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Multifunctional agents for concurrent imaging and therapy in cardiovascular disease

Jason R. McCarthy

Center for Systems Biology and Center for Molecular Imaging Research, Harvard Medical School and Massachusetts General Hospital, 149 13th St., Rm 6229, Charlestown, MA 02129, USA

Abstract

The development of agents for the simultaneous detection and treatment of disease has recently gained significant attention. These multifunctional theranostic agents possess a number of advantages over their monofunctional counterparts, as they potentially allow for the concomitant determination of agent localization, release, and efficacy. Whereas the development of these agents for use in cancers has received the majority of the attention, their use in cardiovascular disease is steadily increasing. As such, this review summarized some of the most poignant recent advances in the development of theranostic agents for the treatment of this class of diseases.

Keywords

Cardiovascular disease; theranostic; nanoparticle; microparticle; imaging; therapy; atherosclerosis; thrombosis; restenosis

1. Introduction

Cardiovascular disease (CVD) encompasses a class of diseases that involves the heart and vasculature. Most often, when people refer to CVD, they are actually making reference to atherosclerotic vascular disease and its sequelae, including myocardial infarction and stroke. For decades, CVD has been the leading cause of mortality in the United States, as diseases of the heart are responsible for more deaths each year than cancer (26.6% versus 22.8%), resulting in almost 1800 deaths per day [1]. When stroke is included, CVD accounts for virtually 1/3 of all deaths. Given these facts there is a pressing need to develop novel techniques for the early detection and treatment of CVD.

One class of compounds that may prove useful in the treatment of CVD incorporates both diagnostic and therapeutic functionalities. These theranostic agents are exceptional, in that they allow for feedback mechanisms to determine the localization, release, and therapeutic efficacy of treatments. In certain circumstances, they may also provide for the simultaneous diagnosis and treatment of disease. While the advent of nanomedicine has facilitated the creation of multifunctional theranostic nanoagents, these compounds can be as simple as the labeling of a drug moiety, such as recombinant tissue plasminogen activator (rtPA), with a fluorophore or radionuclide.

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Tel.: 617-726-9218 Fax.: 617-726-5708 jason_mccarthy@hms.harvard.edu.

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While CVD is responsible for the largest proportion of deaths, it is highly underrepresented in the development of theranostic agents, with the bulk being created for cancers. This is surprising, given the numerous barriers that must be traversed to deliver agents to tumors. Vascular targets, on the other hand, are for the most part readily accessible upon intravenous administration. Due in part to this fact, there has recently been an escalation in the number of reports of theranostic agents for the treatment of CVD. Herein, this review will highlight some of the most poignant advances in the development of theranostic agents for the treatment of this class of diseases.

2. Atherosclerosis

Atherosclerosis is an insidious disease occurring over a number of decades that often goes undetected until the onset of clinical symptoms. Atherosclerotic lesions offer a plethora of potential targets, including specific inflammatory cell types, and the upregulation of a number of cell surface receptors, such as $\alpha_v\beta_3$ -integrin and vascular cell adhesion molecule-1 (VCAM-1). This multitude of targets has allowed for the generation of numerous agents for the detection of the disease, particularly with multimodal nanoagents (see table 1), yet the ability to concomitantly treat the disease has not been fully realized. This section will focus upon the latest methodologies that have been utilized for the development of theranostic nanoagents for atherosclerotic vascular disease.

2.1. Antiangiogenic treatment of atherosclerosis

Angiogenesis is a key component of the progression of atherosclerotic vascular disease, as neovasculature is formed in response to hypoxia [13], proangiogenic growth factors [14], and oxidative stress [15]. Importantly, increased angiogenesis has been correlated with plaque growth, intraplaque hemorrhage, and lesion instability. During the process of neovascularization, integrins, such as $\alpha_v\beta_3$, are dramatically upregulated on endothelial cells [16], and thus serve as an ideal target for the delivery of antiangiogenic drugs. This methodology has also proven useful in other diseases, including cancers [17].

Wickline and Lanza have developed gadolinium-loaded perfluorocarbon (PFC) nanoparticles targeted to $\alpha_v\beta_3$ -integrin via a peptidomimetic vitronectin antagonist, that have previously demonstrated excellent utility as T_1 magnetic resonance (MR) contrast agents for the detection of atherosclerotic lesions [18]. The particles are comprised of perfluorooctylbromide emulsions encapsulated within a lipid-surfactant monolayer. Lipophilic diethylenetriamine pentaacetic acid-bis-oleate chelates of gadolinium were also incorporated in the nanostructure (~ 90,000 per particle) in order to effect MR contrast. These particles are also capable of incorporating lipophilic drugs within their cores for delivery to sites of interest [19]. Drug delivery from this class of nanoparticles has been termed “contact facilitated drug delivery,” as the drug moiety is transferred from the lipid to the target cell via a hemifusional complex with the outer leaf of the cell membrane [20]. In order to generate nanoagents for the inhibition of the neovascularization of atherosclerotic lesions, the authors have incorporated the antiangiogenic drug fumagillin within the targeted PFC nanoparticles [21]. The impetus for this study is the observation in animal models that chronically high systemic doses of a water-soluble version of fumagillin resulted in a decrease in neovascularization and plaque development, thus a targeted nanoagent may allow for localized delivery requiring decreased dosing.

In vivo studies of nanoagent targeting and efficacy were performed in New Zealand white (NZW) rabbits on high cholesterol diet in order to promote atherogenesis. The rabbits were subject to magnetic resonance (MR) imaging prior to administration of the nanoagent, and 4 hours post-administration, in order to compare pre- and post-contrast images (Figure 1 middle). As compared to control particles bearing no targeting ligands, the targeted particles

with or without fumagillin incorporated displayed increased signal in the aortic wall (10.8%, 16.7%, and 16.7% respectively). According to the authors, the increased contrast from the untargeted particles may be due to the slow washout kinetics of the particles (300 minute blood half-life), giving a small degree of background signal. One week following the antiangiogenic treatment, pre- and post-contrast images were again acquired with the non-drug encapsulating targeted particles in order to assess the degree of angiogenesis in the treated and control rabbits (Figure 1 bottom). As was hoped, the targeted particles containing fumagillin resulted in a significant decrease in MR signal enhancement (2.9%) versus the control particles without the drug (18.1%). These results were correlated histologically, with the treated animals exhibiting greater than 50% decrease in PECAM-positive microvessels per section. Importantly, as the authors had originally hypothesized, efficacious nanoparticulate delivery of fumagillin was possible at a dose 50,000 times lower than a comparably effective cumulative oral dose.

More recently, Lanza and Wickline investigated the durability of the antiangiogenic effect that the theranostic nanoagent had exhibited [22]. Surprisingly, after the initial decrease in neovascularization at the 1 week time point, the presence of $\alpha_v\beta_3$ -integrin increased steadily over the next three weeks, until it reached the same level as observed in the control, non-treated animals. In order to facilitate a more durable effect, the authors included a statin (atorvastatin) as an adjuvant to the theranostic nanoparticles. In the initial 8 week study, the animals were administered the fumagillin-containing nanoagent at 0 weeks concomitant with dietary atorvastatin, but the therapeutic regime did not elicit a positive antiangiogenic effect. Yet, when the animals were administered the nanoagent twice, at 0 and 4 weeks, in addition to the dietary atorvastatin, it resulted in a sustained decrease in neovascularization.

Fumagillin has also been incorporated into colloidal iron oxide nanoparticle (CION) emulsions [23]. As opposed to gadolinium, which is an effective T_1 contrast agent, iron oxide nanoparticles are effective at shortening T_2 relaxation times, resulting in negative contrast. Interestingly, when oleic acid-coated iron oxide nanoparticles are suspended in almond oil, and subsequently encapsulated within a crosslinked phospholipid-surfactant co-mixture, the T_2 effect is diminished, and the CION become a highly sensitive T_{1w} contrast agent. These particles were capable of 98–99% drug encapsulation efficiency, with less than 1% release after 3 days, when dialyzed against an infinite sink. Further research to validate the drug delivery effectiveness of these particles is currently underway.

2.2. Focal macrophage ablation

The macrophage is a major component of atherosclerotic lesions, and is responsible for the release of inflammatory cytokines and proteases, which further promote the inflammatory process, and degrade the fibrous cap, respectively, making the plaque prone to rupture [24–27]. Thus the macrophage may serve as an ideal target for the imaging and therapy of atherosclerosis. In particular, the focal ablation of macrophages may produce a durable stabilization of vulnerable atherosclerotic lesions.

Theranostic nanomaterials based upon the conjugation of chlorin-based near infrared light activated therapeutic (NILAT) moieties to macrophage avid magnetofluorescent nanoparticles (MNP) have been developed [28,29]. These particles, based upon crosslinked dextran coated iron oxide nanoparticles, have been utilized in the imaging of numerous diseases and conditions with inflammatory components, including cancers and atherosclerosis [30,31]. In addition to the NILAT, which is photoactivated at 650 nm, a spectrally distinct fluorophore, AlexaFluor 750, was also included in the preparation in order to allow for the optical determination of particle localization prior to the induction of therapy. The phototoxicity of the agent was initially tested in vitro in RAW 264.7 murine

macrophages, and demonstrated LD₅₀ of 14 nM, significantly less than the commonly utilized chlorin e₆ (800 nM).

When injected into atherosclerotic lesion-laden apoE^{-/-} mice, the nanoagent readily localized to atherosclerotic lesions within 24 h, as visualized by intravital fluorescence microscopy in the surgically exposed carotid artery (Figure 2). After the initial imaging session, the carotid artery lesion was irradiated with a 650 nm laser in order to elicit a therapeutic response, at which time the surgical incision was closed. One day after therapy, one cohort of mice was sacrificed and their carotid arteries were subject to histological examination, revealing a dramatic difference in the number of cells undergoing apoptosis. The other cohorts were allowed to recover for one week, at which time the nanoagent was reinjected, the surgical incision was reopened, and the animals imaged. In the treated cohort, there was minimal reuptake of the agent, intimating the focal ablation of the inflammatory macrophages. The plaque stabilizing therapeutic efficacy of this strategy is still under investigation.

2.3. Agents for the prevention of restenosis

Stenosis is the narrowing of a vessel and can be resultant of occlusive atherosclerotic lesions. Clinically, treatment involves percutaneous angioplasty and the placement of a stent. Unfortunately, the damage caused by this intervention can lead to restenosis, due to normal physiological processes for vessel repair, as well as the identification of the stent as a foreign body. Systemic administration of drugs to prevent restenosis have been met by a number of drawbacks, including the inability to localize enough of the agent to site of damage, and systemic toxicity. Drug eluting stents have been developed to ameliorate the observed delivery issues, allowing for the localized delivery of antiproliferative agents, yet have a few potential drawbacks, such as local toxicity and polymer-induced inflammatory responses, which delay vascular healing, thereby increasing the rates of late thrombotic events [32]. It would thus be important to develop therapeutic strategies for the systemic administration of targeted agents for the prevention of restenosis without these deleterious side effects.

A small number of targeted nanoagents have been developed for the prevention of restenosis. Joner *et al.* have developed cationic liposomal nanoparticles that specifically bind to chondroitin sulfate proteoglycans that are expressed within the subendothelial matrix but not vascular endothelial cells [33]. In areas of vascular injury where the endothelial cell barrier is compromised, the anionic proteoglycans are exposed, readily facilitating localization of the nanoagent. The utilized liposomes were comprised of pegylated 3,5-dipentadecyloxybenzamidinium hydrochloride, hydrogenated soy phosphatidylcholine, and cholesterol, incorporating a fluorescent dye, Lissamine rhodamine B, and encapsulating the corticosteroid prednisolone disodium phosphate [34]. The nanoagent was tested in hyperlipidemic NZW rabbits that underwent balloon injury of the iliac artery, followed by artery stenting with a bare metal stent two months later. Initial investigation of the ability of the liposomes to localize to the damaged vessel was performed histologically 24 h after administration. As compared to non-stented vessels, the stented artery demonstrated 100-fold higher accumulation, which remained higher over the course of the study. This localization of the agent resulted in a significant decrease in stenosis (24% reduction as compared to control animals), and inflammation. Importantly, there was no difference in the endothelialization of the vessel, indicating that the therapeutic strategy did not delay healing.

While the above study demonstrated that the synthesis of a theranostic nanoparticle for the treatment of restenosis is feasible, the authors did not take full advantage of the imaging component that was included within the liposome to determine *in vivo* localization. Cyrus *et al.*, on the other hand, have utilized the $\alpha_v\beta_3$ -integrin targeted paramagnetic PFC

nanoparticles described above, and incorporated the antiproliferative agent, rapamycin [35]. Initial studies into the retention of the drug within the nanoparticle revealed only 1% was released per day, when dialyzed against an infinite sink. The nanoagent was next tested in vivo in atherosclerotic NZW rabbits. Five minutes after balloon overstretch injury to the femoral artery, the theranostic agent, a targeted control agent without the drug, or an untargeted particle containing rapamycin were administered. Noninvasive imaging of agent localization 30 minutes after injection revealed significant accumulation within the damaged artery of the targeted particles, as opposed to the non-targeted particle which demonstrated no signal enhancement. The therapeutic efficacy of the particles was ascertained by sacrificing cohorts of the rabbits 2 weeks after the intervention, and analyzing the vessel histologically (Figure 3). As compared to the control particles or saline, the targeted theranostic nanoagent demonstrated an inhibition of stenosis, yet no delay in endothelial healing, thereby illustrating the potential benefit of these antirestenotic agents.

3. Thrombosis

Thrombosis is the formation of a clot within a vessel, which results in the obstruction of blood flow. Upon initial vessel injury, tissue factors promote the coagulation of platelets to form the initial clot, which is subsequently stabilized with fibrin [36]. Commonly, thrombosis is caused by endothelial cell damage, as is seen in the rupture of vulnerable atherosclerotic plaques. When a thrombus occupies more than 75% of surface area of the lumen of an artery, blood flow distal to the obstruction is affected causing hypoxia. When this blockage reaches 90%, this can result in anoxia and infarction. Additionally, if this clot, or pieces thereof become dislodged, they are able to travel through the circulation, eventually becoming lodged and occluding blood flow. In the absence of treatment, this can lead to tissue necrosis.

3.1. Targeted anticoagulants for the prevention of thrombosis

The main cause of the mortality associated with atherosclerotic vascular disease is plaque rupture, and subsequent thrombosis. Throughout atherogenesis, subtle clotting occurs within lesions, especially in the shoulder regions where the plaques are known to be prone to rupture. Peters *et al.* have developed modular multifunctional micelles targeted to fibrin and incorporating the anticoagulant drug hirulog [37]. Hirulog is a small synthetic peptide derived from the active site of the natural thrombin inhibitor hirudin, and is a direct thrombin inhibitor. The micelles were synthesized from a lipopeptide monomer consisting of a 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) tail, a PEG2000 spacer, and a variable head group. This head group can include each of the following alone or in combination: a near infrared fluorophore, the hirulog peptide, or a targeting peptide CREKA, which was originally identified via in vivo phage screening for tumor homing, and later identified to bind to clotted plasma proteins.

When injected into atherosclerotic lesion-laden apolipoprotein E deficient mice (apoE^{-/-}), the CREKA-targeted micelles readily localized to plaques, as determined by ex vivo fluorescence reflectance imaging in excised aortas, and correlate histologically, with no binding to the healthy vasculature (Figure 4). In fact, the micelles were found to be concentrated in the rupture prone shoulder regions of the lesions. When the micelles containing all three head groups were injected and allowed to circulate for 3 hours, they demonstrated identical accumulation to micelles containing only the CREKA targeting moiety. The excised aortic tree of the treated rabbits was further examined, and demonstrated significantly higher antithrombin activity than in mice with untargeted micelles (1.8 and 1.2 µg/mg of tissue, respectively). According to the authors, the targeted localization of an anticoagulant to the rupture prone shoulder region may be potentially useful in reducing the risk of thrombus formation.

3.2. Thrombolytic therapies

In thrombosis syndromes, it is of the utmost importance to restore blood flow in order to prevent end-organ tissue death due to hypoxia. Pharmacologically, this is attempted via the administration of thrombolytic agents that activate the fibrinolytic system in order to effect the dissolution of the blood clot. These plasminogen activators (PA) convert the proenzyme plasminogen into an active enzyme, plasmin, allowing for the digestion of fibrin into its soluble degradation products. Plasminogen is released from the liver and is normally found in circulation. During thrombogenesis, fibrin presents binding sites for plasminogen, promoting its localization within thrombi.

Thrombolytics can be classified into two groups, namely fibrin-selective and fibrin non-selective [38]. The fibrin non-selective agents, such as streptokinase and two-chain u-PA (tcu-PA), activate both plasminogen in the circulating blood and fibrin-bound plasminogen. When generated in the blood, this plasmin is rapidly inactivated by α_2 -antiplasmin until such time that the inhibitor is saturated, at which time it begins to degrade blood proteins. Fibrin-selective agents, such as recombinant tissue plasminogen activator (rtPA), single chain urokinase-PA, and staphylokinase, on the other hand, only activate the zymogen present within thrombus. The generated plasmin remains within the thrombus, shielded from inhibition, allowing for an increased therapeutic effect.

In addition to lack of efficacy, additional limitations of existing fibrinolytic therapies include bleeding complications that can be life-threatening [39]. In a pivotal stroke trial, treatment with rtPA resulted in symptomatic intracerebral hemorrhage (ICH) rate in 6.4% of patients, as compared to 0.6% of those given placebo. In a more recent analysis of rtPA therapies for acute myocardial in the United States, the ICH rate was 0.93% [40]. From this group, 55% of these patients died and among those surviving to discharge, 55% had a residual neurological deficit. This data further confirmed the findings from prior clinical trials that ICH is a serious complication of thrombolytic therapies. It is therefore necessary to focus on the creation of novel thrombolytics with increased efficacy, greater therapeutic windows, and vastly improved safety profiles.

3.2.1. Thrombolytics as imaging agents—As in most diseases, the simplest theranostic agents for the treatment of thrombosis are based upon the labeling of a therapeutic molecule with an imaging moiety. For example, several radiolabeled rtPA analogs have been reported, including those labeled with ^{99m}Tc and ^{123}I , although their full potential as theranostic agents has not been utilized [41–44]. rtPA possesses two functional sites; one which binds to fibrin, and another which is responsible for the conversion of plasminogen to plasmin. Thus, rtPA can be utilized to image fibrin within thrombi. Two methodologies have been utilized to create these agents. In the first case, the radionuclide ^{123}I was appended to the tripeptide chloromethyl ketone (YPACK), which is an irreversible inhibitor of the catalytic rtPA active site [44]. Thus, incubation of rtPA with the inhibitor yielded a fibrin nuclear imaging agent that was unable to perform thrombolysis. Butler and coworkers also utilized chloromethyl ketone (PPACK) inactivated rtPA in the creation of their imaging agents, yet further modified the enzyme in order to append a ^{99m}Tc chelate [41–43]. In their most recent work, the authors were able to illustrate how the synthesized imaging agent could be utilized to determine the age of acute deep vein thrombi, a cause of significant morbidity and mortality due to the increased likelihood of pulmonary embolism, or other associated complications [41].

3.2.2. Theranostic micro- and nanoparticulate agents—A number of strategies to deliver thrombolytics to sites of action, while concomitantly diminishing the associated detrimental side effects such as ICH, have been developed. With regard to being able to

simultaneously detecting agent localization, these theranostic nanoagents have mainly fallen into two categories; ultrasound contrast or MR imaging agents.

Ultrasound contrast agents, such as microbubbles of perfluorocarbon nanoparticles are well suited for vascular delivery of drugs, including thrombolytics. Echogenic liposomes (ELIP) are commonly comprised of mixtures of phospholipids, such as dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, dipalmitoylphosphatidylglycerol, and cholesterol, and are formed by a sonication-lyophilization-rehydration methodology. In this methodology, the lipids are initially dissolved in an organic solvent, such as chloroform, which is allowed to evaporate, leaving behind a lipid film, which is subsequently hydrated under sonication to form the liposomes. The echogenicity of the liposomes is derived from the entrapment of gases, such as air or a perfluorinated gas (e.g. perfluoropropane) within the aqueous compartment. This is also where thrombolytic drugs, such as rtPA are expected to be localized upon encapsulation within the liposome. One of the more interesting facets of these microbubble formulations is the ability to use insonification to induce liposomal disruption, and subsequently localized release of the encapsulated drug.

Holland and coworkers have synthesized air-entrapping ELIP containing rtPA via the hydration of the lipid film with a solution of the drug under sonication [45,46]. This resulted in a maximum loading efficiency of 50%, with 15% located within the core and an additional 35% associated with the lipid bilayer. In their initial report, the authors demonstrated in vitro that the liposomal formulation of the drug was readily detectable by ultrasound imaging, and that, in the absence of ultrasound, that the liposomes were as efficacious as free rtPA at lysing clots. When the clots, in the presence of the rtPA-loaded ELIPs, were subject to 2 minutes of insonification, there was an almost 50% increase in thrombolysis. The authors subsequently followed up on this initial report with a study designed to look at the ultrasound energies required to effect release of rtPA from the liposomes [45]. Interestingly, they found that two different mechanisms can be utilized, with one arising from the rapid fragmentation of the particles at higher mechanical indexes, and the other from acoustically driven diffusion at lower energies. The former mechanism resulted in a rapid and more complete release of the thrombolytic drug, with a concomitant cessation of echogenicity. The latter retained some degree of echogenicity for up to 60 minutes of insonification, with a lesser degree of total rtPA release.

Hua *et al.* have taken the above methodologies one step further by developing targeted perfluoropropane-containing liposomes targeted to activated platelets via a peptide (RGDS) derived from the α -chain of fibrinogen [47]. Initially, the peptide, appended to the terminus of an amine-modified polyethylene glycol, and rtPA were each modified with distinct fluorescent labels to enable tracking of the microbubbles and thrombolytic drug, which were subsequently encapsulated within the liposome via the sonication-lyophilization-rehydration methodology described above. After the microbubbles were formed, the vial was purged of air, and saturated with perfluoropropane in order to increase the echogenicity. When injected into healthy rabbits, the agent was readily visualized within the liver using ultrasound imaging. When the thrombolytic potential of the synthesized agent was examined, it proved to be superior to the administration of rtPA alone, yet demonstrated comparable efficacy to a mixture of free rtPA and microbubbles, all under insonification. This may demonstrate the additive effect of microbubble rupture on the ultimate lysis of clots.

A number of theranostic nanoparticulate agents have also been reported. Marsh *et al.* have developed fibrin-targeted perfluorocarbon nanoparticles encapsulating streptokinase [48]. These particles, synthesized by an evaporation/dispersion technique resulted in the formation of nanoparticles ~250 nm in diameter that are echogenic, and thus useful for

ultrasound imaging. After their initial synthesis, the particles were further modified by covalent attachment of the thrombolytic drug streptokinase. The synthesized particles were initially examined for their binding to plasma clots via acoustic microscopy, which illustrated their binding to the clot surface. Penetration of the particles into the clot was not observed, presumably due to the density of the fibrin fibrils. Clots were next incubated with the streptokinase nanoparticles in the presence of, or in the absence of plasminogen, as well as control particles that were not streptokinase modified. As expected, the thrombolytic particles were capable of converting the plasminogen to plasmin, thereby lysing the clots, whereas the particles without streptokinase, or in the absence of plasminogen were not.

Thrombolytics have also been conjugated to the surface of superparamagnetic iron oxide nanoparticles. This nanopatform is interesting, in that it allows for the detection of agent localization via magnetic resonance imaging, as well as magnetic targeting via the application of external magnetic fields to trap the agents at sites of interest. This may have the added advantage of limiting the systemic activation of plasminogen, thereby minimizing side effects. Ma *et al.* have synthesized polyacrylic acid coated magnetic nanoparticles (MNP) to which was covalently conjugated rtPA [49]. The conjugate was initially characterized in vitro for its amidolytic and fibrinolytic activity, and demonstrated a minor decrease in activity versus the free thrombolytic drug. The magnetic targeting and therapeutic efficacy was next examined in a rat hind limb ischemia model (Figure 5). In this model, clots were administered into the left iliac artery in order to reduce the hind limb perfusion. Free rtPA, MNP-bound rtPA, and unmodified MNP were each administered to the rats, under magnetic guidance, and the skin perfusion was monitored by a laser Doppler perfusion imager (Figure 5). As compared to the controls, the magnetically targeted agent demonstrated a significantly greater degree of perfusion after only 30 minutes.

Bi *et al.* have also conjugated a thrombolytic to the surface of a MNP [50]. In their construct, epichlorohydrin-crosslinked dextran coated iron oxide nanoparticles were initially modified with glutaraldehyde, followed by reaction with urokinase (UK). Initial investigation of the enzyme activity using a chromogenic assay demonstrated that a significant amount the UK was associated with the nanoparticles, and that it retained its amidolytic activity. The particle was next assayed in vivo in a rat arteriovenous shunt thrombosis model, into which a silk thread was placed to promote thrombus formation. After 15 min of blood circulation through the shunt, the targeted particle, with and without magnetic targeting, or saline were injected into the rats and allowed to circulate for an additional 30 min, at which time the silk thread was removed, and the weight of the residual thrombus measured and compared. The authors found that the magnetically targeted particle was 5-fold more effective than saline, and 2.6-fold more effective than the untargeted particle. Importantly, the off-target effects of the therapy were also examined. This was accomplished by assaying the amount of residual fibrinogen in the blood, a marker for systemic plasminogen activation, and demonstrated that UK, by itself, resulted in a decrease to 72% of the value observed in non-treated mice, whereas magnetically targeted UK demonstrated a negligible decrease. Additionally, the authors examined the tail bleeding time of the animals to determine the propensity of the therapeutic strategy to promote systemic hemorrhage. As was demonstrated above, the delivery of the agent under magnetic targeting caused a negligible systemic activation of plasminogen, thus the bleeding time did not show a significant increase over baseline.

4. Future perspectives

The ability to detect and treat disease is the main concern of clinical medicine. With the advent of nanotechnology, and the generation of multifunctional agents, it becomes possible to perform both actions simultaneously. There are many advantages to this approach, such as

the ability to determine agent localization, release, or efficacy. There are also several drawbacks, such as a mismatch in the dosing required for imaging and therapy, and the fact that theranostic agents will only be applicable in certain circumstances as there is no need to administer a diagnostic moiety every time a patient receives a therapeutic drug. Reconciling these impediments will be the first major challenge for the field, which must be accomplished prior to this class of agents becoming clinically viable.

Thus far, the majority of research that has been performed has focused upon either the diagnosis of cardiovascular disease or the development of novel therapeutic strategies for its treatment, with few examples of truly theranostic agents. As is demonstrated by the recent articles contained within this review, this paradigm is shifting toward the generation of multifunctional agents, and can be expected to drive a renaissance in the treatment of disease.

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Figure 1. MRI of abdominal aorta showing outline of segmented region of interest (ROI) (top), false-colored overlay of percent signal enhancement at time of treatment (middle), and 1 week post-treatment (bottom). The color overlays are thresholded at 10% enhancement to show some anatomic detail within the ROI. Reproduced with permission from [21].

Figure 2.

Focal macrophage ablation. In vivo localization of the phototoxic nanoagent to carotid atheroma, as determined by intravital fluorescence microscopy. A) Fluorescence image in the AF750 channel demonstrating particle uptake by a carotid plaque. B) Fluorescence angiogram utilizing fluorescein-labeled dextran outlining the vasculature. C) Merged image of the two fluorescence channels.

Figure 3. Serial histological analysis of femoral arteries 2 weeks after balloon overstretch injury. Top, Treatment with $\alpha_v\beta_3$ -integrin-targeted rapamycin nanoparticles. Bottom, Serial sections of injured femoral segment after exposure to $\alpha_v\beta_3$ -integrin-targeted nanoparticles without drug. Serial cryosections stained with H&E represent 2-mm segments starting proximal to the injury (left) and ending distally (right). Lesion areas are depicted in green/orange and illustrate an irregular pattern of stenosis development and remodeling response along the injured vessel segments. Reproduced with permission from [35].

Figure 4.

Localization of CREKA micelles in atherosclerotic plaques. (A) Serial cross-sections (5 μm thick) were stained with antibodies against CD31 (endothelial cells; Top), CD68 (macrophages and other lymphocytes; Middle), and fibrin(ogen) (Bottom). Representative microscopic fields are shown to illustrate the localization of micelle nanoparticles in the atherosclerotic plaque. Micelles are bound to the entire surface of the plaque with no apparent binding to the healthy portion of the vessel. CREKA targeted micelles also penetrate under the endothelial layer (CD31 staining) in the shoulder of the plaque (Inset) where there is high inflammation (CD68 staining) and the plaque is prone to rupture. Clotted plasma proteins are seen throughout the plaque and its surface [fibrin(ogen) staining]. (Left) Images were taken at a 10 $\times$ magnification. (Scale bar, 200 μm .) (Right) Images were taken at a 150 $\times$ magnification. (Scale bar, 20 μm .) (B) Fluorescence was not observed in the heart or lung, and only a small amount was seen in the kidney, spleen, and liver. Images were taken at a 20 $\times$ magnification. (Scale bar, 100 μm .) Reproduced with permission from [37].

Figure 5.

MNP-bound rtPA improved tissue perfusion in a rat embolic model. Hind limb skin tissue perfusion of the rat was measured by a laser Doppler perfusion imager. After clot lodging into the left iliac artery, rtPA (0.2 mg/kg; 0.27 U/kg), MNP-bound rtPA (0.2 mg/kg; 0.22 U/kg) or equivalent MNP (2.5 mg/kg) was administered from the right iliac arterial 5 min after introducing the clot. Reproduced with permission from [49].

Table 1

Targeted nanoagents for the detection of atherosclerosis.

Target	Nanoagent	Imaging Modality	References
Macrophage	MFNP	Optical	[2,3]
	Radiolabeled MFNP	Nuclear/optical	[4]
	Iodinated nanoparticles	X-ray CT	[5,6]
	Nanocrystal Core HDL	MRI/CT/optical	[7]
Angiogenesis	Gd-labeled liposome	MRI	[8]
	Gd-labeled PFC nanoparticle	MRI	[9]
VCAM-1	MFNP	Optical/MRI	[10,11]
E-selectin	MFNP	MRI	[12]

MFNP - Magnetofluorescent nanoparticle; CT - computed tomography; HDL - high-density lipoprotein; MRI - magnetic resonance imaging; PFC - perfluorocarbon