherosclerosis. *Bioconjug Chem* 2016; 27: 569–575.
88. Dong Y *et al.* Polymer-lipid hybrid theranostic nanoparticles co-delivering ultrasmall superparamagnetic iron oxide and paclitaxel for targeted magnetic resonance imaging and therapy in atherosclerotic plaque. *J Biomed Nanotechnol* 2016; 12: 1245–1257.
89. Wu Y *et al.* Novel iron oxide-cerium oxide core-shell nanoparticle as a potential theranostic material for inflammatory diseases. *J Mater Chem B* 2018; 6: 4937–4951.
90. Zhao H-X *et al.* Theranostic metal–organic framework core–shell composites for magnetic resonance imaging and drug delivery. *Chem Sci* 2016; 7: 5294–5301.