

Review

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Nanomedicine in diagnostics and therapy of cardiovascular diseases: beyond atherosclerotic plaque imaging

Abstract: Atherosclerosis results from the accumulation of the modified lipoproteins within the artery walls, which triggers complex vascular inflammatory processes. Although the pharmacologic agents for the treatment of clinical manifestations of atherosclerosis are available, their systemic delivery has serious disadvantages, such as considerable side effects or low efficacy at tolerated doses. Moreover, the treatment of atherosclerosis using the interventional techniques bears further shortcomings: the implanted stents require a lengthy antiplatelet therapy and carry the risk of in-stent restenosis. In the surgical approach to atherosclerosis, apart from the overall risk of open heart surgery, the lack of adequate venous material for bypasses constitutes a common problem. The nanotechnology has the potential to overcome the disadvantages of the current therapy of atherosclerosis, e.g., by the formation of nanosized assemblies for the earlier detection of atherosclerotic lesions and for cell-specific delivery of therapeutics. Replacing the current systemic pharmacological approach by a locally targeted treatment of plaques can substantially minimize the adverse effects, by lowering the drug cytotoxicity and reducing the required dosage. Moreover, a new generation of nanotechnological approaches to the revascularization procedures is now emerging, e.g., vascular tissue engineering utilizing the magnetic nanoparticles or the design of stents with the reduced risk of thrombosis and restenosis. This review discusses the possible applications of the nanomedical approaches in the treatment of cardiovascular diseases.

Keywords: atherosclerosis; nanoparticles; plaque imaging; stent design; vascular tissue engineering.

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1 Introduction

The cardiovascular diseases are responsible for the majority of deaths in the developed countries (acute coronary syndrome, sudden cardiac death, and stroke), and despite the enormous diagnostic and therapeutic advances, remain one of the biggest global public health problems [1]. A real improvement of the primary prevention of the cardiovascular morbidity and mortality requires the development of new diagnostic tools and therapeutic strategies, which focus on the suppression of disease development in the early, subclinical stages. In the later stages of atherosclerosis, more effective risk-stratification and patient-specific treatment strategies are necessary. Nanomedicine, which merges the research areas of medicine, chemistry, biology, physics, and engineering, utilizes the nanosystems for sophisticated diagnosis and therapy of various diseases. The application of the nanomedical strategies for cardiovascular medicine will have significant impact on the outcomes of atherosclerosis. In cardiovascular medicine, it should provide *molecular imaging* for the early disease detection [2], and the region- and target-specific modalities for the therapeutic interventions in advanced disease [3].

In molecular imaging, in order to visualize the particular pathogenic process at the molecular level, the magnetic/optical imaging nanoparticles are bound to the specific affinity ligands, e.g., the monoclonal antibodies. The molecular targeting of the imaging nanoparticles will, thus, enable the noninvasive phenotypic characterization of the atherosclerotic plaques [4, 5], thereby, improving the

current risk stratification. For the *therapeutic approaches*, these nanoparticles can simultaneously be used as the transport vehicles that allow the targeted drug delivery to the atherosclerosis-specific cells or tissues. For example, the meaningful therapeutic interventions might include the suppression of the endothelial dysfunction in the atherosclerosis-prone regions to prevent the development of the plaques or the antiangiogenic therapy with the specifically targeted drug delivery systems to arrest the neovascularization of the advanced plaques.

Despite the extensive efforts in the field of nanomedicine, no specific nanoparticle system has been approved in the vascular medicine for diagnostics or therapy, neither in the USA nor in Europe. Currently, some contrast agents as well as about 30 nanodrugs are available in nanoparticulate form for other indications, such as cancer treatment. But the integration of a carrier, a targeting modality, and an active molecule into one single nanosystem has not yet been achieved in the humans for the diagnosis and treatment of atherosclerosis [3]. As with all the newly introduced medicinal products, extreme caution must be paid to the potential health hazards and long-term adverse effects. Many of the nanosystems tested preclinically are based on the clinically approved contrast agents. However, the potential risk of the cyto- and genotoxicity, as well as the possible immunogenic reactions induced by the targeting ligands must be carefully considered and excluded before each particular nanosystem enters the clinical trials.

Apart from the multiple applications in cardiovascular imaging and the drug delivery systems, nanotechnology offers a platform for the novel approaches to vascular tissue engineering or for improving the safety profiles of the stents following the cardiovascular interventions. The recent years brought about an intensification of research focusing on the cardiovascular applications of the nanotechnologies, resulting in a vast number of published reports. This review discusses some of the efforts to implement the nanomedical approaches to the diagnosis and treatment of atherosclerosis. We apologize to the scientists whose work was not cited due to the spatial limits of our review.

2 Nanoparticles for imaging of atherosclerosis: detection of plaques

Atherosclerosis begins with endothelial activation by the hemodynamic and inflammatory factors, which leads to

an increased permeability of the endothelial barrier for the blood lipids and immune cells. While the earliest type of atherosclerotic plaque consists mainly of lipids and some lymphocytes, the advanced lesions are characterized by a lipid-rich necrotic core, encapsulated by a cap consisting of fibrous matrix. The presence of the advanced lesions is initially compensated by the so-called positive remodeling – the diseased arteries enlarge and, thereby, preserve the normal lumen dimensions. As the disease progresses, the lipid core grows and the atherosclerotic plaques start narrowing the arteries, which can result in blocking the blood flow and reducing the oxygen supply to the heart or brain. Molecular imaging, capable of visualizing a particular pathogenic process at the cellular or molecular level, is thus particularly useful for the detection of both the early disease stages characterized by endothelial dysfunction and of the advanced plaques prone to rupture.

2.1 Imaging modalities and nanoprobe

Many of the molecular imaging techniques routinely used in cardiovascular medicine are constantly being optimized to better detect the atherosclerotic plaques. However, each of the modalities has its own advantages and limitations [6]. For example, magnetic resonance imaging (MRI), a noninvasive and nonionizing imaging technique has an excellent resolution but low sensitivity. Positron emission tomography (PET), on the contrary, has the highest sensitivity of all the imaging modalities and an unlimited penetration depth. These advantages are counterbalanced by its low resolution, very high cost, and radioactivity. Optical fluorescence imaging, suitable for, e.g., imaging the plaque endothelium, can be difficult to quantify in the tissues more than a few millimeters in depth. As a solution to this problem, the multimodal contrast agents or imaging probes detectable with multiple molecular imaging techniques are being developed to improve the sensitivity and accuracy of the vascular surveys.

In order to detect the cell- or tissue-specific antigens, the various constructs combining the affinity ligands (e.g., the monoclonal antibodies) and the contrast agents serving as the reporter molecules are possible. The paramagnetic gadolinium chelates are commonly used as extracellular contrast agents for MRI. The chelators, such as diethylene triamine pentaacetic acid (DTPA), reduce the toxicity of free gadolinium [7], whereas the short half-life of this metal can be improved by producing the micellar structures with albumin, high-density lipoprotein, or

liposomes [8–11]. Another class of contrast agents used for the detection of the plaque characteristics are the superparamagnetic iron oxide nanoparticles (SPIONs), which offer multiple possibilities of imaging intracellular targets due to their longer half-life and smaller diameter [12, 13]. To stabilize the iron oxide nanoparticles, polymer coating is required, which additionally allows the stable conjugation of the specific affinity ligands, thus increasing the ability to target the particular molecules. The SPIONs can be further conjugated to the fluorochromes, which allows the detection of those particles by the optical fluorescence methods both *in vitro* [14] and *in vivo* [12, 15]. Moreover, the atherosclerosis-related intracellular proteases can be detected with the SPIONs by employing the enzyme-triggered nanoparticle self-assembly [16]. In this technique, the SPIONs are inhibited by the attachment of the polyethylene glycol (PEG) chains that are anchored by the protease-cleavable peptide substrates. Upon the enzymatic removal of the PEG via the cleavage of the peptides, the particles self-assemble into larger structures, which acquire enhanced magnetic properties. This, in turn, generates a change in the relaxivity time (R_2) of the nanoassemblies, which is detectable using the T2-weighted MRI [16]. However, also the unconjugated SPIONs can serve as very useful markers of plaque growth and progression by targeting the plaque macrophages, which internalize these nanoparticles with particularly high avidity.

Apart from the diagnostic imaging by MRI, the dextran-coated SPION constitute a versatile platform for the combined-modality imaging, such as MRI/PET/computed tomography (CT) and optical fluorescence imaging. As an example, a trimodality reporter based on the dextranated SPION was described by [17]. In that study, the monocrystalline SPIONs labeled with the near-infrared fluorochrome were further chelated with DTPA to allow the attachment of the PET radiotracer ^{64}Cu . Whereas the iron oxide core provided contrast in MRI, the fluorochrome served for the fluorescence imaging (fluorescence microscopy, flow cytometry, and fluorescence-mediated tomography), and the radiotracer allowed PET imaging. Combined with CT imaging for anatomic coregistration, this approach was highly accurate for the detection of the inflamed plaques in the murine arteries and may serve to survey the lesion severity among the different vascular beds.

Thus far, the preclinical studies performed in order to investigate the diagnostic and therapeutic benefit of the nanoparticles in atherosclerosis mostly utilized the MRI contrast agents. However, increasing numbers of reports are now emerging that employed other imaging modalities, such as PET or optical coherence tomography (OCT),

and the multimodality nanoprobe for atherosclerotic plaque detection and classification. One of the recent examples is a study by Wang et al. [18] that combined photothermal wave (PTW) imaging and OCT to detect and characterize the distribution of the macrophages in the aortic atherosclerotic plaques harvested from the rabbits treated with the gold nanoroses. Detected with this method, the nanorose-loaded macrophages were distributed at the upstream shoulder of the atherosclerotic plaques, at the edges of the lipid deposits, indicating that the combined PTW-OCT imaging can simultaneously reveal the plaque structure and composition.

2.2 Targets for detection: dysfunctional endothelium in the early stages of the disease

The vascular endothelium, located at the interface between the atherosclerotic plaque and the flowing blood, and critically involved in lesion progression [19], constitutes a good candidate for nanoparticle targeting. In the early stages of the disease, the endothelium becomes activated by the hemodynamic and inflammatory factors, leading to the increased expression of cell adhesion molecules (CAMs) and selectins (E- and P-selectin). These pro-inflammatory cell surface proteins steer the recruitment of the circulating immune cells into the vessel intima, thus playing an important role in the pathogenesis of atherosclerosis. Conjugating the nanoparticles to the specific ligands that target the endothelial activation markers may thus serve as a useful approach to the noninvasive imaging of the early stages of atherosclerosis. It can also prove therapeutically beneficial by inhibiting leukocyte binding to the endothelial receptors and, consequently, reduce the accumulation of the inflammatory cells in athero-prone regions. Several endothelial adhesion molecules have, thus far, been tested as diagnostic and potentially also therapeutic targets (see Table 1), among them vascular cell adhesion molecule (VCAM-1), as well as E- and P-selectins.

2.2.1 VCAM-1

In 2005, Kelly et al. reported the successful targeting of the magnetofluorescent iron oxide nanoparticles to VCAM-1-expressing endothelial cells in the atherosclerotic lesions of the cholesterol-fed apolipoprotein E (ApoE)-deficient mice [20]. In that study, the nanoparticles were conjugated with a peptide selected by the phage display

Table 1 The diagnostic targets of the nanoparticles in the cardiovascular disease.

Target cell/tissue	Label	Nanoparticle type	Imaging agent	References
Endothelium	VCAM-1-targeting peptides	Crosslinked iron oxide NPs	Iron oxide/Cy5.5	[20, 21]
	Anti-VCAM-1 Ab	p-Toluene-sulfonylated iron oxide microparticles	Iron oxide	[22]
	Anti-E-selectin Ab	Aminated ultrasmall iron oxide NPs	Iron oxide	[23]
	Anti-P-selectin Ab	p-Toluene-sulfonylated iron oxide microparticles	Iron oxide	[22]
Platelet deposits	Anti-P-selectin Ab	Albumin-Gd-DTPA NPs	Gadolinium	[24]
	Anti-P-selectin Ab	PEGylated dextran NPs	Iron oxide	[25]
Macrophages	Unlabeled	Monocrystalline iron oxide	Iron oxide	[26]
		MION-47 (dextran-coated magnetite-like NPs)	Iron oxide	[5, 27]
		P904 (Guerbet, France)	Iron oxide	[28]
		Dextranated DTPA-iron oxide NPs	Iron oxide/ ⁶⁴ Cu/VT680	[17]
	Homing peptide LyP-1	Aminated iron oxide NPs	Iron oxide/fluorescein	[29]
	Anti-CD36 Ab	Lipid-based NPs	Gadolinium	[30]
	CD36 ligand (oxidized phosphocholine)	Liposomes containing carbon-caged Gd NPs	Gadolinium	[11]
	SR-A ligand dextran sulfate	Dextran-coated iron oxide NPs	Iron oxide	[31]
	ApoE-derived lipopeptide P2fA2	Reconstituted HDL-like NPs	Gadolinium/fluorescein	[32]
	Anti-OxLDL Ab	PEG-coated ultrasmall iron oxide NPs	Iron oxide	[33]
Apoptotic cells	Annexin A5		Iron oxide	[4]
	Annexin A5	Aminated PEG-Gd-DTPA disterylamide NPs	Gadolinium	[34]
	Phosphatidylserine-targeting peptide (Leu-Ile-Lys-Lys-Pro-Phe)	PEGylated ultrasmall iron oxide NPs	Iron oxide	[35]
Neovasculature	Peptidomimetic vitronectin antagonist (Arg-Gly-Asp)	Perfluorocarbon Gd-DTPA-bis-oleate NPs	Gadolinium	[36, 37]
	C-type atrial natriuretic factor	PEGylated DOTA-poly (methylmethacrylate) NPs	⁶⁴ Cu	[38]
Fibrin clots	Anti-fibrin Ab	Lipid-encapsulated perfluorocarbon NPs	Gadolinium	[39]
	Fibrin-binding peptide (Cys-Arg-Glu-Lys-Ala)	Lipopeptide NPs	Fluorescein	[40]
	Fibrin-binding peptide EP-2104R	Gd-DOTA-chelated NPs	Gadolinium	[41]

Ab, antibody; DOTA, tetraazacyclo-dodecane; HDL, high-density lipoprotein; NPs, nanoparticles; Ox-LDL, oxidized low-density lipoprotein; SR-A; scavenger receptor type A; VCAM-1, vascular cell adhesion molecule-1; VT680, near-infrared fluorochrome Vivotag 680.

screening, which contained the VHSPNKK motif that has a homology to the α -chain of the very late antigen-4 (VLA-4, a known ligand for VCAM-1) and showed 12-fold higher target-to-background ratios compared with the VCAM-1 monoclonal antibodies. Such VCAM-1-targeting nanoparticles showed a high affinity for the endothelial cells expressing VCAM-1 and a low affinity for the macrophages, in contrast to the control unconjugated nanoparticles, which did not bind to the endothelial cells [20].

Conjugating the nanoparticles to yet another peptide homologous to VLA-4, VHPKQHR, further increased their affinity to VCAM-1 in the aortic roots of the ApoE-deficient mice [21]. This nanoconstruct was also shown to specifically target the VCAM-1-expressing cells in the *ex vivo* human carotid endarterectomy samples by

immunohistochemistry and MRI. In the *in vivo* pharmacotherapy experiments, the ApoE-deficient mice treated with atorvastatin showed a reduced accumulation of the probe in the aortic root lesions, corresponding to the decreased VCAM-1 expression. This supports the notion that utilizing a probe of sufficient specificity should not just facilitate the monitoring of the disease progression, but also enable the noninvasive verification of the therapeutic effects.

More recently, the monoclonal antibodies against VCAM-1 were conjugated to the iron oxide microparticles and used to target the atherosclerotic lesions of the ApoE-deficient mice. These nanoconstructs were capable of detecting the activated endothelial cells, and their affinity to the endothelium could be further significantly

improved by adding an additional P-selectin-targeting moiety [22].

2.2.2 E-selectin

Considering its important role in leukocyte rolling and inflammation, E-selectin rapidly upregulated on the endothelial cells upon their activation constitutes another potential target for the molecular imaging of the vascular inflammation and early atherosclerotic lesions. The expression of E-selectin in the TNF- α -activated endothelial cells was detected *in vitro* by MRI using the paramagnetic liposomes containing gadolinium-DTPA and conjugated with anti-E-selectin antibodies [42]. In a mouse model of inflammation, an anti-E-selectin monoclonal antibody was conjugated to the ultrasmall SPION for targeting E-selectin *in vivo*. The injection of the targeted nanoparticles resulted in the distinct changes in the R2 relaxation rate (1/T2) characteristics in the inflamed regions compared with the control regions, which were subsequently confirmed by histologic analysis, indicating that the E-selectin-targeted probe detects the specific pattern of the vascular inflammation [23].

2.2.3 P-selectin

Platelets precede monocytes in the interactions with the activated endothelium at the athero-prone regions. P-selectin, expressed both by the platelets and the activated endothelium, thus, plays an important role in the initial contact between the platelets and diseased vessel walls. Although the nanoparticles targeted to P-selectin are not specific to the activated endothelium, the dual targeting of P-selectin and VCAM-1 with the micron-sized iron oxide particles has been successfully employed for MR imaging of the atherosclerotic plaques in the ApoE-deficient mice [22]. Additionally, several reports indicate that P-selectin constitutes a suitable target for the molecular imaging of the platelet deposits and superficial thrombi in the initial stages of thrombus formation. The P-selectin antibody-conjugated gadolinium nanoparticles were originally used to detect the microthrombi *in vitro* and *in vivo* on the intimal surface of the dog vein endothelium, showing a good correlation of the histologic and MRI data [24]. In a recent study by Jacobin-Valat et al., the PEGylated dextran/iron oxide nanoparticles were labeled with rhodamine and coupled to the anti-human P-selectin antibodies [25]. *In vitro*, the strong labeling of the activated vs. resting platelets was detected by flow cytometry and

microscopy, which highly correlated with MRI. Furthermore, the *in vivo* imaging of the atherosclerotic plaques in the ApoE-deficient mice at 4.7 T showed the accumulation of the P-selectin targeting nanoparticles in the thickened intima [25].

The above studies indicate that the adhesion molecule-targeted paramagnetic nanoparticles bind specifically to the activated endothelium. The molecular imaging of such nanoparticle constructs can, therefore, represent the sensitive detection method for the direct identification of early atherosclerotic lesions.

2.3 Targets for detection: vulnerable plaque

The rupture of the vulnerable plaque is a life-threatening event – with the most dramatic clinical manifestations being sudden cardiac death, myocardial infarction, or stroke [1]. About 70%–80% of those acute cardiovascular events can be traced back to the ruptured “culprit” plaques [43]. In the USA alone, >900 000 patients per year suffer myocardial infarction, and nearly 800,000 patients undergo stroke. About 40% of these patients do not survive the acute ischemic event [1]. Because the majority of the victims die suddenly without prior symptoms, the identification of the patients predisposed to the plaque rupture is a major focus of cardiovascular research. Currently, many vulnerable plaques go unnoticed – improvement of their detection, both with the targeted modality-specific nanoparticles and with the multimodality nanoprobe, will reduce the risk of the acute cardiovascular events. As discussed below, the molecular imaging approaches to the vulnerable plaque identification focused, thus far, mainly on the inflammatory status of the plaque (in particular, the macrophage content and their activation), the lipid content of the lesions, thrombus detection, and imaging of the intimal neovascularization (Table 1).

2.3.1 Macrophages

The activated macrophages are an early marker of the atherosclerotic processes in the arterial wall and specifically incorporate the polymer-coated SPIONs. Following the animal studies in rabbits, an MRI study on the 11 symptomatic patients scheduled for carotid endarterectomy demonstrated that the ultrasmall SPIONs accumulated predominantly in the ruptured and rupture-prone atherosclerotic lesions, causing significant signal decreases in

the *in vivo* MR images, whereas hardly any SPIONs were taken up in the stable plaques [13].

In vitro, the uptake of the SPION by the macrophages was shown to be regulated both by the endogenous cytokines and exogenous factors. Whereas the endocytosis of the SPIONs was significantly reduced by the pretreatment of the cells with lovastatin, interferon- γ and interleukin-4 strongly increased the SPION uptake, indicating that the MRI signal changes after the SPION administration may reflect both the macrophage numbers and their phagocytic capacity [44].

The suitability of the monocrystalline iron oxide nanoparticles for the detection of the macrophage-rich atherosclerotic tissue was recently confirmed in several further studies in the hyperlipidemic rabbits by the differential phase OCT [26] and MRI [5, 27]. The study by Morishige et al. [27] demonstrated, moreover, that the macrophage imaging may serve not only as a biomarker to identify the vulnerable lesions but also can provide a tool to monitor the therapeutic effectiveness of the anti-atherosclerotic medication. The *in vivo* application of the nanoparticles resulted in the MRI visualization of the thickened abdominal aortas and a significant reduction in the T2 signal intensity, which correlated with the macrophage-rich areas in the lesions. The treatment of the rabbits with rosuvastatin for 3 months significantly diminished the macrophage content and reversed the T2 signal intensity changes [27]. A very recent study by Sigovan et al. applied a similar approach using the ultrasmall SPION to noninvasively monitor the therapeutic effect of the irbesartan therapy on the macrophage burden in the atherosclerotic plaques of the ApoE-deficient mice [28].

It must be noted, however, that the uptake of the SPION is not specific to the plaque macrophages, and thus far, they have been used for the imaging of the plaques exclusively in the research settings. Clinically, the ultrasmall SPIONs are used as a contrast agent for the MRI of the lymph nodes [45]. te Boekhorst et al. [46] showed that the signal loss in the aortic walls of the ApoE-deficient mice and ApoE/eNOS-double knockout mice did not correspond with the histological evidence of the ultrasmall SPION uptake by the aortic wall but rather by the peri-aortic lymph nodes. The authors concluded that the use of the SPIONs for the detection of the atherosclerotic lesions may be limited in the vessels where the lymph nodes are highly prevalent. Therefore, the specific targeting of macrophages and macrophage receptors may be helpful to improve the labeling efficiency of the particles *in vivo*. For example, a report has been published recently in which the homing peptide LyP-1 was used to deliver the

SPION to the plaques in the ApoE-deficient mice. As demonstrated by MRI, the LyP-1 coating improved the ability of the particles to traverse through the arterial endothelial wall and to accumulate in the plaque macrophages. The LyP-1 was also suitable for PET imaging of the atherosclerotic plaques, as the accumulation of the fluorobenzoic acid-labeled LyP-1 was significantly greater in the plaque-containing aortas than in the control vessels [29]. Nearly in parallel, another study was published demonstrating the ability of the LyP-1 to enhance the plaque visualization by fluorescence imaging [47]. That study utilized the 12-nm-small cage-like protein nanoparticles with LyP-1 incorporated onto the exterior surface and the fluorescent stain conjugated to the interior cavity, demonstrating that the specific targeting improves the delivery of the imaging agents to the atherosclerotic plaques.

2.3.2 Macrophage-specific scavenger receptors

The macrophage scavenger receptors mediate oxLDL uptake and initiate the inflammation cascade. The scavenger receptor type A (SR-A) and CD36 (macrophage scavenger receptor B), predominately expressed on the macrophages, are both involved in atherogenesis and atheroinflammation, thus constituting the targets for the noninvasive detection of the macrophage-rich lesions.

An approach utilizing the gadolinium-containing lipid-based nanoparticles targeting CD36 in the macrophages *in vitro* and in the human aortic specimens *ex vivo* was reported by Lipinski et al. [30]. Compared with the nontargeted nanoparticles, the CD36-targeting agents were avidly scavenged by the macrophages *in vitro* and targeted the resident macrophages in the aortic lesions. Dellinger et al. [11] showed that the CD36-targeting gadolinium-containing liposomes exhibited a time-dependent accumulation in the atherosclerotic lesions of the ApoE-deficient mice, whereas no accumulation was observed in the vessels of the wild-type animals. The nontargeting nanoparticles did not bind plaques *in vivo*. In a recent study by Tu et al. [31], the iron oxide nanoparticles were coated with dextran sulfate, a ligand of SR-A. The targeting to the SR-A was confirmed *in vitro*. Following the intravenous injection of the sulfated nanoparticles into an ApoE-deficient mouse carotid ligation model, MRI showed a substantial signal loss on the injured carotid at 4 and 24 h post-injection, compared with the control arteries and with the nonsulfated nanoparticles. The macrophage-specific nanoparticles could thus facilitate the detection and diagnosis of the vulnerable plaques.

2.3.3 Lipoproteins

The lipoproteins, the natural nanoparticles of 5–20 nm diameter, can be easily modified to create multifunctional nanoparticles both for imaging, as well as for the delivery of therapeutics to the atherosclerotic lesions. The high-density lipoproteins (HDLs), which play a key role in removing the excess cholesterol from the plaques are a good candidate for the transfer of the imaging nanoparticles into the lesions, as their small size allows them to cross the endothelium and penetrate the underlying tissue.

The HDL-like nanoparticles enriched with gadolinium have been reported to specifically image the plaques *in vivo* [9]. Further studies utilizing gadolinium-HDL nanoparticles bound to the carboxyfluorescein-labeled ApoE-derived lipopeptide P2fA2 showed an increased *in vitro* association of the targeted nanoparticles with the murine macrophages compared with the untargeted ones. In a carotid constriction collar model in the ApoE-deficient mice, a significantly higher signal enhancement of the atherosclerotic wall was observed 24 h after the administration of HDL-P2fA2 relative to the unlabeled HDL. By the confocal laser scanning microscopy, the HDL-P2fA2 nanoparticles colocalized primarily with the intraplaque macrophages [32].

Another possible target for the detection of the vulnerable plaques is oxidized LDL. Conjugating the anti-mouse OxLDL polyclonal antibodies to the PEGylated ultrasmall SPION is one of the recent approaches used to image the atherosclerotic plaques in the ApoE-deficient mice undergoing carotid constriction. After the administration of the targeted anti-OxLDL nanoparticles, the MRI signal loss in the carotid atherosclerotic lesions, consistent with the presence of the nanoparticles, was observed at 8 h and 24 h, which was subsequently confirmed by immunohistochemistry showing colocalization of the OxLDL/macrophages and iron oxide nanoparticles. This strategy can thus be used to directly detect the OxLDL and estimate the macrophage burden in the atherosclerotic plaques *in vivo* [33].

2.3.4 Apoptosis

Imaging apoptotic cells is another possible approach to detect the vulnerable plaques. The characteristic feature of the cells undergoing apoptosis is the exposure of phosphatidylserine (PS) at their surface. In a study by Burtea et al., a PS-targeting peptide (Leu-Ile-Lys-Lys-Pro-Phe) was conjugated to the ultrasmall SPIONs and subsequently used for the plaque MRI in the ApoE-deficient

mice [35]. Those nanoparticles, able to reach their target in low doses and as fast as 30 min after the administration, concentrated inside the plaque producing a negative enhancement of the plaques rich in the apoptotic macrophages and neutral fats. The SPION derivatives targeting the apoptotic cells colocalized with their target cells detected by histology and were able to identify the plaques with a vulnerable morphology.

The PS-exposing cells in the atherosclerotic lesions can also be detected with the annexin A5-labeled nanoparticles. As an example, Smith et al. utilized the SPION labeled with annexin A5 to detect the apoptotic macrophages in a rabbit model of atherosclerosis [4]. The targeted SPIONs accumulated in the atherosclerotic lesions, but not in the healthy arteries, and allowed MRI differentiation of the occlusive and mural plaques. In a more recent study, gadolinium nanoparticles functionalized with the fluorescent annexin A5 were used for the MRI of the atherosclerotic plaques in the abdominal aorta of the ApoE-deficient mice. An enhanced uptake of annexin A5 nanoparticles, compared to the controls, was observed, and the targeted agent colocalized with the apoptotic cells and with the plaque macrophages, a fraction of which were presumably apoptotic [34]. Those results indicate that annexin-A5-targeted nanoparticles can contribute to the noninvasive assessment of the plaque progression.

2.3.5 Angiogenesis

In the advanced plaques, growing metabolic needs, inflammatory cell infiltration, and concomitant release of the numerous pro-angiogenic cytokines may lead to the induction of an uncontrolled intimal neovascularization, resulting in the production of the immature and fragile (leaky) vasa vasorum [48], similar to the blood vessels seen in the tumor development. The nanoparticles circulating in the bloodstream approach the lesion both through the lumen of the arteries and through the vasa vasorum in the adventitia. The latter route allows a rapid detection of the microvessels sprouting into the intimal layer of the diseased arteries, the presence of which contributes to plaque instability and enhances the risk of rupture.

The intimal neovascularization is associated with the increased expression of integrin $\alpha_v\beta_3$, one of the key mediators of neovessel formation [49]. The perfluorocarbon nanoparticles containing gadolinium chelates and targeted to the $\alpha_v\beta_3$ -integrin by the conjugation with the peptidomimetic vitronectin receptor antagonist (Arg-Gly-Asp) were previously used to image this integrin in the atherosclerotic rabbits. Such nanoparticles provided a specific

detection of the neovasculature within 2 h by routine MRI at 1.5 T [36]. In a more recent study from the same group, the $\alpha_v\beta_3$ -integrin-targeting nanoparticles were tested for monitoring the angiogenic therapy in a rabbit model of peripheral vascular disease. Following the femoral artery ligation, the hypercholesterolemic animals received pro-angiogenic L-arginine. Compared to the untreated rabbits, the MRI at 10 days postligation revealed an increased signal enhancement in the L-arginine-treated animals. The MRI signal enhancement observed with the $\alpha_v\beta_3$ -integrin-targeting nanoparticles was two times higher as with the nontargeted particles, indicating that the molecular targeting improves the identification of the angiogenic vasculature [37].

Another approach to detect angiogenesis was reported by Liu et al. [38], who used PET imaging to detect the angiogenesis-related upregulation of the natriuretic peptide clearance receptor. For this purpose, the ^{64}Cu -labeled nanoparticles conjugated to the C-type atrial natriuretic factor were applied in a mouse model of hind limb ischemia. Compared with the nontargeted particles, the PET imaging showed an enhanced accumulation of the targeted nanoparticles in the angiogenic regions, colocalizing with the endothelial cells and SMCs. This indicates that a sensitive detection of angiogenesis with PET is possible by applying the radioactive tracer-labeled nanoparticles conjugated with the angiogenic markers.

2.3.6 Fibrin

The thrombus formation that occurs on the luminal surface of the atherosclerotic plaques presents a target for the nanoparticle-based diagnostics and therapeutics. In 2000, a report was published on the *in vitro* thrombus detection using the anti-fibrin monoclonal antibodies conjugated to the lipid-encapsulated perfluorocarbon nanoparticles with gadolinium-DTPA complexes incorporated into their outer surface [50]. A dense accumulation of the nanoparticles on the clot surfaces was observed by scanning electron microscopy, and the MRI detectability of all the clots was dramatically increased by the targeted nanoparticles. In a further study from the same group, these nanoparticles were also evaluated by MRI *in vivo* under open-circulation conditions in dogs and *ex vivo* in the carotid artery endarterectomy specimens [39]. The study showed that the detectability of the clots *in vivo* was markedly enhanced by the fibrin-specific paramagnetic nanoparticles relative to the control nanoparticles. The fibrin-targeting nanoparticles formed a thin surface layer, which also dramatically enhanced the *ex vivo* detectability

of the thrombotic deposits in the ruptured carotid plaque treated with the targeted paramagnetic nanoparticles compared to the control nanoparticles and to the control arteries [39].

More recently, targeting of atherosclerotic plaques in the ApoE-deficient mice fed a high-fat diet was accomplished with the pentapeptide Cys-Arg-Glu-Lys-Ala (CREKA), which binds to the clotted plasma proteins in the blood vessels. Those fibrin-targeting PEGylated lipopeptide nanoparticles were observed on the entire surface of the plaque and, notably, concentrated at the shoulders of the plaque, a location that is prone to rupture [40]. A similar approach to the intraplaque and endothelial fibrin detection in the ApoE-deficient mice on a high-fat diet was reported in 2012 by Makowski et al. using a commercially available gadolinium-based fibrin-binding peptide EP-2104R [41]. The MRI of the brachiocephalic artery performed 90 min after the fibrin-targeted nanoparticle administration demonstrated a significant increase in the contrast agent uptake in the atherosclerotic plaques, in particular, in the late-stage lesions, as confirmed by *ex vivo* fibrin staining and gadolinium concentration measurements [41]. Those studies demonstrate that the nanoparticles that target fibrin can improve the identification of the advanced and potentially vulnerable atherosclerotic plaques.

2.3.7 Anti-inflammatory pathways

The recent years brought about some novel molecular imaging approaches to the atherosclerotic plaque detection and characterization. An illustrative example of those is a method applied by Almer et al. who used two types of anti-inflammatory agents to deliver an increased amount of fluorescently labeled nanoparticles to the atherosclerotic plaques of the ApoE-deficient mice [51–53]. The fluorescently labeled, sterically stabilized liposomal nanoparticles were conjugated either to the globular domain of adiponectin (atheroprotective adipocytokine, which accumulates in the injured endothelium and has plaque-stabilizing effect) or to the recombinant interleukin-10, the most prominent anti-inflammatory cytokine, expressed by the atherosclerosis-related immune cells, e.g., the macrophages and dendritic cells. The results of those studies showed that both cytokines were suitable to enhance the detection of the atherosclerotic plaques *ex vivo* by confocal microscopy, but exhibited remarkably distinct staining patterns: While the nanoparticles conjugated with the globular adiponectin accumulated at the surface of the atherosclerotic plaques [52], those linked to interleukin-10 penetrated the plaques and localized to the

macrophage-rich areas [53]. The combined use of a specific anti-inflammatory protein bound to the contrast agent-containing liposomes should thus improve the detection and characterization of the atherosclerotic plaques and may potentially contribute to plaque stabilization.

2.4 Conclusion

The timely detection of atherogenesis in a given patient will allow establishing an in-time-treatment with the possibility to reverse the pathological process. Particularly, in the high-risk patients, the early identification and aggressive pharmaceutical and/or interventional treatment of the vulnerable plaques can help to reduce the incidence of acute coronary syndromes and sudden cardiac death. Thus far, most invasive and noninvasive modalities for plaque detection fail to reliably identify the vulnerable plaques in the clinical settings, due to their insufficient sensitivity and specificity. Employing the targeted nanoparticles, in particular, the multimodality imaging nanoparticles, to refine the available noninvasive techniques should improve the detection of the atherosclerotic plaques and reduce the risk of acute cardiovascular events.

3 Nanocarriers for treatment of atherosclerosis

Whereas many pharmacologic agents for the treatment of the clinical manifestations of atherosclerosis are available, the systemic administration of these drugs has several serious drawbacks. The percentage of the applied dose that reaches the diseased site is usually low, resulting in a low treatment efficacy or in considerable adverse effects upon the dosage increase. As the efficacy and safety profiles of the existing atherosclerosis therapies are far from ideal, the alternative strategies to deliver the patient-specific treatment are urgently required. To overcome the problems associated with the traditional therapeutic approaches, the targeted nanoparticles can be used as transport vehicles that allow the local targeted drug delivery to the disease-specific cells or tissues and, thus, concentrate the therapeutic agent at the site of action. Such nanocarriers, being larger than 5 nm in diameter (ca. 10–200 nm), evade the renal clearance, thus increasing the circulation half-life of the transported drugs, and are small enough to bind their target receptors with high affinity. The application of such nanocarriers for the therapy of atherosclerosis is moreover expected to lower

the drug cytotoxicity by the (a) locally targeted tissue accumulation and (b) reduction of the required doses, and to allow the monitoring of a patient's response to treatment.

The atherosclerotic plaque formation is a complex process consisting of many sequential steps, each of them being a potential therapeutic target (Figure 1). Several experimental approaches to the nanoparticle-targeted treatment of the different stages of the disease have been reported thus far (see Table 2), most of them focusing on the inhibition of endothelial dysfunction, plaque macrophages, angiogenesis, intimal hyperplasia, and thrombus formation. Those studies are summarized below.

3.1 Targets for therapy: endothelium

One of the key underlying processes in atherosclerosis is the dysfunction of the vascular endothelium [19], which consequently constitutes the major target for pharmacological therapies of coronary artery disease in the acute and chronic settings. Thus far, the platelet-endothelial cell adhesion molecule-1 (PECAM)-directed delivery of the antioxidant therapeutics has been tested as a feasible approach to many vascular disease conditions characterized by the endothelial injury. In a murine model of endotoxemia, the anti-PECAM/superoxide dismutase conjugates, but not the unconjugated enzyme, accumulated in the vascular endothelium after intravenous injection, localized in the endothelial endosomes, and inhibited the lipopolysaccharide-induced VCAM-1 expression [66]. Basing on these findings, the same group employed the PECAM-targeting liposomal nanoparticles encapsulating a NADPH oxidase (nicotinamide adenine dinucleotide

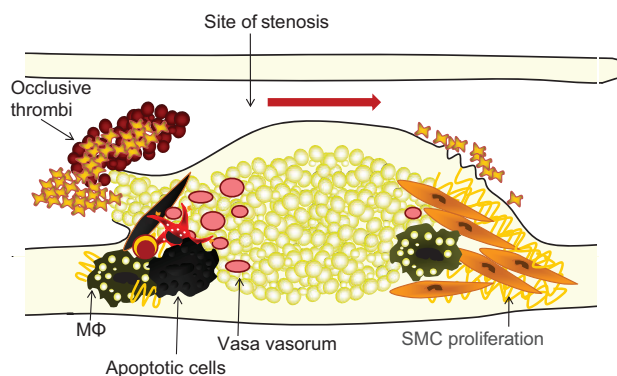


Figure 1 The possible targets for the nanomedical approaches to the treatment of the advanced atherosclerotic plaques. The schematic presentation of the longitudinal section of a vessel-narrowing atherosclerotic lesion based on the histologic observations. The block arrow indicates the blood flow direction. MΦ, macrophage; SMC, smooth muscle cell.

Table 2 The therapeutic targets of the nanoparticles in cardiovascular disease.

Target	Therapeutic compound	Nanoparticle/target protein	References
Endothelium	NADPH oxidase (NOX) inhibitor	PECAM-targeting liposomal NPs	[54]
Macrophages	Pravastatin	SR-A-targeting polymer NPs	[55]
	Light-activated therapeutic moieties	Magnetofluorescent NPs, unlabeled	[56]
Angiogenesis	Fumagillin	$\alpha_v\beta_3$ Integrin-targeting iron oxide NPs	[57, 58]
SMC proliferation	Rapamycin	Polymer NPs, unlabeled	[59]
	Rapamycin	Gel-like NPs, unlabeled	[60]
	Rapamycin	$\alpha_v\beta_3$ -Targeting Gd-DTPA NPs	[61]
	Paclitaxel	Collagen IV-targeting PEGylated-lipid NPs	[62]
	NOX2-targeting siRNA	Amino acid-based NPs, unlabeled	[63]
	Prednisolone	Chondroitin sulfate proteoglycans-targeting polymer liposome NPs	[64]
Coagulation cascade	Hirulog	Fibrin-targeting lipopeptide NPs	[40]
	Tissue plasminogen activator	Shear-activated microaggregates of polymer NPs	[65]

NPs, nanoparticles; NOX, NADPH oxidase; PECAM, platelet-endothelial cell adhesion molecule; SMC, smooth muscle cell; SR-A, scavenger receptor type A.

phosphate oxidase, NOX) inhibitor in order to suppress the vascular oxidative stress and inflammation *in vitro* and *in vivo*, in the murine pulmonary endothelium. The targeted therapeutic nanoparticles were specifically bound to the endothelium and attenuated the angiotensin-induced reactive-oxygen species production, inhibited the TNF- α -induced endothelial expression of VCAM-1, and prevented the VEGF-induced increase in endothelial permeability *in vitro* and *in vivo* [54].

Ikuta et al. developed a polymeric drug carrier containing the Evans blue dye as a probe for endothelial injury, which spontaneously formed the stable micelle-like nanoparticles encapsulating doxorubicin [67]. This carrier specifically targeted the region of the injured endothelium in the extracted porcine aortas [67]. However, in the context of vascular injury, the delivery of cytostatics which delay endothelialization, has been associated with the late adverse events among the stent recipients treated with the different antiproliferative therapies [68, 69]. Thus, incorporating the endothelium-protective anti-inflammatory agents (e.g., statins) or endothelialization-inducing substances may constitute a more feasible approach to the treatment of the endothelial dysfunction in atherosclerosis.

3.2 Targets for therapy: macrophages and their receptors

Inflammation is the dominant process in all the stages of atherothrombotic disease. Diagnostic studies using PET-CT showed that the plaque inflammation was

associated with the lesion progression and an increased cardiovascular event risk [70]. The targeted pharmacologic interventions with an inhibitory effect on the macrophages should improve the efficacy of the anti-atherosclerotic therapies.

The pravastatin-loaded polymer nanoparticles functionalized with an oligonucleotide sequence (polyG) targeting the macrophage scavenger receptor SR-A1 were tested *in vitro* in a study by Broz et al. [55]. The nanoparticles labeled with the polyG ligands showed a strong uptake into the active macrophages, but were not endocytosed in significant numbers by the skeletal muscle cells, which do not express SR-A and, therefore, did not affect the muscle cell survival. After the uptake into the macrophages, the polymer vesicles were gradually degraded in the intracellular compartments and released their encapsulated load in a time-dependent way. Compared to free drug, the pravastatin-containing nanoparticles inhibited the endocytosis of acetylated LDL by the macrophages in a concentration-dependent manner. A 50% reduction in the uptake was observed at the 0.25- $\mu\text{mol/l}$ final pravastatin concentration in the culture medium. To achieve the same degree of inhibition with the free drug, the concentration of 11 $\mu\text{mol/l}$ pravastatin (>40 times higher) was necessary [55].

The macrophage-targeted delivery of the therapeutics via the drug-loaded HDL-like nanoparticles *in vitro* was also described by Zhang et al. [71]. The differently formulated Tanshinone IIA-loaded reconstituted HDL nanoparticles had targeting effect for the foam cells through the CD36 scavenger receptor, with the disc-shaped constructs

showing a higher targeting efficiency than the spheroids [71]. Such basic studies, testing the influence of the formulation and structure of the nanoparticles on their targeting efficiency, are important in order to select the optimal nanocarriers for delivering the cardiovascular drugs to the atherosclerosis plaque.

As another approach, McCarthy et al. designed the magnetofluorescent nanoparticles modified with the light-activated therapeutic moieties, which allow the multimodal noninvasive monitoring of the nanoparticle localization and phototoxic activation at the spectrally distinct wavelengths [56]. The intravenous administration of the nanoparticles into the ApoE-deficient mice fed high-cholesterol resulted in their localization within the macrophage-rich atherosclerotic lesions and led to an efficient focal ablation of inflammatory macrophages upon irradiation of the plaques. Such nanoparticles, inducing the focal phototoxicity confined to the macrophages without affecting the endothelial or smooth muscle cells, could have a durable plaque-stabilizing effect.

3.3 Targets for therapy: angiogenesis

The recent studies strengthen the concept that the intra-plaque neovascularization and bleeding are events that play a major role in leukocyte infiltration and plaque progression and may serve to stratify the risk for the occurrence of future cardiovascular events [72]. The antiangiogenic therapies are thus expected to stabilize and/or reduce the atherosclerotic plaques. In 2006, Winter et al. reported the application of $\alpha_v\beta_3$ integrin-targeting SPIONs for the site-specific delivery of the antiangiogenic drug fumagillin and quantitative monitoring of the response in a rabbit model of atherosclerosis [57]. The study showed that a single intravenous administration of $\alpha_v\beta_3$ -targeting, fumagillin-carrying nanoparticles, at a total dosage approximately 50,000 times lower than the oral dose reported previously, effectively reduced the number of microvessels in the aorta as determined by MRI and immunohistochemistry [57]. The dose-related toxicity observed by the systemic drug application could thus be overcome by the dosage reduction upon the targeted drug delivery. In a further study, the $\alpha_v\beta_3$ -targeting fumagillin nanoparticles reduced the neovascular signal by 50%–75% at 1 week post administration and maintained this effect for 3 weeks, with the readministration on week 4 reproducing the 3-week antiangiogenic response. Moreover, the combination of two doses of $\alpha_v\beta_3$ -targeting fumagillin nanoparticles with oral atorvastatin resulted in a sustained inhibition of angiogenesis over 8 weeks [58]. These results

indicate that a single nanosystem combining imaging and the therapeutic agents may not only monitor the specificity of the targeted drug delivery to the cells/tissues, but can also offer valuable information about a patient's response to treatment.

3.4 Targets for therapy: smooth muscle cell proliferation and intimal hyperplasia

The smooth muscle cell proliferation and migration into the atherosclerotic intima is one of the processes contributing to plaque formation, as well as intimal hyperplasia and subsequent stenosis following balloon angioplasty. The nanoparticle-mediated delivery of the antiproliferative drugs promises region-specific inhibition of intimal thickening and restenosis.

The release of the antiproliferative drugs, dexamethasone and rapamycin, from the polymeric nanoparticles was investigated *in vitro* by Zweers et al., showing that the drug release can be substantially reduced by increasing the amount of protein (e.g., gelatin or albumin) associated with the nanoparticles: after the gelatin treatment of the drug-loaded nanoparticles, the sustained release of dexamethasone for 17 days and of rapamycin for 50 days was achieved compared with 100% release within 5 h from the gelatin-untreated nanoparticles [73]. A more recent study investigated the drug release of sirolimus (rapamycin) from the biodegradable polymeric nanoparticles and their *in vitro* biocompatibility using the human coronary SMCs and endothelial cells [59]. The nanoparticles showed biphasic release kinetics, consisting of a short, initial burst release phase of 5 h and a slower continuous release period of at least 30 days, indicating their suitability as a drug depot. The sirolimus-loaded nanoparticles were endocytosed, inhibiting the viability and proliferation of the human coronary SMCs and endothelial cells. In a rat carotid injury model, the local infusion of rapamycin-containing gel-like nanoparticles in the injured arterial region immediately after balloon angioplasty resulted in a significant inhibition of hyperplasia 3 weeks postangioplasty and improved natural reendothelialization of the injured artery [60]. A local delivery of rapamycin with the $\alpha_v\beta_3$ -targeting paramagnetic nanoparticles containing gadolinium-DTPA was also tested in a rabbit femoral artery model of stenosis formation following balloon injury [61]. A significant inhibition of stenosis (reduced neointima/media ratio) 2 weeks post-injury was observed in the nanoparticle-treated arteries compared with the control vessels. Importantly, the endothelial healing at 4 weeks

post-injury was not delayed in the vessels treated with $\alpha_v\beta_3$ -targeting rapamycin nanoparticles compared with the control nanoparticles.

As another approach to inhibit SMC hyperproliferation, the nanoparticles with a polymer core loaded with slow-eluting conjugates of paclitaxel were designed allowing for a controlled drug release over approximately 12 days [62]. The nanoparticle core was surrounded by a PEGylated-lipid monolayer with the targeting peptide specific to collagen IV (KLWVLPK), a key matrix protein of the vessel basement membrane. The release of paclitaxel from the nanoparticle core inhibited the vascular SMC proliferation *in vitro*. The *in vivo* studies in the balloon-injured rat arteries showed that fourfold higher numbers of the targeted nanoparticles were observed on the injured carotid artery compared with the non-injured control. Equal numbers of the nontargeted nanoparticles attached to both the arteries, and those numbers were markedly reduced compared with the collagen IV-targeting nanoparticles [62].

Recently, Li et al. developed a novel approach to inhibit NOX (NADPH oxidases) that have a pivotal role in the development of neointimal hyperplasia and restenosis [63]. The lysine-based HB-OLD7 nanoparticles, which bind to the siRNA through the electrostatic interaction, were used for the local delivery of NOX2 siRNA to the carotid artery walls after angioplasty in an atherosclerotic rat model. The NOX2 gene expression was reduced by >87% 2 weeks post angioplasty compared to the controls. The local delivery of the siRNA-nanoparticles significantly decreased the neointima-to-media-area ratio and preserved the vessel lumen without inducing systemic toxicity [63].

In order to prevent the neointimal hyperplasia following stent implantation, Joner et al. developed the polymer liposome nanoparticles targeted to the chondroitin sulfate proteoglycans that encapsulated prednisolone. These nanoparticle constructs were administered intravenously to the atherosclerotic rabbits 3 times a week for 6 weeks following the bare metal stent implantation [64]. The presence of the nanoparticles was exclusively observed at the sites of the stent-induced injury, whereas none were detected in the contralateral nonstented arteries. The tissue concentration of prednisolone was 100-fold higher in the stented arteries compared to the contralateral nonstented arteries 24 h after drug administration and resulted in a significant suppression of the in-stent neointimal growth. This indicates that the site-specific targeting of the steroid-containing nanoparticles to the stent-injured regions can constitute a suitable approach for the prevention of the in-stent restenosis.

3.5 Targets for therapy: coagulation cascade

The rupture or erosion of the atherosclerotic plaques leads to the exposure of a highly thrombogenic material to the bloodstream, triggering platelet activation and fibrin formation within the arterial lumen. The ischemic strokes and transient ischemic attacks are commonly caused by a cerebral embolism originating from the platelet-rich thrombi induced by the atherosclerotic plaques. The activation of the coagulation cascade in response to the plaque disruption thus represents a target pathway for the therapy of the clinical manifestations of atherosclerosis and for the prevention of the acute cardiovascular events. Peters et al. constructed the fluorescent PEGylated lipid nanoparticles containing a fibrin-binding peptide (CREKA) and the anticoagulant agent, hirulog. Hirulog, a 20-amino acid synthetic peptide combining the active sites of the natural thrombin inhibitor hirudin, inhibits thrombin even after its binding to fibrin. Upon the administration of the fibrin-targeting hirulog particles, significantly higher levels of antithrombin activity were achieved in the aortic tree of the ApoE-deficient mice compared with the non-targeted particles [40].

The single-chain antibody conjugation to the nanoparticles constitutes yet another approach to the targeted molecular imaging, drug delivery, and cell homing in cardiovascular disease. This was previously shown as an efficient approach to the targeted delivery of medication in the form of the fusion proteins. As an example, the urokinase-type plasminogen activator (uPA) fused with a single-chain antibody directed to PECAM-1 was shown to accumulate in the brain after the intravenous application in a mouse model of cerebrovascular thrombosis [74]. Compared to the unconjugated drug, the PECAM-targeting uPA efficiently lysed the clots blocking the cerebral arterial vasculature, thus providing a rapid and stable cerebral reperfusion and alleviating the post-thrombotic brain edema.

In a recent study, Ta et al. produced a single-chain antibody that specifically binds to the ligand-induced binding sites (LIBS) on the glycoprotein IIb/IIIa (CD41/CD61), the most highly expressed molecules on the surface of the activated platelets [75]. This single-chain antibody was subsequently used to label the iron oxide particles and living cells, e.g., the human mononuclear cells and the Chinese hamster ovarian cell line. Using this approach, a strong and specific binding of the single-chain-coupled cells and nanoparticles to the activated platelets was demonstrated *in vitro*, as well as *in vivo*, by intravital microscopy and MRI of the mouse carotid arteries [75]. This method may, in the future, find application in the cell-based regenerative therapies in the cardiovascular disease.

Whereas targeting was, thus far, accomplished by binding the specific-affinity ligands to the nanoparticles, a novel and extremely promising nanomedical strategy of the targeted drug delivery to the stenotic arteries was recently described by Korin et al. [65]. The new approach employs hemodynamic forces to direct the therapeutic agents to the diseased sites. As the occlusions of blood vessels result in the local increases in shear stress leading to platelet activation and clotting, the authors designed microaggregates of poly(lactic-co-glycolic acid) nanoparticles coated with the fibrinolytic tissue plasminogen activator (tPA). These microaggregates, comparable in size to the platelets, are not affected by the physiologic flow conditions with the shear stress values up to 70 dyn/cm². However, in the regions of vascular occlusion/stenosis, the abnormally high shear stress induces their break up (Figure 2A). Following dissociation, the smaller particles being pushed toward the vessel wall by the hemodynamic forces adhere to the narrowed region and release the drug locally. This concept was tested *in vitro* and *in vivo*, in the mouse models of the mesenteric injury and pulmonary embolism. Compared with the free drug, the shear-activated tPA-coated nanoparticles induced a rapid dissolution of the arterial thrombi induced by the exposure of the mesenteric arteries to ferric chloride, with the complete clearance of the occluding thrombi within 5 min after the application (Figure 2B–C) [65]. Moreover, upon the infusion of large fibrin clots, which accumulate in the major pulmonary arteries resulting in the death of the animals within 1 h, an immediate application of the shear-activated tPA-coated nanoparticles increased the survival by 80%. The doses of the shear-activated tPA nanoparticles required for the clot dissolution were about 100 times lower than the doses required for achieving comparable effects with the free drug, which, in addition to the locally exerted activity, can minimize the risk of bleeding and neurotoxicity [65]. This strategy, utilizing a universal hemodynamic phenomenon of shear stress increase upon the reduction in the vessel diameter, should result in a broad applicability for all the occlusive vascular conditions, including, e.g., the treatment of the stenotic atherosclerotic plaques, pulmonary emboli, and ischemic stroke.

3.6 Conclusion

Although several pharmacologic agents for the treatment of the clinical manifestations of atherosclerosis are available, the conventional treatment using the systemic delivery methods has several serious disadvantages, such as the considerable side effects or low efficacy at

the tolerated doses. Replacing the systemic with the locally targeted treatment can substantially minimize the adverse effects, by lowering the drug cytotoxicity and reducing the required dosage. The targeted drug nanocarriers constitute a versatile platform to develop novel treatment approaches and have the potential to transform the therapy of cardiovascular diseases.

4 Vascular tissue engineering with nanoparticles

Nanotechnology provides an attractive platform for vascular tissue engineering. With the aging of the society, an increasing part of the older population demands surgical revascularization of their coronary vessels. However, substantial numbers of the patients undergoing coronary artery bypass surgery (CABG) lack the venous material for the bypass placement. In addition, about 50%–60% of the implanted venous coronary bypasses become occluded within 10 years. This clinical background calls for intensifying the research in order to produce the artery-like vessel substitutes. Although the tissue-engineered prostheses are getting widespread in the clinical use for the treatment of the large-diameter blood vessel disorders, thus far, the synthetic vascular grafts have failed to outperform the autologous grafts for CABG. The interaction of a synthetic material with the circulating blood triggers the thrombogenic responses characterized by platelet adhesion and fibrin deposition on the grafts' luminal surface. The poor long-term patency of the synthetic grafts precludes their use for coronary bypassing, where the vessels with the diameters ranging between 2 and 4 mm are needed. To date, only the synthetic grafts >6 mm in diameter are frequently used in the peripheral bypass surgery with a, nevertheless, moderate success. For example, for the above-knee femoropopliteal bypass, the primary patency rate of the polytetrafluoroethylene (PTFE) grafts after 2 and 5 years is 69% and 39%, respectively [76]. The poor outcome of the plain synthetic grafts results in a higher incidence of the revision surgeries and amputations of the affected limbs. Yet, the synthetic grafts are the only alternative in the patients with no adequate autologous vessel substitutes for transplantation.

Therefore, the development of the vessel substitutes with an endothelial lining, which limits the thrombogenicity and stenoses by creating a physiological interface with the circulating blood, is one of the most urgent tasks in tissue engineering. Ideally, such grafts should also reduce the necessity of the post-graft

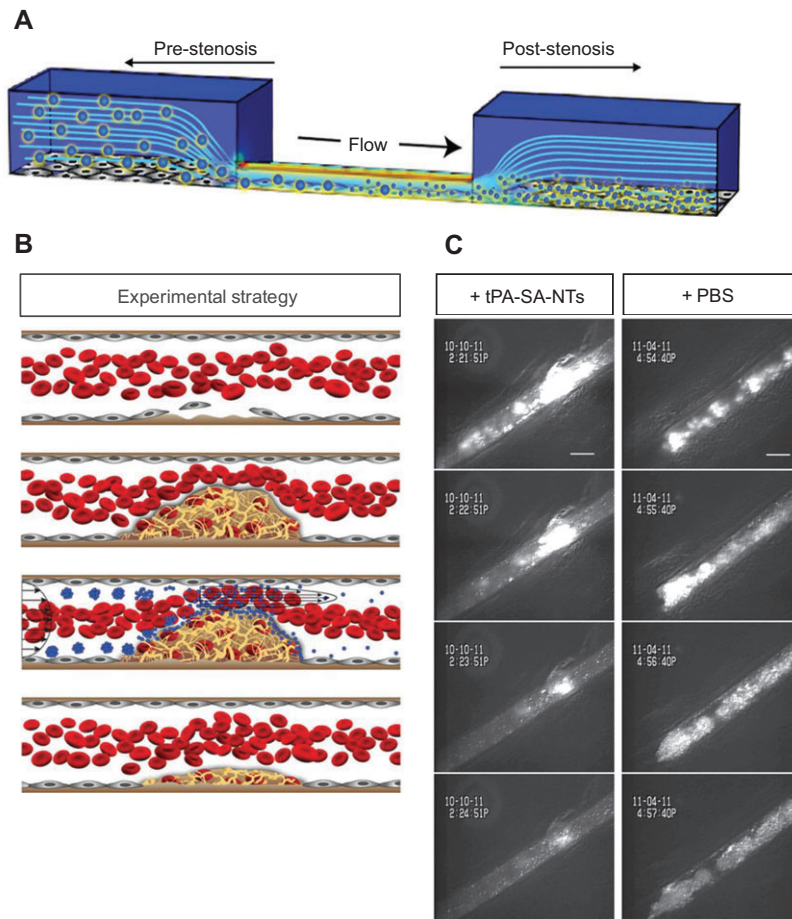


Figure 2 The shear-activated nanotherapeutics. (A) A microfluidic model of the vascular stenosis (90% lumen narrowing) showing the shear-induced dissociation of the microaggregates into the nanoparticles and their targeting to the stenotic and poststenotic region. (B) Experimental strategy: ferric chloride injury initiates the formation of a thrombus (top) that grows to partially obstruct blood flow (top middle). The intravenously injected microaggregates dissociate into the NPs at the thrombus site because of the rise in the local shear stress (bottom middle). The accumulation of the tPA-coated NPs and the binding to the clot at the occlusion site progressively dissolve the obstruction (bottom); (C) The intravital fluorescence microscopic images showing the fluorescently labeled platelets accumulated within a thrombus that partially occludes a mesenteric artery 8 min after injury. The clot on the left, treated with injection of the tPA-carrying microaggregates (50 ng tPA), is greatly reduced in size within 5 min after injection. The control vessel receiving the PBS injection (right) fully occludes over the same time period. Scale bar, 100 μ m; tPA-SA-NTs, tissue plasminogen activator-carrying shear-activated nanotherapeutics. From Korin et al. [65] modified with the authors' permission. Reprinted with permission from the American Association for the Advancement of Science.

immunosuppression by containing either the patient's own or patient-histocompatible endothelial cells. The first clinical trials with the endothelialized vascular grafts have shown that they exceeded the performance of the unseeded custom-made ePTFE grafts [77, 78]. Despite these documented successes, the endothelialized vascular transplants have not found widespread access into the clinical practice. The reasons for that are mainly technical. The tubular geometry of the grafts hinders an efficient delivery of the cells onto the scaffold. The prolonged cell culture protocols and elaborated *in vitro* handling procedures further hamper the scale-up of the production

and the timely supply of the tissue-engineered grafts of clinical quality. For example, the tissue-engineered blood vessels produced by the method of L'Heureux et al. [79], based exclusively on the use of the cultured human cells, i.e., without synthetic or exogenous biomaterials, contain numerous ECM proteins, including elastin, which results in an excellent mechanical strength of the tubular vascular constructs: burst strength >2000 mmHg and a wall thickness of 680 μ m. The major disadvantage of this method is the time required to produce such a vessel construct, which involves a cell culture period of 3 months, including 3 weeks for sheet formation. Optimizing the

efficiency and homogeneity of seeding, reducing the extended cell culture periods, and mimicking the native tissue architecture are thus crucial challenges in vascular tissue engineering.

The use of the magnetic nanoparticles to create the tubular vascular constructs was reported in 2005 and 2006. Nearly in parallel, two independent groups applied different approaches to this process. In 2005, Ito et al. [80] used the cationic liposomes to load endothelial cells, smooth muscle cells, and fibroblasts with the magnetite nanoparticles. To construct a blood vessel, the magnetically labeled endothelial cells were seeded on the planar plates and placed over a magnet for 24 h. Within this time, the cells formed a cell sheet, which was subsequently wrapped around a silicone tube containing a cylindrical magnet. The endothelial cell sheet was followed by the addition of an SMC sheet and a fibroblast sheet. The cell constructs were then supported with collagen I for 24 h, before the silicone tube and the cylindrical magnet were pulled out of the tubular structures. Histologically, the vascular constructs had well-defined tissue layers: intima, media, and adventitia, and the wall thickness of 400 μm . This indicated that magnetic seeding is a useful method to produce the tubular vascular constructs with the native vessel architecture in a relatively short time.

Another approach to the magnetic tissue engineering was reported in 2006 by Perea et al. [81], who applied radial magnetic force from the outside to deliver the magnetically labeled cells directly on the luminal side of a tubular scaffold. The radial magnetic force prevents the undesired sedimentation effects due to gravity and provides the necessary mechanical support for establishing the cell-scaffold contacts. As soon as cell attachment to the surface occurs, the magnetic field is no longer required, and the culture may continue according to the customary physiological conditions. This approach was first tested on human SMCs and endothelial cells which, by successive seeding steps, were assembled into the organized multilayers mimicking the vessel architecture. The tubular collagen scaffolds containing the suspension of SMCs labeled with CD44 magnetic Dynabeads (4.4- μm -diameter spheres consisting of an agglomeration of the submicron-sized iron oxide particles incorporated in a polymeric binder) were placed inside the iron core of an electromagnet and exposed to the radial magnetic field for 20 min. After 5 h, one additional layer of human umbilical vein endothelial cells (HUVECs) labeled with CD31 Dynabeads was seeded onto the luminal SMCs. After 5 days of coculture, the histological examination of the constructs showed densely packed multiple layers of elongated SMCs covered by a monolayer of endothelial cells. The seeding

efficiency of HUVECs and SMCs exceeded 90% after 20 and 40 min magnetic exposure, respectively. In a second study by Perea et al. [82], HUVECs labeled with the clinically approved 50-nm-diameter carboxydextran-coated SPIONs (Resovist, Schering) were seeded on the 7-mm-diameter PTFE tubular grafts coated with fibronectin. The use of the superparamagnetic particles did not affect the cell viability or eNOS expression, and rendered the endothelial monolayer detectable in a 1.5-T MRI scanner. Compared to a conventional rotational bioreactor for the vascular tissue-engineering applications, this technique was shown to considerably reduce the seeding time and enhance both the cell homogeneity and reproducibility of cell seeding.

In a recent study by Gonzalez-Molina et al. [83], this approach was also tested on the fibroblasts tagged with the SPIONs, which were directed onto the luminal surface of large tubular scaffolds by a magnetic field generated by a k4-type Halbach cylinder device. In comparison to a conventional rotational cell-seeding technique for the tubular constructs, a significantly more uniform distribution of cells on the luminal surface of the scaffold was achieved with the radial magnetic field.

The functionalized tissue-engineered vascular grafts do not only possess the potential for the applications in the peripheral and coronary bypass surgery but are also attractive to the pediatric surgery for the congenital heart defects. Moreover, the tissue fabrication technique utilizing the magnetic nanoparticles described by the group of Ito et al. [84] was recently tested *in vitro* for yet another cardiovascular application, namely, the cardiac tissue engineering. A mixture of collagen, Matrigel basement matrix, and cardiomyocytes labeled with the magnetic nanoparticles was added into a well containing a central polycarbonate cylinder. With the use of a magnet, the cells were attracted to the bottom of the well and allowed to form a layer. During cultivation, the cell layer shrank toward the cylinder, leading to the formation of a ring-shaped tissue that possessed a multilayered cell structure and contractile properties. These results indicate that magnetic tissue fabrication is a promising approach also for the cardiac tissue engineering.

4.1 Conclusion

The delivery of the cells into the tubular tissue constructs poses significant spatial and temporal challenges. The nanomedical strategies described above overcome the limitations associated with the existing cell-delivery techniques and raise hope for engineering the vascular

constructs in a rapid, reproducible, and monitorable fashion. Labeling the cells with the superparamagnetic nanoparticles has two major advantages: First of all, it allows the remotely controlled manipulation of the cells by applying an external magnetic field. Second, the intracellular presence of the magnetic nanoparticles renders the cells visible in a MRI scanner, thus, enabling a noninvasive imaging of the seeded constructs, which provides an essential quality control before the implantation as well as the assessment of the graft integration into the physiological environment *in situ*.

5 Nanomedical approaches for improving stent safety profiles

In coronary artery disease, the highly stenosed arteries are frequently treated by stent implantation. The stents are lattice-like scaffolds that are inserted into the stenosed arteries to reestablish the blood flow. Originally, the stents served as the mechanical support systems to the vessels, but the success of the implantation procedure was limited by the complications, such as stent thrombosis and restenosis. The stent insertion leads to the disruption of the endothelial monolayer, leaving a highly prothrombotic intimal surface exposed to the bloodstream. In order to prevent stent thrombosis, a lengthy dual-antiplatelet therapy (DAPT) is necessary. Moreover, the vascular injury induced by the stent implantation results in an excessive SMC proliferation and tissue growth into the arterial lumen, which in the longer term can cause restenosis and vessel occlusion [85, 86]. So far, the most successful solution to the stent restenosis has been the drug-eluting stent (DES), which contains antiproliferative drugs in order to reduce the hyperplastic and immune responses induced by the stent implantation. In the clinical trials, the DES has been shown to significantly reduce restenosis compared to the bare metal stents [87, 88]. However, maintaining the adequate drug concentrations on the DES represents an obstacle to a long-term prevention of the SMC proliferation and restenosis of the coronary arteries after stent implantation. This problem could be bypassed using nanotechnological approaches.

5.1 Application of nanoparticles to prevent restenosis

Nakano et al. [89] designed, developed, and tested a novel bioabsorbable polymeric nanoparticle-eluting

stent (NES). It was hypothesized that such NES provides a drug delivery system that shows more sustained delivery of the therapeutic agents than the common dip-coated stents. As a proof of concept, the cationic nanoparticles encapsulated with a fluorescence marker were transferred to the stent using an electrodeposition coating technology. A substantial fluorescence signal was observed for 4 weeks in the neointima and media of the porcine coronary artery segments that received the NES. The stent-induced injury, inflammation, endothelial recovery, and neointima formation were comparable for the NES and the control stents.

Another approach to extend the life and effectiveness of the implanted DES employs the biodegradable, superparamagnetic nanoparticles as the transport vehicles that can be guided to the stents by high gradient magnetic fields for their redosing with antiproliferative drugs [90]. In their study, Johnson et al. determined the characteristics of the polymeric magnetite nanoparticles releasing paclitaxel *in vitro*. Upon treatment with the paclitaxel-containing nanoparticles, the cell growth arrest of 80% in vascular SMCs and 100% in endothelial cells over 8 days was observed compared with the untreated cells and unconjugated nanoparticles. Such biodegradable drug-releasing nanoparticles loaded with magnetite could potentially function as the drug carriers for redosing the depleted stents, thereby prolonging the efficiency of the implant.

However, the drug-induced inhibition of the SMC proliferation also inhibits the reestablishment of a healthy endothelium, thus increasing the risk of stent-related thrombosis. There is growing evidence that the incidence of stent thrombosis is higher in the DES than the bare metal stents, particularly after the cessation or premature discontinuation of DAPT [91–93]. Therefore, a new DES system targeting the SMCs without adverse effects on the endothelial cells is urgently needed.

Masuda et al. [94] have tested the suitability of the platelet-derived growth factor (PDGF) inhibition for reducing the in-stent neointima formation. For this purpose, the nanoparticles containing imatinib mesylate (PDGF receptor tyrosine kinase inhibitor) were delivered using the bioabsorbable polymeric NES. The imatinib nanoparticles suppressed the proliferation of SMCs via the inhibition of PDGF receptor- β , but did not affect the endothelial proliferation *in vitro*. In a pig coronary artery stent model, imatinib-NES reduced the in-stent neointima formation and stenosis by approximately 50% compared with the fluorochrome-eluting stent and bare metal stent and did not affect the reendothelialization. In a further study from the same

group [95], the statin-incorporated NES was used to inhibit the in-stent stenosis without delaying endothelial healing. Based on its potent inhibitory effects on the SMC proliferation *in vitro*, pitavastatin was selected for producing NES. In a pig coronary artery model, the effectivity of the in-stent stenosis inhibition by the statin-containing NES equaled that of the polymer-coated sirolimus-eluting stents, but was not accompanied by the delayed endothelialization as observed in the sirolimus group.

5.2 Nanoscale structure modifications

As an alternative to the DES, the recent studies focus on the strategies that enhance the endothelialization of the stented arteries, rather than inhibit the uncontrolled SMC proliferation. In this context, growing attention has been paid to the role that stent design and material composition can play on the outcomes of the patients undergoing stenting. Among other characteristics, the surface topography either in the micrometer or in the nanometer range has been attributed an important role in the stent performance.

A smooth stent surface was previously believed to prevent the activation and aggregation of platelets, consequently leading to the reduced thrombus formation and decreased neointimal hyperplasia of the coronary stents. In 1998, de Scheerder et al. [96] found that the stents with a surface smoothed by electrochemical polishing caused less clot formation compared to the nonpolished stents after their implantation in a rat carotid arteriovenous shunt model. In the same study, when the two stent types were implanted in the right coronary arteries of the healthy pigs, the mural thrombi at 7 days were less frequently found among the smooth-surface stents. In addition, at 6 weeks after implantation, the neointimal hyperplasia decreased by 40% in the smooth-surface stents compared to the stents with a rougher surface [96]. In 2002, in an *in vitro* model with fresh human whole blood, Tepe et al. [97] evaluated the thrombogenicity of the different stent types. They reported that smoothing the stent surface by electropolishing reduced their thrombogenicity. These studies constituted the rationale for recommending the use of the stents with the smooth surface to improve the patients' outcomes. However, the recent work suggests that apart from thrombogenicity, another factor that critically determines the fate of the implanted stents is the rate of endothelialization, which may be enhanced by a rough surface. In 2005, Dibra et al. [98] conducted the first clinical study on 200 patients to

evaluate the relationship between stent surface topography and the patients' outcomes following the implantation of the smooth and rough surface stents. The 316-L stainless steel coronary stents were electrochemically polished to obtain a smooth surface. The rough surface was produced by sand blasting of the electrochemically polished stent surfaces. The results showed that both types of stents were equivalent with respect to the safety profile and late lumen loss. However, the rough-surface stents had a reduced angiographic restenosis compared to the smooth electrochemically polished stents.

In an *in vitro* study from 2010, the e-beam evaporation was used to generate the various titanium stent structures with the different roughness: submicron rough surface features with the lateral dimensions larger than 100 nm; nanorough surface features with the lateral and vertical dimensions <50 nm; and the flat surface features as the controls [99]. The cell growth as well as the intracellular collagen and elastin synthesis were determined in rat aortic endothelial cells and SMCs grown individually or in cocultures on the various substrates. Compared to the control flat titanium surfaces, the nanometer rough-surface features enhanced the endothelial proliferation, which was further improved on the submicron surfaces. Simultaneously, the SMC proliferation was inhibited in parallel with the increasing surface roughness. The collagen and elastin production by the endothelial cells was increased with the growing roughness of the titanium substrates compared to the flat control substrates. The surface characterization results showed that the submicron rough titanium was most hydrophilic, which may have promoted the selective protein adsorption to increase the endothelial cell attachment and proliferation [99]. The results of the above studies suggested that modifying the stent surface topography in the nano- or submicroscale can enhance the stent endothelialization. In parallel with the stent technology development, a question arose whether the endothelialization induced by the random roughness of the substrate can be further improved by the application of the rationally designed structures. Considering that the endothelial monolayer consists of endothelial cells aligned with the direction of the blood flow, it has been speculated that a pattern of the parallel grooves could further enhance the attachment and growth of endothelial cells and improve their physiologic (anti-inflammatory and antithrombotic) functions. Several *in vitro* studies carried out between 1999 and 2002 by the investigators from the group of E. Sprague showed that creating the surfaces with the parallel microgrooves accelerated the migration rate of endothelial cells compared to the smooth controls, suggesting a potential

effect of the grooved endovascular stent surfaces on the faster endothelialization times [100]. In 2008, the patterned titanium surfaces consisting of the periodic arrays of the grooves with spacings ranging from 750 nm to 100 μm have been produced by Khang et al. [101]. The adhesion and growth assays using rat aortic endothelial cells were performed on these substrates, showing the enhanced endothelial cell coverage on the nanometer-scale titanium patterns (750 nm) compared with the larger micrometer-scale titanium patterns (5–100 μm), as well as the controls with a randomly nanostructured surface. Furthermore, the nanometer-patterned titanium surfaces induced the endothelial cell alignment similar to the natural endothelium. A further study from the same group investigated the contribution of the pure nanometer (grooves <100 nm in both the lateral and vertical scale) and submicron (grooves larger than 100 nm in the lateral scale) surface structures on the adhesion of endothelial cells [101]. Compared with the flat titanium surfaces, the submicron surface structures led to an increase in the surface energy and promoted endothelial cell adhesion density by 200%. In comparison, the nanometer surface structures led to a 50% increase in the endothelial cell adhesion density. The study by Biela et al. [102] essentially confirmed the above findings using the nano-microstructured silicon substrates with rectangular parallel grooves of different depths (50, 100, and 200 nm), and groove widths between 2 and 10 μm . The cell adhesion, orientation, and migration of fibroblasts, SMCs and endothelial cells were influenced by the different surface topographies. The endothelial orientation response and change of cell shape was induced by the grooves of a minimum of 100 nm depth, with little effect observed on the shallower grooves (50 nm).

However, an *in vivo* study confirming those findings was missing until very recently. In their paper from 2012, the group of E. Sprague investigated the effects of the stents with the microscopic parallel grooves on the early endothelialization and restenosis in a porcine coronary injury model. The authors reported that already at 3 days post-implantation, the coronary artery stent endothelialization was significantly increased in the microgrooved stents (81.3%) compared with the smooth surface stents (67.5%). Moreover, at 28 days, the neointimal thickness was significantly lower in the microgrooved stent group compared with the smooth surface controls [103]. These results indicate that an enhanced rate of early endothelialization and significant inhibition of the neointimal hyperplasia can be achieved by the application of an appropriate surface structure to the bare metal stents.

5.3 Conclusion

The rapid stent endothelialization reduces in-stent thrombosis and shortens the treatment time with the dual antiplatelet therapy. Moreover, the delayed endothelial healing among the patients receiving the DES is associated with the late adverse events. To overcome the safety concerns related to the currently used DES, nanotechnology offers several alternative approaches that reduce in-stent stenosis and/or enhance endothelialization. The nanoparticle-eluting stents represent a novel platform to deliver the endothelium-protective, antiproliferative drugs to the injured sites. Considering the strong influence of the stent topography on the endothelial cell growth and functions, which are decisive for the subsequent biological response to the implant, creating the nano/micro-scale structures on the bare metal stent surfaces constitutes a promising approach to accelerate endothelialization and reduce stent thrombogenicity.

6 Challenges and safety considerations

Although the potential benefits of the nanoparticles in cardiovascular medicine are considerable, several issues regarding nanoparticle delivery, biodistribution, cytotoxicity, and clearance demand attention to ensure the patients' safety. For example, in the patients with impaired renal function, a delayed serious adverse reaction known as nephrogenic systemic fibrosis was reported after the administration of the low-stability gadolinium-based contrast agents, which led to the withdrawal of these MRI agents from clinical practice. Within the last 3 years, two new molecular MRI agents have been approved for clinical use, exhibiting excellent safety profiles [104]. A recent report from the RESCUE study showed that gadolinium-DOTA did not affect the renal function and was a safe contrast agent in the patients with chronic kidney disease [105]. It must be noted, however, that even in the case of the targeted nanoparticles, most of the applied dose accumulates in the liver, spleen, kidneys and bladder. Although the metal or metal oxide-containing nanoparticles have generally favorable safety profiles, their retention in the human body can lead to the delayed toxicity due to an increased inflammation and oxidative stress. The accumulation of the metal-based nanoparticles in the body could also, in a longer term, affect other medical tests (e.g., radiological and MRI examinations). Compared to the free drugs, the nanosystems are extremely complex

constructs, and the lack of their comprehensive standardized characterization (structural and physicochemical properties, stability and breakdown products, behavior in the biological environment, as well as the coating characteristics, and interactions between the targeting moiety and its ligand) is a serious obstacle to overcome before they can enter the clinical practice. The stability of the surface coating and the potential immunogenicity of a ligand must also be investigated in detail, both *in vitro* and *in vivo*. With respect to the iron-containing nanoparticles, an additional issue is their fate following the delivery to the atherosclerotic plaques. The high iron concentrations localized to the plaque area can have toxic implications, particularly as the high levels of free iron ions can increase the oxidative stress and lipid peroxidation [106].

Owing to their hydrodynamic diameter of 10–200 nm, the therapeutic nanoparticles will evade renal clearance, which, on one hand, constitutes an advantage over the free drugs. On the other hand, for the nanoparticles that are not renally cleared, the only other major route of excretion is through the liver into the bile, which is an extremely slow and inefficient process. As the treatment with the therapeutic nanoparticles is likely to involve repeated administrations, the long-term retention of the nanoparticles in the liver can result in an increased risk of toxicity. These considerations should be made already at the earliest steps of the nanosystem design, and the

demonstration of their safety and efficacy should be given high priority even in the preclinical studies.

7 Concluding remarks

Nanotechnology offers a unique platform for the novel approaches to cardiovascular imaging and drug delivery, as well as the vascular tissue engineering and improving the safety of the stents. The extensive efforts in the field of nanomedicine, together with the promising results of the animal studies, raise hope that before long, the nanoparticle assemblies integrating a carrier system, a targeting modality, and an active drug will be approved for the diagnosis and therapy of cardiovascular diseases. Moreover, the emerging nanotechnological approaches to the revascularization procedures should, in the near future, help overcome the shortcomings of the current interventional and surgical techniques used in the treatment of atherosclerosis.

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Christoph D. Garlichs, an experienced clinician specialized in interventional and experimental cardiology, studied Medicine and Philosophy in Berlin, and completed his doctoral thesis in 1996. He headed the Laboratories of Molecular Cardiovascular Research at the University of Dresden before becoming the Head of the Laboratory of Molecular Cardiology at the University of Erlangen-Nuremberg in 1998. He obtained his habilitation in Internal Medicine in 2002 and became Professor of Cardiology in 2008. Since 2007, he is the Vice Director in the Medical Clinic 2 (Cardiology, Angiology) of the University Hospital Erlangen.

Over the last 15 years, the Laboratory of Molecular Cardiology has carried out multiple research projects concerning cardiovascular diseases. The main topics of the studies have been vascular inflammation, pathomechanisms of atherosclerosis, as well as hypertension and vascular calcification research. The research team covers a broad spectrum of the experimental methods. Large biobanks have been established, comprising, e.g., >1500 specimens of the human atherosclerotic plaques; serum, plasma, and the DNA samples from >5000 patients with coronary heart disease.

The Section of Experimental Oncology (SEON) emerged from the working group of Prof. Alexiou, after he received the first chair for Nanomedicine in Germany, endowed by the Else Kröner-Fresenius Stiftung in 2009. The group has more than 15 years of experience in the application of iron oxide nanoparticles in cancer treatment. The favored therapy approach is “Magnetic Drug Targeting.” The main goal of SEON is to enhance cancer treatment and simultaneously reduce the side effects of chemotherapy, by accumulating the nanoparticle-bound drug with strong external magnetic forces. Recently, the SEON has broadened its activities to include the application of the iron oxide particles in regenerative medicine and in the treatment of atherosclerosis.

The Laboratory of Molecular Cardiology and SEON cooperate on several projects involving the application of the nanomedical strategies for the treatment of cardiovascular disease.