Review

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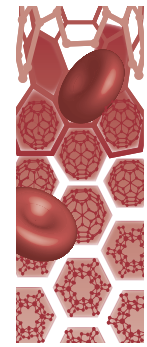
Nanomedicine



Nanotechnology in diagnosis and treatment of coronary artery disease

Nanotechnology could provide a new complementary approach to treat coronary artery disease (CAD) which is now one of the biggest killers in the Western world. The course of events, which leads to atherosclerosis and CAD, involves many biological factors and cellular disease processes which may be mitigated by therapeutic methods enhanced by nanotechnology. Nanoparticles can provide a variety of delivery systems for cargoes such as drugs and genes that can address many problems within the arteries. In order to improve the performance of current stents, nanotechnology provides different nanomaterial coatings, in addition to controlled-release nanocarriers, to prevent in-stent restenosis. Nanotechnology can increase the efficiency of drugs, improve local and systematic delivery to atherosclerotic plaques and reduce the inflammatory or angiogenic response after intravascular intervention. Nanocarriers have potential for delivery of imaging and diagnostic agents to precisely targeted destinations. This review paper will cover the current applications and future outlook of nanotechnology, as well as the main diagnostic methods, in the treatment of CAD.

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Coronary artery disease (CAD) describes a disease process in which atherosclerotic plaque accumulates in the lining of coronary artery, producing a narrowing of the lumen of the artery, reducing the compliance of the vessel wall and gradually or suddenly causing a loss of blood supply to a portion of the myocardium [1]. Angina or chest pain that can occur either after exercise or even at rest results, when blood supply to the heart muscle is seriously restricted. Another major problem occurs when a coronary thrombosis suddenly blocks the blood supply to the heart, precipitating a heart attack or a myocardial infarction. The commonest cause of this sudden thrombosis is the rupture of an atherosclerotic plaque in a coronary artery [2]. The plaques that are prone to rupture have certain characteristics which are described as 'vulnerable'. These characteristics include the presence of a thin collagen cap, a lipid-rich interior, a high metabolic rate, many activated macrophages, a high degree of inflammation, a necrotic core resulting from macrophage apoptosis and a content rich in tissue factor that precipitates the actual thrombosis [3]. Recent reviews have widened the concept of vulnerability to heart attack to include vulnerable blood (prone to thrombosis) and vulnerable myocardium (prone to fatal arrhythmia). Therefore, the term 'vulnerable patient' may be more appropriate to describe the high likelihood of developing cardiac events in the near future [4].

Atherosclerosis is a thickening of the arterial vessel wall that becomes inflamed due to atheromatous plaque formation [5]. The propagation of the lesions in atherosclerosis leads

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to angiogenesis or the formation of new blood vessels within the artery wall similar to that seen in growth of cancerous tumors. Increased metabolic activity of the growing plaque requires a higher supply of nutrients and oxygen to the underlying proliferating cells. To satisfy this nutritional need, endothelial cells rapidly proliferate and form atypical blood vessels that are defective and immature. This state alters the dynamics of macromolecular transport to and from the lesion, and is responsible for the enhanced permeability and retention (EPR) effect that allows accumulation of systemically delivered macromolecules [6]. Furthermore, tissue inflammation causes constant leukocyte recruitment through the release of proinflammatory cytokines. As leukocytes traverse through the endothelium into the extravascular space, the transport of circulating nanomaterials is facilitated by the leaky endothelial cell-to-cell junctions. This condition increases endothelial permeability and allows for selective delivery of therapeutic nanocarriers in the inflamed area [7].

Restenosis occurs after balloon angioplasty has been employed to widen the arterial lumen, where the vessel closes upon itself again, caused by a different mechanism than the original atherosclerosis [1]. This pathological state caused by balloon angioplasty has been attributed to three different processes: the elastic response that occurs after the overstretching of the vessel, neointimal formation and chronic remodeling [8].

Current therapies for atherosclerosis focus on lessening the burden of atherosclerotic plaque, stabilizing vulnerable plaques defined as those plaques likely to rupture and cause thrombosis [9]. To reduce the risk of in-stent thrombosis and/or restenosis caused by bare metal stents, new types of stents were designed that function as drug-eluting stents (DES). A wide range of dissimilar classes of drugs have been tested with DES in order to prevent smooth muscle cell (SMC) growth and proliferation including anticancer and anti-inflammatory agents. Another approach is the modulation of gene expression with plasmid DNA or RNA interference to generate an imbalance in local concentrations of specific signaling molecules that can inhibit the growth of certain cells, while promoting the growth of others [1].

Therapeutic agents

Drugs that can be used to combat restenosis can be classified into four groups: anti-inflammatory, antithrombogenic, antiproliferative and immunosuppressive. Some common agents that have been loaded onto DES to inhibit restenosis are:

- Sirolimus/rapamycin: these are potent immunosuppressive drugs that can also prevent migration of SMCs;
- Paclitaxel: cytotoxic drug that causes inhibition of SMCs migration and proliferation;
- Zotarolimus and Everolimus: bind to cytosolic FK-506-binding protein-12 and inhibit the proliferation of SMCs and T-cells;
- Tacrolimus: an immunosuppressive agent;
- Actinomycin D: an inhibitor of cellular proliferation like paclitaxel;
- Dexamethasone: the corticosteroids are well established as anti-inflammatory drugs [10].

Several clinical trials have tested oral anti-inflammatory or immunosuppressive methods to prevent instent restenosis (ISR), and these studies have shown a significant decrease of angiographic ISR [11,12].

Furthermore, different specific molecular targets have also been used in treatment of restenosis. For instance, examination of the effect of PDGF receptor specific inhibitors, tyrphostin, AG1295 and AGL-2043 on neointimal formation revealed significant inhibition of restenosis [13]. Some of the important studies including *in vivo* studies with various drugs are reported in Table 1. Additionally, Table 2 looks into couple of clinical trials which are observed related in order to investigate physiological factors such as neointima thickness and stenosis diameter.

Adhesion, activation and accumulation of platelets in injured position is one of the key factors in atherothrombosis [22]. Currently used antiplatelet agents include cyclooxygenase-1 inhibitors (aspirin, indobufen, triflusal), $P2Y_{12}$ inhibitors (prasugrel and ticagrelor [23], clopidogrel, cangrelor), phosphodiesterase inhibitors (dipyridamole, cilostazol), glycoprotein (GP) IIb/IIIa blockers (abciximab, etifibatide, tirofiban), thromboxane receptor and thrombin receptor (PAR-1) antagonists [22].

Aspirin is responsible for interrupting the production of thromboxane A_2, one of the influential agents on platelet activation, by blockage of cyclooxygenase-1 enzyme [24]. GP IIb/IIIa blockers attach to GP IIb/IIIa receptors instead of fibrinogen and Von Willebrand factor at the last stage of platelet accumulation and are considered as fast and effective antiplatelet agents [24]. $P2Y_{12}$-receptor inhibitors are activated via a hepatic metabolism to joint the adenosine diphosphate-binding positions on $P2Y_{12}$-receptor which are in charge of inducing signaling cascades in platelet accumulation process [23].

Dual antiplatelet therapy (DAPT) is the simultaneous application of aspirin and a $P2Y_{12}$-receptor inhibitor [25] (e.g., ticlopidine, clopidogrel, prasugrel, ticagrelor [26]). After myocardial infraction, all the patients

should follow a DAPT prescription for a year and a single agent (commonly aspirin) afterward [27]. Nevertheless, application of this method over a long time span escalates bleeding risk [28]. Mauri *et al.* studied the application of DAPT for 12 or 30 months after DES implantation. They reported the risk of ISR was decreased by utilizing this method follow-up to 16 months compared with merely aspirin, however, the risk of bleeding was high [29].

Plasmonic and photothermal therapies are uprising in the field of atherosclerosis therapy. They consist of a dialectric silica core covered by a metallic shell (commonly gold or silver). While these NPs irradiated with near-infrared (NIR) laser, absorbed energy leads to irreparable damage of the tissue. This approach is like excimer laser angioplasty which uses monochromatic light energy source to excite molecules within atheroma. Acoustic dissection happens when water content is superheated to produce vapor bubbles. So, acoustic energy can be applied for plaque elimination. High-energy UV rays penetrate in the tissue at small depths causes injury of internal elastic lamina, leading to exuberant response of intima and media. On the other hand, NIR laser has little impact on elastic lamina. Three groups of plasmonics are available which could be used in atherosclerosis therapy: microbubble overlapping mode, nanocluster aggregation mode and thermal explosion mode (nanobombs) [30]. Regression of plaque burden was achieved in two different approaches: first, transplantation of bioengineered on-artery patch advanced with stem cells bearing NP; second, trancatheter intravascular infusion or injection of NP loaded with iron containing stem cells or targeted microbubbles coated with protein and delivery to the specific site applying magnetic fields [31].

Nanoparticle delivery systems

Nanotechnology can be used in therapies for atherosclerosis by increasing systemic agent circulation time, lowering off-target cytotoxicity of drugs, improving drug solubility, decreasing the required dosage, combining diagnostic and therapeutic agents to form theranostics and increasing accumulation of agents at specific sites [6]. Targeted drug delivery can be categorized as either active or passive targeting. Active targeting involves the conjugation of tissue or cell-specific ligands to either the nanocarriers or to the drugs themselves. In the passive targeting strategy, therapeutic agents or drugs are coupled with macromolecules to take advantage of the EPR effect [32]. Recently, stimuli-responsive delivery systems have been emerged based on various triggers such as light irradiation, pH alteration, application of magnetic or electric fields, a change in temperature or response to redox potentials.

Table 1. Drugs that have been tested for restenosis treatment *in vivo*/clinical trials.

Drug/gene		Animal	Region of treatment	Internal elastic lamina (luminal area; mm^2)	Endotheliaziation (%)	Neointima	Stenosis (%)	Inhibition of proliferation (%)	Ref.
Imatinib mesylate		Pig	Coronary artery	–	Not affected	50%	–	–	[14]
Paclitaxel	Low dose 0.2 μg/mm^2	Pig	–	5.331 ± 0.717	99.524 ± 1.289	1.432 ± 0.632 (mm^2)	27.648 ± 12.980	–	[15]
	Medium dose 0.4 μg/mm^2	Pig	–	5.560 ± 0.780	99.381 ± 1.024	1.845 ± 0.950 (mm^2)	33.052 ± 16.428	–	
	High dose† (0.7 μg/mm^2)	Pig	–	7.305 ± 0.912	86.708 ± 16.956	2.930 ± 1.144 (mm^2)	40.143 ± 15.547	–	
Sirolimus (rapamycin)		Pig	Porcine aortic endothelial cells	–	–	–	–	100	[16]
			Porcine vascular smooth muscle cells	–	–	–	–	31	
TRM-484	Control	Rabbit	–	–	–	–	29.5 ± 8.1	–	[17]
	1 mg/kg	Rabbit	–	–	–	–	22.5 ± 4.4	–	
Adinoviral vectors containing human inducible nitric oxide synthase		Rat	Carotid	–	–	0.248 ± 0.028	17.54 ± 1.8	–	[18]

†Cobalt chromium stents coated with a poly-lactide and poly lactide-co-glycolide biodegradable polymer and high dose.

Table 2. Relative physiological factors in coronary artery disease observed by optical coherence tomography in clinical therapies before and after applying drug-eluting stents (human studies).

Drug	Region of treatment	Age (years)	Total patients (n)	Minimal lumen diameter (mm)	Diameter stenosis (%)	Neointima thickness	Evaluation method	Ref.
Silrolimus	Coronary artery	63 ± 11	26	In-sten: 2.22 In-segment: 1.95	In-stent: 15.18 In-segment: 22.90	57.1 (μm)	OCT	[19]
Biolimus	Coronary artery	64.9 ± 10	20	In-stent: 2.40 In-segment: 2.06	In-stent: 13.13 In-segment: 23.24	67.6 (μm)	OCT	[19]
Sirolimus	Coronary artery	66 ± 10	34	–	–	52.5 (μm)	OCT	[20]
Sirolimous	Coronary artery	67 ± 10	46	(Follow-up) 2.03 ± 0.42	(Follow-up) 23.5 ± 10.6	77 ± 53 (μm)	OCT	[21]
Paclitaxel	Coronary artery	67 ± 10	37	(Follow-up) 2.00 ± 0.53	(Follow-up) 22.8 ± 15.7	201 ± 136 (μm)	OCT	[21]

OCT: Optical coherence tomography.

These smart NPs could be potentially applied in DES for therapy of CAD. For example, Tang *et al.* tested pH-responsive delivery of antioxidants for treatment of cardiovascular disease such as atherosclerosis [33]. On the other hand, localized NP delivery via stents may be a promising strategy to combat restenosis, because it could provide a sustained drug release in the target region of the artery [32]. DES can be used to localize delivery of drugs and avoid the potential toxicity of systemic drug administration [34].

NPs used to inhibit restenosis are reviewed in the following paragraphs, and Figure 1 shows the schematic structure of important NPs.

Liposomes

Liposomes are small vesicles, have a spherical shape and are composed of a lipid-bilayer formed from natural and nontoxic phospholipids and cholesterol [35]. The features of liposomes such as biocompatibility (because of using natural biologically safe lipids), nanometer size, the ability to tailor the hydrophobicity and hydrophilicity can provide enhanced tissue specificity for delivery of hydrophobic drugs in the lipid environment, and for hydrophilic drugs in the aqueous core [36]. The revascularization of occluded arteries *in vivo* was enhanced, along with a reduction in the risk of hemorrhagic side-effects. The effect of peptide-modified liposomes with good potential for vascular-targeted delivery of therapeutic and diagnostic agents has been studied. Ligands that recognize surface receptors on activated platelets (e.g., integrin GP IIb/IIIa and P-selectin) have been attached to liposomes to demonstrate the vital role of activated platelets in atherogenesis, atherosclerotic lesion progression and thrombosis in vascular diseases [37]. Phase 1 results of one study show that after 28 days of follow-up in rabbit carotid artery, liposomal alendronate can reduce ISR to 40.1% in comparison to 73.5% in empty liposomal [38]. Figures 2 & 3 demonstrate examples of liposomal delivery in CAD therapy.

Micelles

Micelles are formed when amphiphilic molecules undergo self-assembly due to the energy minimization that occurs when the hydrophobic portions bunch together to form the interior. The hydrophilic shell provides the long circulation time and accounts for the relative stability *in vivo*. The hydrophobic core of micelles can be used for encapsulation and delivery of either bioactive therapeutic molecules, diagnostic agents or both. Attachment of moieties such as targeting ligands to the outer shell of micelles can increase active binding to disease-relevant tissues and cells [36]. There are results that suggest phospholipid-based micelles provide better antirestenotic effects (neointimal area 0.034 in comparison to 0.046 mm^2 after 14 days of implantation in rat carotid arteries) than the PEGylated liposomes; probably due to the distinctly smaller size of the phospholipid-based micelles [41]. Micelles that had been surface-modified with anti-CD36 antibodies were loaded with Gd. These micelles could target macrophages in specimens from atherosclerotic human aortas [42].

Polymeric nanoparticles

Polymeric NP may be constructed in the form of solid, dense but porous structures (e.g., nanospheres and

nanorods) or hollow structures (e.g., nanoshells and nanocapsules) [1,43]. Results of one study on 60-nm diameter lipid–polymeric NP functionalized by collagen IV-targeting peptides and enriched with paclitaxel demonstrate efficacious improvement. Injection of paclitaxel (0.3 mg/kg or 1 mg/kg) in a rat carotid arteries followed on days 0 and 5 resulted in lower neointima-to-media (N/M) ratio for the targeted NP group at 2 week versus the control groups. Compared with controlled groups, a 50% reduction in arterial stenosis was observed with targeted NP delivery [44]. Another study reported that PLGA NPs containing alendronate reduced neointimal formation and restenosis up to 64% for a dose of 3 mg/kg by systemic transient depletion of monocytes in a hypercholesterolemic rabbit model [45]. Delivery of imatinib (used as an inhibitor of PGDF receptor) by means of bioabsorbable polymeric NPs reduced the occurrence of ISR up to 50% compared with bare metal stents [14]. Statins have been shown to prevent the proliferation of vascular SMCs (VSMC) and also to stimulate vascular healing. Researchers formulated an NP-coated DES with 20 µg pitavastatin dosage per stent and tested it in a pig coronary artery model. This coated stent inhibited ISR as effectively as a polymer-coated sirolimus-eluting stent. There was a delay in endothelial healing with the conventional sirolimus-eluting stent, whereas no delay in re-endothelialization was observed in the pitavastatin NP-eluting stent [46].

Dendrimers

Dendrimers consist of a single molecule constructed from an original inner core with a series of macromolecular branches built up by successive additions of discrete units (generations). The ability to display multiple copies of functional groups on their surface makes them a unique structure for drug-delivery applications [47]. Dendrimers are more used in cell-labeling rather than in ISR therapy. For instance, manganese G8 dendrimers [48] have been successfully applied in atherosclerosis detection. One study described the development of 'tadpole' dendrimeric materials for siRNA delivery in a rat ischemia-reperfusion model. Angiotensin II (Ang II) type 1 receptor (AT1R) has been investigated since it is the major receptor that mediates most adverse effects of Ang II. Among those tadpole dendrimers evaluated, significant effective downregulation in AT1R expression in cardiomyocytes was related to the oligo-arginine-conjugated dendrimer loaded with siRNA *in vitro*. Delivery of the siRNA *in vivo*, inhibited AT1R levels to be increased, and meaningfully cardiac function recovery was improved compared with saline injection or empty dendrimer treated groups [49]. Additionally, polyamidoamine (PAMAM) dendrimers have been favored in recent years in CVD therapies. PAMAM zero generation dendrimers (G0) were tested as nanocarriers in drug delivery and conjugated G0 PAMAM dendrimers with a ZnPc photosensitizer were chosen to study their effects on the diseased and normal tissues extracted from human carotid arteries. Statistical analysis was carried out based on AFM images extracted through fractal analytical methodologies and Minkowski functionals. The affinity of the nanocarriers for healthy tissue and atheromatous tissue was different. Dissimilar aggregation behaviors between G0 and G0/ZnPc nanomateri-

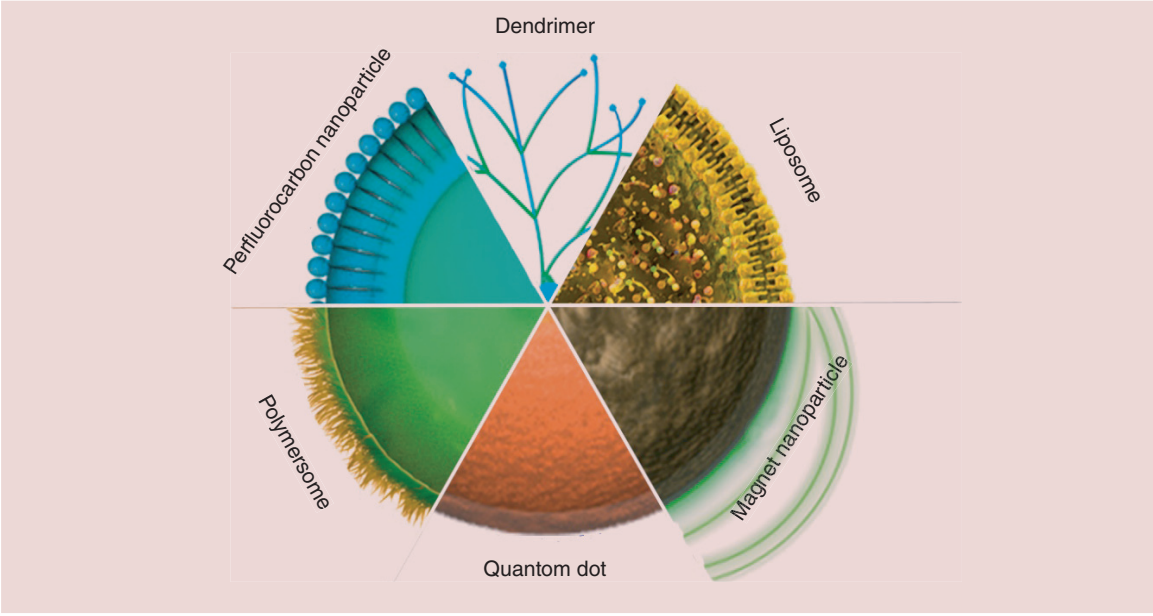

Figure 1. Schematic structure of important nanoparticles.

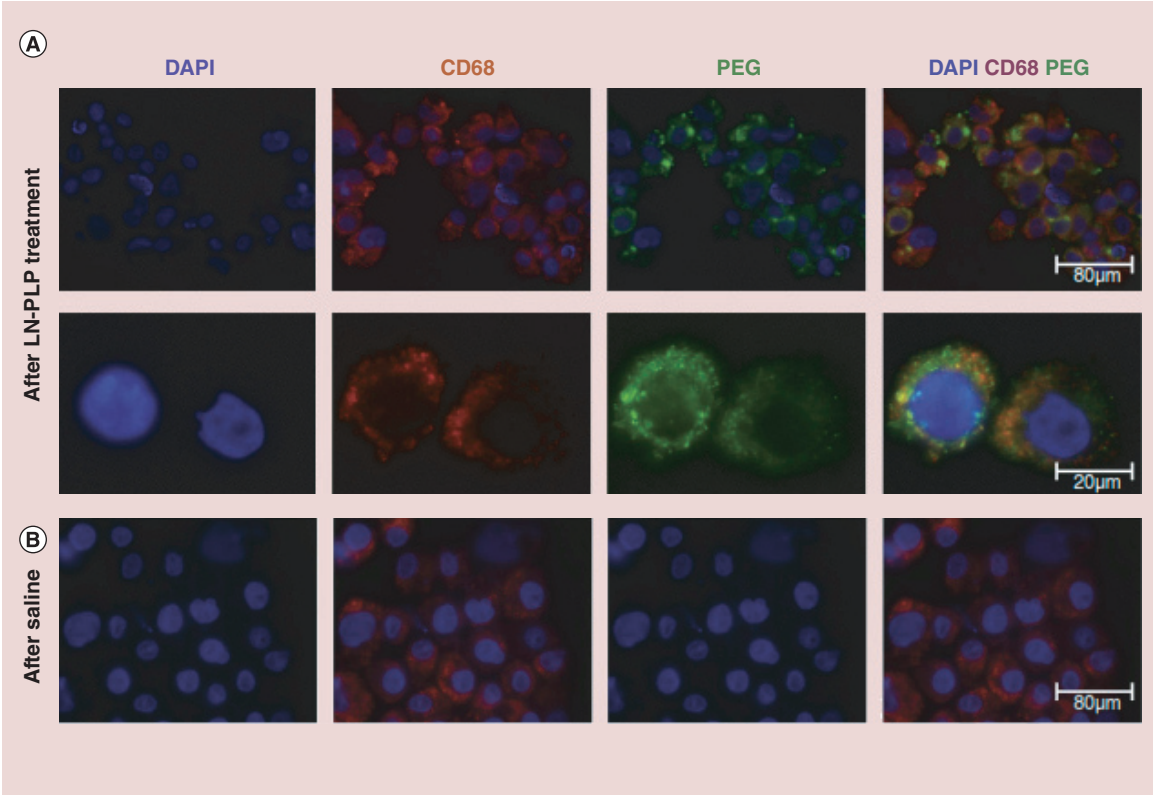

Figure 2. The liposomal nanoparticle with prednisolone phosphate stored in a macrophages of iliofemoral plaques. (A) First row illustrates the plaque cells cured by LN-PLP marked for cell nuclei (DAPI), macrophages (CD68) and liposome-coating PEG. In the second row, magnified images of isolated cells are shown. **(B)** Third row shows CD68 cells from a plaque cured by saline, but there is no positivity for PEG.
LN-PLP: Liposomal nanoparticle with prednisolone phosphate.
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als were observed. Larger G0/ZnPc aggregation on the atheromatous plaque were reported [50]. Photodynamic therapy with PAMAM dendrimers could have a bright future in therapy of atherosclerosis.

Gel-like nanoparticles

It was demonstrated that hydrogel nanospheres (100 nm) made of poly (N-isopropylacrylamide) were internalized by endothelial cells and VSMC to a greater degree than microspheres (1 μm), although the cellular uptake was dependent on the incubation time, nanosphere concentration and applied shear stress levels in the medium. By contrast, microspheres were rapidly taken up by phagocytes, especially at high concentrations. These findings suggest that hydrogel nanospheres were more effective as an intravascular delivery system in terms of vascular uptake and biocompatibility [51]. Since significant number of VSMC undergo rapid apoptosis following balloon angioplasty, Reddy and co-workers [52] tested the hypothesis that preventing VSMC from undergoing apoptosis could prevent intimal hyperplasia. They used rapamycin (which has antiapoptotic and antiproliferative properties) loaded into gel NPs with a mean diameter of 54 nm. When infused into a rat carotid artery model of vascular injury the authors reported significant inhibition of hyperplasia and improved re-endothelialisation of the injured artery. Furthermore, the group reported inhibition of the caspase 3/7 enzyme systems in the treated artery, thus preventing VSMC from undergoing apoptosis.

Magnetic nanoparticles

Magnetic targeting is a promising possibility for efficacious guidance of therapeutic agents to CAD diseased sites, elimination from nontargeted propagation (i.e., safety concern) and deep and long-term tissue targeting, furthermore it has suggested benefits for anti-ISR therapies, delivery of cells, gene vectors and therapeutic proteins and stented artery delivery of paclitaxel by utilization of magnetic field-guided magnetic carriers for specific-vascular delivery [53]. Functional MNP-loaded primary endothelial cells as vectors targeting vascular stents induced gene expression related to EC growth and survival, and suppressed gene-related coagulation and suggested them for re-endothelialization by the implant and decreasing neointimal hyperplasia [54].

Metallic MNPs, made of iron, cobalt or nickel, are typically prepared with a core–shell structure in which gold or silica is applied as a coating material. Iron MNP composed of nanocrystalline magnetite (Fe_3O_4) or maghemite (γFe_2O_3) form a close-packed cubic lattice [55]. Furthermore, gold shell NPs (~120 nm) have been used for both imaging and therapy applications [56]. Another study used paclitaxel-loaded magnetic NP with a uniform magnetic field that allowed the attachment of NP to the stent, and also drug release in order for it to be taken up by the target cells. In this case inhibition of ISR occurred with 7.5 μg paclitaxel,

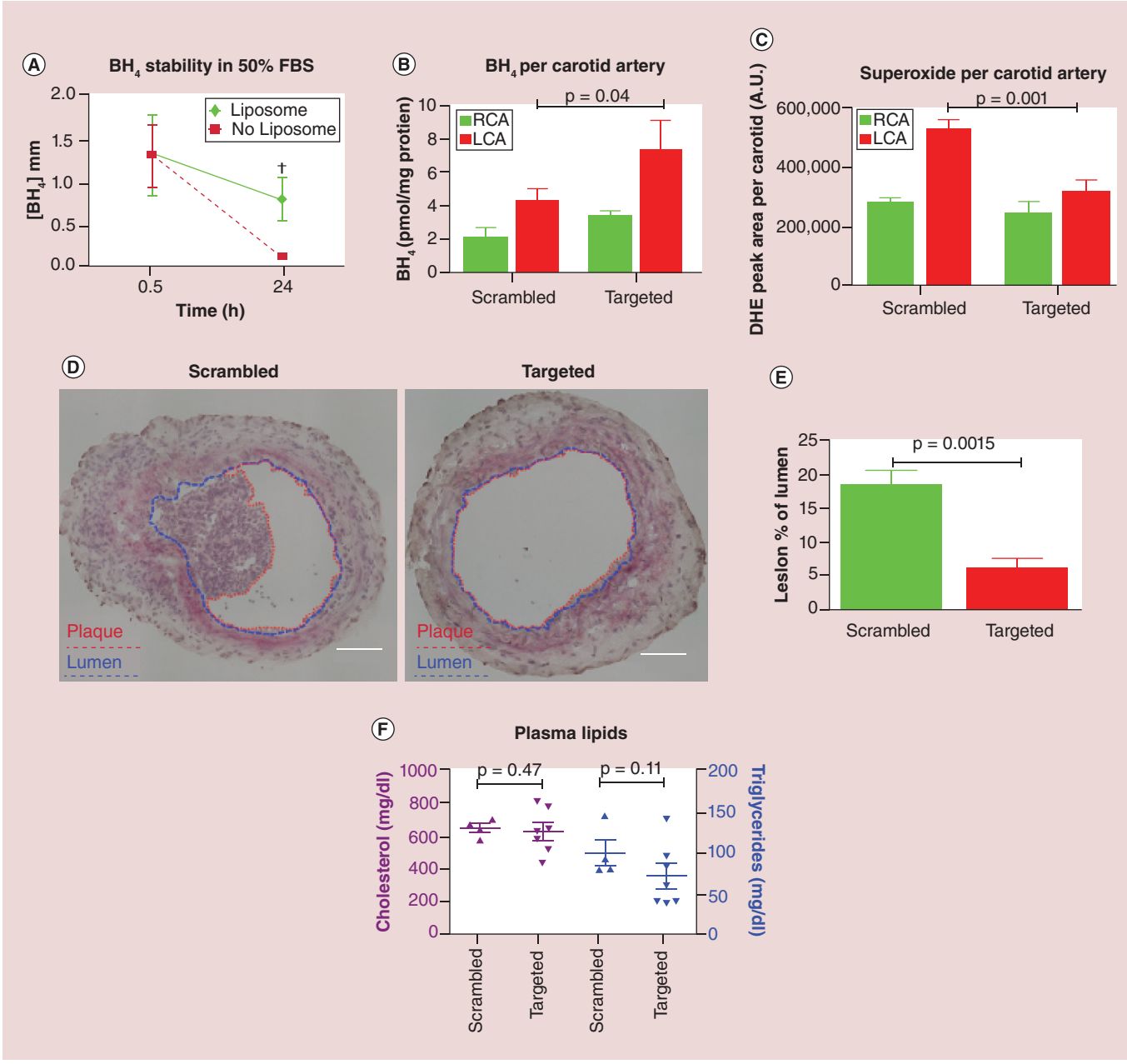

Figure 3. Delivery of tetrahydrobiopterin (BH_4) by liposome nanocarrier. (A) Improved stability of BH_4 in comparison with unencapsulated BH_4 after 24 h; p = 0.017; **(B)** BH_4 concentration in ligated artery increased by liposomal delivery; p = 0.04; **(C)** superoxide concentration in ligated artery with targeted liposomes decreased; **(D)** the plaque burden was decreased by BH_4 liposomal delivery in the mice ligated left carotid artery fed by 7-day high fat diet (plaque and lumen are marked by red and blue, respectively) scale bars = 100 μm; **(E)** the area of plaque; p = 0.0015; **(F)** there is no alteration in lipid metabolism via liposome deliver; p = 0.47 and 0.11, respectively).

BH_4: Tetrahydrobiopterin; DHE: Dihydroethidium; FBS: Fetal bovine serum; LCA: Left common carotid artery; RCA: Right common carotid artery.

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together with a noticeable reduction in the ratio of neointima/media that was only 63 ± 13% of that of the control group [57]. Figure 4 reports the results of the mentioned study.

Additionally, quantum dots have been used as florescent labels to prepare traceable NP due to their tunable physicochemical features, high photostability, broad absorption spectra and narrow emission bands. Quantum dots have been proposed to monitor disease-associated events, such as macrophage cell infiltration into arterial tissues and the processes of angiogenesis and vascular remodeling [58]. Figure 5 shows using of quantum dots to monitor monocyte-macrophages in atherosclerosis plaque. More details in plaque imaging can be found in the section 'Molecular imaging and atheroslerosis detection'.

Nanocoatings

Nanocoatings on DES release drugs locally at the site of plaque accumulation. Polymeric coatings are more common rather than ceramic ones. In following paragraphs, both of them are reviewed briefly.

Stent polymeric coatings

Polymer-based coatings have been used to control drug release rates from stents. However, numerous side effects could be caused by the polymer coatings themselves such as irritation, inflammation [60], blood protein adsorption, allergic reactions and in-stent thrombosis [61]. Based on bench tests and animal studies, it was found that stent implantation could cause significant problems, such as webbing and bonding, with the resulting possibility of inhomogeneous drug elution and increased thrombogenicity despite the lack of mechanical damage or deformity at the time of stent implantation [62].

Various polymers, such as PLGA, polyethylene glycol (PEG) and polycaprolactone (PCL), have been coated on stents by using the solvent evaporation technique. Improved sustained drug release profiles and a lower platelet adhesion rate were seen with stents coated with PCL matrix because of the improved surface morphology [63]. PLGA which is considered to be a biocompatible polymer has been applied widely in recent years. PLGA NP were constructed to carry paclitaxel, using an emulsion-solvent evaporation method to encapsulate drug molecules in the polymer. Moreover, the amount of paclitaxel was readily controlled by varying the preparation procedure. *In vitro* investigations demonstrated controlled paclitaxel release from the NP layers without any initial burst [64].

Tan *et al.* suggested that a nanocomposite polymer, containing polyhedral oligomeric silsesquioxane poly-(carbonate-urea) urethane (POSS-PCU), could be suitable for stent coating [65]. Antiproliferative, antithrombogenic and antiplatelet agents could be incorporated into POSS-PCU to lessen problems with BMS and DES. POSS-PCU was recently employed as the scaffold material in the first synthetic trachea transplant in a human patient [66]. Another study investigated POSS-PCU, as a stent-coating because PCU is blood compatible and antithrombogenic, particularly when POSS is added further reducing the thrombogenicity of the material. Human endothelial progenitor cells adhered and proliferated well on this nanocomposite [67].

Another strategy reported for surface modification is the preparation of nanoroughened surfaces by nitrogen ion implantation plasma deposition to promote stent performance. In addition to the deposition of nanometer sized particles, thin layers of polymers can be deposited onto metal surfaces by the IPD method. Various polymeric and metallic substrates have been modified with nanostructured Ti using the IPD technique. Rat aortic endothelial cells were used to evaluate the cytocompatibility properties [68].

Porous DES functions as a reservoir to continuously release antirestenosis agents. Nanoporous coatings may be better able to promote VSMC and endothelial cell adherence and encourage proliferation on the surface topography [69].

Table 3 summarizes some of the important advances in polymeric stent coatings in recent years.

Nanotextured ceramic coatings

Nanoporous aluminum oxide (Al_2O_3) was one of the first nanoporous ceramic coatings used by Jomed International (recently acquired by Abbott Vascular) on DES. In the process of surface manufacturing, 316L stainless steel stents were firstly coated inside and outside with a thin layer of aluminum via a physical vapor deposition process in order to ensure proper adhesion of the coating to the base metal. In the next step, the metallic layer was electrochemically converted into a nanoporous ceramic (Al_2O_3) using a bath of 2% oxalic acid in water. The pore diameter and pore density of the resultant surface was reported to be 5–15 nm and 109 pores/cm^2, respectively. In order to deliver tacrolimus to rabbit carotid arteries *in vivo*, the antirestenosis drug was applied by dipping the stents into a defined solution of tacrolimus and subsequent drying steps [76]. However, the clinical performance of this system in present trials was disappointing since the coating 'flaked off' the stent surface and the resulting particulate debris led to a proinflammatory response producing a thicker neointima [77].

Furthermore, a hydroxyapatite-coated stent with thickness of 0.30–1 μm and porosity of 40–60% by

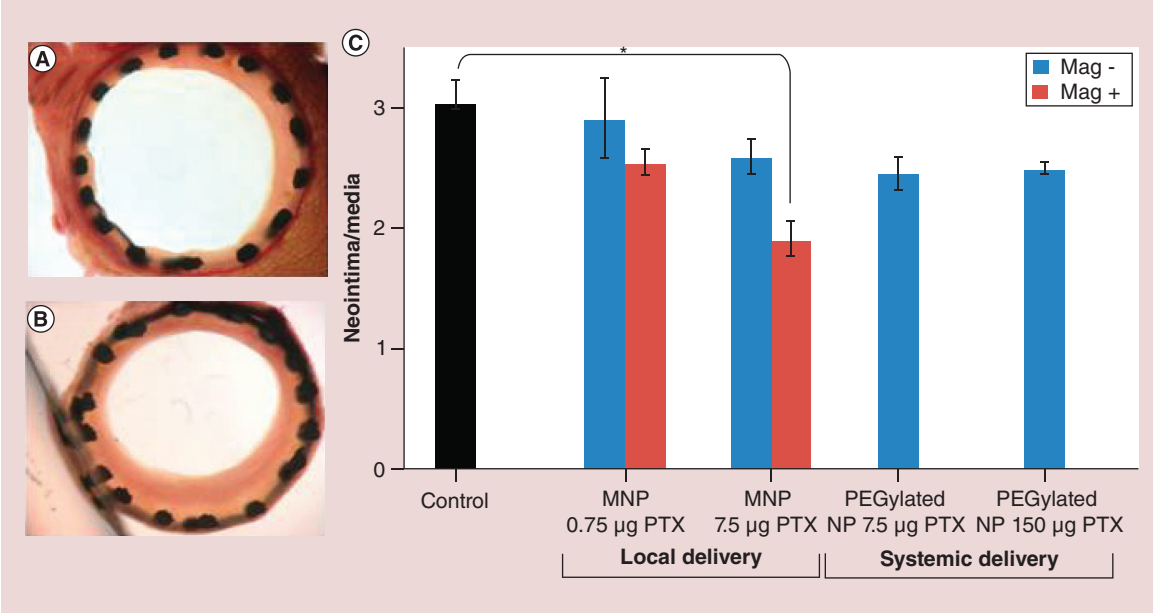

Figure 4. Paclitaxel-loaded magnetic nanoparticles applied to coronary stents with a uniform magnetic field. MNPs with PTX doses of 7.5 and 0.75 µg entered into animal bodies under magnetic versus nonmagnetic conditions. The animals sacrificed and the stented carotid segments were harvested 14 days after surgery. The control group did not receive MNP but stented. Verhoeff–van Gieson-stained section of an artery lumen treated with 7.5 µg PTX under magnetic conditions **(A)** demonstrated versus 'no treatment' control **(B)** ($p < 0.05$, Dunn's Test Q statistic = 3.7). Original magnification 100×. Morphometric results as neointima/media ratios **(C)** pictured as a function of the magnetic field application and PTX dose ($n \geq 6$). Data are presented as mean ± standard error. MNP: Magnetic nanoparticle; NP: Nanoparticle; PTX: Paclitaxel.
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volume has been developed by MIV Therapeutics Inc., Vancouver, British Columbia, Canada [78]. Another approach was the combination of a stainless steel platform with a nanosized microporous hydroxyapatite coating. This DES system, which was also impregnated with a small amount of sirolimus, demonstrated promising results in the first year of clinical trials [79].

Recently, it was shown that nanoporous titanium dioxide (TiO_2), produced via a sol–gel synthesis process, could function as a drug-eluting surface. The main problem is loading the drugs into the nanoporous TiO_2 surface. These surfaces have a large internal surface area, and the behavior of fluids is controlled by surface forces, while the ability of drugs in solution to penetrate into the surface is determined by the contact angle (surface tension) of the solution at the nanoporous surface. Modified surface methods, which enhance the hydrophilicity of the surface and the use of low viscosity solvents, can assist penetration of the solvent into the nanopores [80].

More recently, a novel bioinert paclitaxel-eluting porous carbon NP, showed excellent elastomechanical properties, and provided the possibility of tailoring drug release kinetics by adjusting the pore size, was introduced to coat stents. This surface consists of a porous composite matrix synthesized from amorphous carbon NPs embedded in glassy polymeric carbon [15]. Karagkiozaki *et al.* [81] prepared carbon nanocoating of stents via a manufacturing process using a radio-frequency magnetron sputtering deposition technique, with variation in surface roughness as the main design variable to control the platelet response *in vivo*. They showed that the surface nanotopography of the coating can influence the behavior of the platelets, and this is an important factor for controlling the thrombogenicity of the biomaterials. Among the carbon nanocoatings studied, those with a higher value of surface roughness were less thrombogenic in terms of platelet adhesion.

Rajender *et al.* [82] showed in a study involving pharmacological coating of stents that sustained local delivery of an antiproliferative agent provided higher tissue concentrations compared with systemic administration. Their LC/MS/MS results demonstrated consistency in the drug content on the stent surface. They also measured *in-vitro* release kinetics of sirolimus over 41 days from the nanoporous carbon-coated stents.

Mesoporous silica nanoparticles function as an excellent drug carrier due to their tunable pore size, high-specific surface area, large pore volume and favorable biocompatibility [83–85]. For the first time, magnetic mesoporous silica nanoparticles (MMSN)

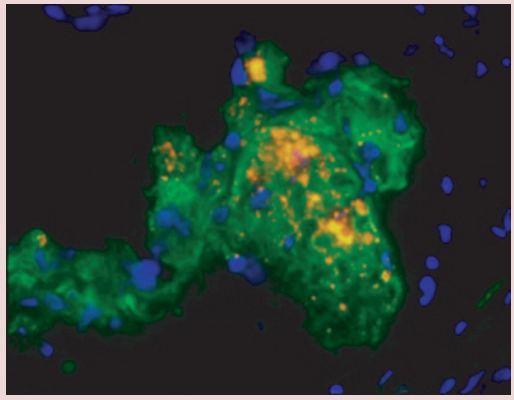

Figure 5. Using quantum dots to image the monocyte-macrophages in atherosclerosis plaque. The monocyte-macrophages loaded by cell penetrating quantum dots were injected to mice. Injected cells and macrophage marker CD68 are portrayed as orange and green, respectively.
Reproduced with permission from [59], © (2010) *Current Atherosclerosis Reports*.

with a core–shell structure were tested as an effective rapamycin (RAPA)-loading vehicle for cardiovascular stents *in vivo*. This group designed a novel two-layered CNT@MMSN/CNT composite coating employing the EPD method to overcome the probable brittleness of the coatings assembled from MMSN alone. By regulating the structures composition, a crack-free two-layered coating with a remarkable network nanotopology was obtained. The thin CNT film acted as an inner buffer layer, and the MMSN/CNT composite coating acted as a functional layer. They also proposed a possible mechanism of the CO-EPD process to combine MMSN and CNT. Moreover, this polymer-free coating exhibited excellent mechanical flexibility and good blood compatibility *in vitro*. The RAPA-loading capability of 316L-BMS@CNT@M/C-3 was 60.10 ± 2.43 μg/mg, and the drugs could be continuously released for up to 2 weeks. Finally, an *in vivo* study showed that this nanostructured DES had the benefit of rapid re-endotheliazation in the early stages in comparison with the commercial P-FBII DES, in order to reduce the risk of thrombosis [86]. Figure 6 illustrates the porous structure of ceramic coatings and the chemical structure of polymers applied to DES. Furthermore, Table 4 summarizes some of the important advances in ceramic stent coatings in recent years.

Gene therapy strategies

The use of DES can be accompanied by side effects such as in-stent stenosis, and thrombosis which could be effectively eliminated by developing new stent materials and coatings. Adverse local hypersensitivity reactions and an inflammatory response to polymeric coatings [88] or delayed or incomplete re-endothelialization might result in late stent thrombosis (associated with high mortality) due to delayed healing of the DES-induced endothelial wound. This important side effect of DES could be avoided by strategies including miRNA gene delivery that improves healing of the vessel wall injury [89], optimizing the DES design for inhibition of restenosis while not affecting the endothelial healing [90] and using fully bioabsorbable/degradable stents as well as newly described polymer-free DES with dual drug-eluting capability [91]. A variety of different gene targets have been used to treat restenosis [18,92,93]. For instance, a gene-eluting metallic stent was prepared by first laying down a robust surface layer of hyaluronic acid followed by deposition of plasmid DNA (pDNA) on the surface. The hyaluronic acid-coated surface improved deposition of pDNA/polyethylenimine polyplexes, and led to enhanced gene expression [94]. In another attempt, Paul *et al.* developed an endovascular stent based on a nanobiohybrid hydrogel. The hydrogel included assembled fibrin matrices built up layer-by-layer on the stent surface, with alternate layers carrying the endosomolytic Tat peptide together with DNA (NCS). Other NPs were hybridized with polyacrylic acid wrapped single-walled carbon nanotubes (NP-CNT). Six weeks after stenting in a beagle dog model, the re-endothelialization area in the NCS (+) group carrying the VEGF + angiopoetin 1 transgene was 87.2 ± 13.2%, in the null-transgene NCS (-) was 54.7 ± 9.3%, and in the bare metal stent (BMS) group was 46.44 ± 4.6%. Furthermore the degree of restenosis in the NCS (+) group was 28.5 ± 9.03%, in NCS (-) group was 39.56 ± 13.8% and in BMS group was 45.34 ± 8.3% [95]. In an injured rat artery, the use of PLGA NPs loaded with PDGF receptor-β antisense RNA was reported [43]. These NPs displayed efficient intracellular delivery and sustained intracytoplasmic release [73].

Molecular imaging & atherosclerosis detection

One of the goals in diagnosis of atherosclerosis is to distinguish the so-called 'vulnerable plaques' that are likely to rupture and precipitate a heart attack from the stable plaques that do not present any sudden threat. Current imaging methodologies that typically image plaque anatomy do not identify such high-risk lesions. Identification of these lesions in relevant vascular beds (coronary and carotid arteries) could alter systemic therapies (i.e., prescribing higher doses of statins or adjunctive treatments despite supposedly normal 'target' serum lipid levels), and also to possibly guide local therapies (e.g., intracoronary stenting of high-risk

lesions) in patients at very high risk. The most commonly used targets in CVD molecular imaging are: macrophages; annexin V for phosphatidylserine (PS) in apoptosis, angiogenesis, fibrin, etc.

The metabolic activity of macrophages in inflamed lesions can be traced through uptake of glucose analogs. Cells take up fluorine-labeled 2-deoxy-D-glucose (FDG) at the same rate as glucose. After phosphorylation, FDG accumulates inside the cells. 18F-FDG imaging combined with positron emission tomography (PET) has been used in atherosclerotic patients to evaluate inflammation and macrophage load in the symptomatic carotid artery compared with the contralateral asymptomatic control vessel [96]. Cross-linked iron oxide fluorescent NPs [97] and MNP with a central iron oxide core (3–5 nm in diameter) coated with carbohydrate [98] have been used for detection of macrophage. Additionally, ^{19}F perfluorocarbon NPs recruited in MRI spectroscopy instead of ^{1}H signals due to better detection of ^{19}F (since, there is no background signal from ^{19}F in the host tissues at the final image) [99]. While monitoring cell proliferation mainly depends on *ex vivo* measurements, patients with atherosclerosis need to be assessed through their biopsy samples. Finding a biomarker which represents cells proliferation would be suitable to diagnose disease as soon as possible. 18F-FLT, a PET isotope-labeled thymidine have the potential to be that goal since atherosclerotic plaques in knock out mice, rabbits and humans accumulate 18F-FLT according to the report of Xiang [100]. Preventing the macrophage accumulation would be a suitable approach before atherosclerotic plaque progression and 3-hydroxy-3-methylglutaryl coenzyme A which also called statins in association with high-density lipoprotein nanoparticle showed to be effective in reducing macrophage proliferation [101]. IVUS/IVPA can be utilized to monitor the presence of systematically gold NPs within plaques in atherosclerosis as shown by Yeager *et al.* [102]. IVPA-assessed, selective plasmonic photothermal heating delivered through the integrated imaging catheter. Silica-coated gold nanorods tendency toward endocytosis by macrophages makes this method specific targeted [102]. Circulation half-life of prednisolone provided with a liposomal nanocarrier shown to be appropriate in atherosclerotic lesions. These liposomal NPs accumulate in macrophages. Nanomedicinal delivery of drugs to atherosclerotic lesions is feasible in humans [39].

During apoptosis, activated flippases quickly redistribute PS molecules from the inner layer into the outer layer of the cell membrane. Therefore annexin V, a protein that binds to PS, has been labeled with radionuclides (^{123}I, ^{124}I, ^{99m}Tc and ^{18}F) [103] to detect apoptosis. A combination of annexin 5 labeled with ^{99m}Tc for

Table 3. Nanotextured polymeric coatings.

Coating material	Stent body	Processing technique	Biodegradability	Type of drug	*In vivo* tests	Ref.
PLGA	Platinum	Sputter coating	Yes	Paclitaxel	–	[70]
PLGA- collagen	316L stainless steel	Electrophoretic deposition/ dip-coating	Yes	N-nitro-somelatonin	On rabbit	[71]
HA-*g*-PLGA	Cobalt-chromium alloy (L605)	Layer-by-layer	–	Paclitaxel	–	[72]
PLGA	316L stainless steel	Electrodeposition	Yes	–	On domestic male pigs (porcine)	[73]
PLA	–	Solvent evaporation technique	Yes	Sirolimus	–	[74]
PLGA, PEG and PCL	316L stainless steel	Solvent evaporation technique	Yes	S-Nitrosoglutathione	–	[63]
Chitosan	316L stainless steel	Spray-coating	Yes	DNA	On rabbit	[75]
POSS-PCU and POSS-PCL	–	Layer-by-layer	No	No drug (anti-CD34 antibodies attached to the surface)	–	[65]
POSS-PCU	NiTi	Electrohydrodynamic spraying	No	–	–	[67]

HA: Hyaluronic acid; PCL: Polycaprolactone; PCU: Poly (carbonate-urea) urethane; PLA: Polylactide or polylactic acid; PLGA: Poly (lactic-co-glycolic acid); POSS: Polyhedral oligomeric silsesquioxane.

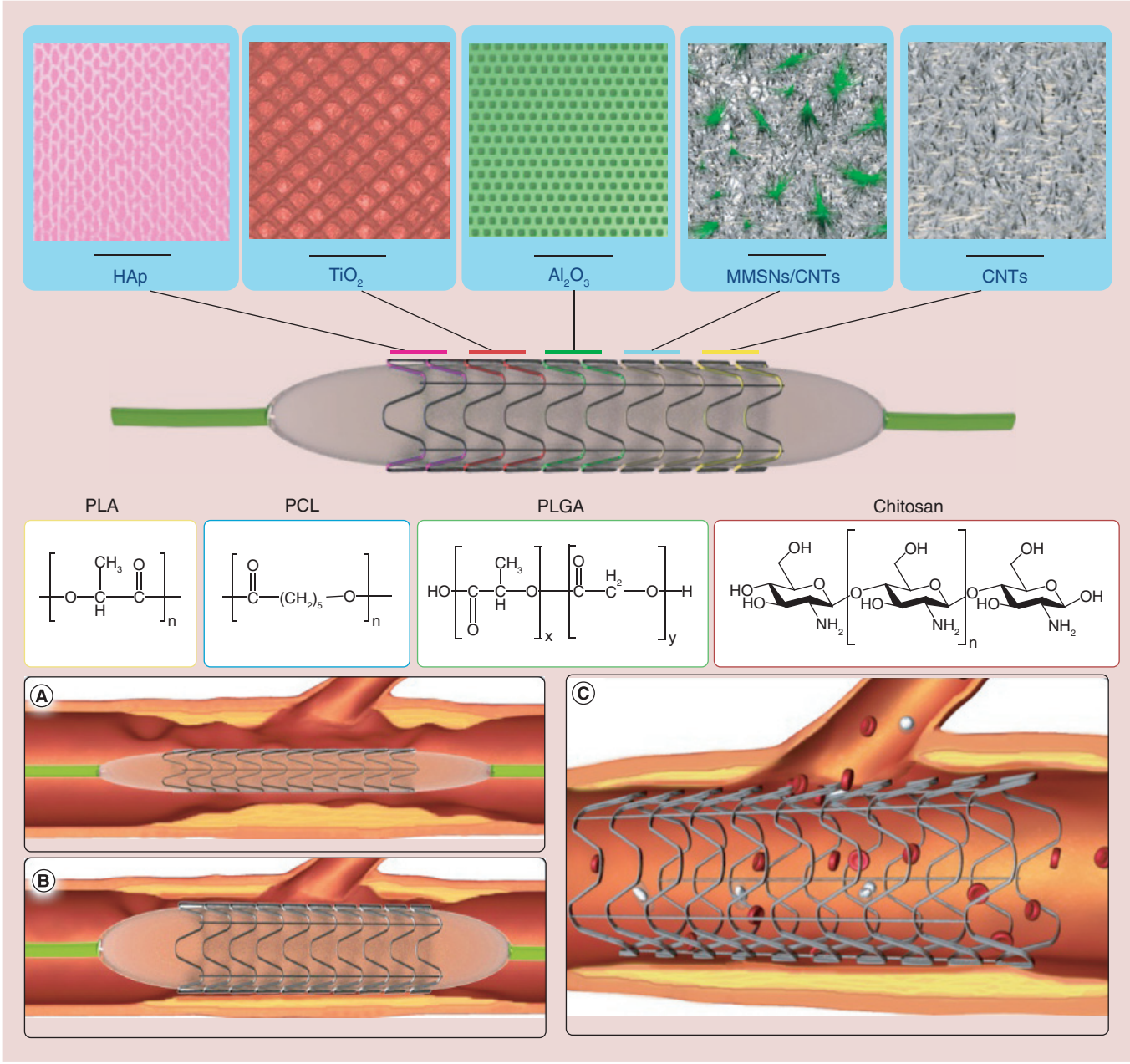

Figure 6. Stent coating structures and positioning in the coronary artery. Top: porous structure of various ceramic coatings and chemical structures of polymers that have been applied on stents; **(A)** plaque accumulation in the lumen of coronary artery and delivery of nonexpanded stent; **(B)** expanded stent via pressure of balloon catheter; **(C)** expanded stent remains at the site of plaque allowing good blood flow.
CNT: Carbon nanotube; HAp: Hydroxyapatite; MMSN: Magnetic mesoporous silica nanoparticle.

SPECT imaging of apoptosis and together with hypericin labeled with ^{124}I for PET imaging of necrosis was used to image plaques in apoE$^{-/-}$ mice [104]. Imaging of apoptotic cells in atheroma may demarcate plaques at risk for future complications [105].

Oxidized low-density lipoprotein can trigger local inflammation that is related to expression of adhesion molecules such as vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 which could be detected by superparamagnetic iron oxide and Gd particles, respectively [106].

Microvessels underlying plaques may cause intraplaque hemorrhage, and thus could identify high-risk atherosclerotic lesions. Therefore, imaging of angiogenesis can be used as a target in molecular imaging. In particular, integrin αvβ3 has been recognized as a key mediator of angiogenesis and thus may represent an important diagnostic and therapeutic target for

diseases characterized by neovascularization. Recent studies have used a gadolinium coated perfluorocarbon nanomaterial (containing 90,000 individual gadolinium chelates) derivatized with an arginine-glycine-aspartic acid (RGD) peptidomimetic to target αvβ3 [107].

During the clotting process, thrombin-mediated cleavage of fibrinogen gives rise to fibrin monomers. The fibrin clot is then cross-linked via factor XIII to stabilize the clot and generate obstructive thrombi. Each of these stages contributes to subocclusive thrombosis and intraplaque hemorrhages and could represent a target for imaging [106]. One way to detect fibrin monomers *in vivo* is to target activated platelets via P-selectin or GP IIb/IIIa. Imaging probes targeting P-selectin can be designed using a specific antibody (VH10), peptides or polysaccharides that bind efficiently both *in vitro* and *in vivo* to platelets and thrombi and detected by MRI [108].

Another method has been studied for imaging of thrombogenesis targets factor XIII, using peptide substrates recognizing factor XIII [109]. These fluorescent-labeled peptide agents are covalently cross-linked into the clots being formed by factor XIII, and show a time-dependent increase in fluorescence signal.

Magneto-photo-acoustic imaging is helpful in assessing the delivery and determine endocytosis for magnetic nanoparticles. For this purpose, photoacoustic and magneto-motive ultrasound (MMUS) signals are linearly proportional. However, gathering NPs within cells causes to nonlinear relationship between MMUS-PA because of MMUS signal nonlinear amplification. Therefore, longitudinal magneto-photo-acoustic imaging can make it possible the delivery of NPs and identifying the endocytosis of NPs in living cells [110].

Conclusion & future perspective

The effectiveness of DES was demonstrated in numerous clinical trials, and their introduction has led to significant improvement in CAD therapy. Other effective therapies for CAD such as atherosclerosis, thrombosis, restenosis and in-stent neointimal hyperplasia have been investigated in recent years and tested in clinical trials. Nanotechnology with its superior advantages can provide innovative solutions to CAD by exploiting various nano/micro carrier platforms that can offer improved delivery of therapeutic agents to diseased sites. These sites include inflammatory cells in CAD-related diseases mediated by specific targeting and receptor-specific biomolecules. Nanotechnology can improve the function of stents coated with polymers, loaded with biomolecules (e.g., PDGF-receptor inhibitors, paclitaxel and rapamycin), leading to reduction of inflammation and angiogenesis and encouraging healing of the injured endothelium. Many antirestenosis strategies have been investigated using anti-inflammatory, antithrombogenic and antiproliferative agents. Immunosuppressive agents can be carried on DES, and a resulting reduction in ISR can be demonstrated. These novel CAD therapies have resulted in advantages such as reduced cytotoxicity, enhanced blood circulation time of agents and reduced overall dosage. Various surface modification techniques that have been applied to DES can improve the performance of intravascular stents. Stents have

Table 4. Nanotextured ceramic coatings.

Coating material	Stent body	Processing technique	Pore size	Type of drug	*In vivo* tests	Clinical trials	Ref.
Al_2O_3	316L stainless steel	Electrochemical conversion of aluminum layer	5–15 nm	Tracrolimus	On rabbit carotid arteries	Inflammatory caused by release of particle debris	[77]
HAp	316L stainless steel	Sol-Gel	40–70 nm	Sirolimus	N/A	Promising results in the first year of clinical trial	[78, 79, 87]
TiO_2	316L stainless steel	Sol-Gel	55 Å	N/A	N/A	N/A	[80]
CNTs	Cobalt–chromium stent	Rf magnetron sputtering deposition technique	Nano size	Sirolimus	N/A	N/A	[81]
MMSNs/CNTs	316L stainless steel	Electrophoretic deposition method	Nano size distribution	Sirolimus	On rabbit carotid arteries	N/A	[86]

CNT: Carbon nanotube; HAp: Hydroxyapatite; MMSN: Magnetic mesoporous silica nanoparticle; N/A: Not available.

been coated with NP, and stent-coating materials have been formulated from polymers and ceramics and their fabrication methods have been investigated. Despite many advantages such as improved controlled/sustained release rates, enhanced re-endotheliazation, reduced platelet adhesion and the biosafety of drug-loaded NP (polymeric, nanoporous ceramic, nanoporous carbon based, etc.), concerns remain. These concerns center on allergic reactions, inflammation, increased neointimia formation. Moreover deformities and damage may be triggered by DES and some clinical catastrophes have occurred. Gene therapy approaches (e.g., pDNA, RNAi) have been used to modify gene expression, and to generate signaling molecules to inhibit growth of diseased cells and hyperplasia, and to cause apoptosis.

The diagnostic and imaging capability of nanoplatforms in CAD therapy is another area of investigation, and magnetic and metallic NP and fluorescent QD have been developed as contrast agents (besides their drug delivery capability). Combinations of diagnostic and therapeutic agents comprise the new field of theranostics. However there are still several challenges to be solved, concerning the design of balanced magnetic properties of MNP, modification of the composition of the stent material that may include magnetic responsive materials for increased targeting and loading capability. There are biocompatibility and toxicity issues due to presence of biological–NP interactions. MNP-based systems have been considered for enhanced nucleic acid transfection in cells via application of magnetic fields, in a process named 'magnetofection'.

In future directions, MNP-based magnetic scaffold implants could be considered tissue engineering using materials containing multicellular cultures (e.g., cardiomyocytes, fibroblasts and endothelial cells) within the scaffolds.

Future approaches will undoubtedly concentrate on personalized medicine taking into account specific features of individualized patients and their particular disease site. This approach requires tailoring various parameters including the stent-design and intervention strategy, and these should be specifically designed for each patient. Moreover, cost–benefit analysis cannot be ignored in these innovative approaches. The ever-rising cost of healthcare cannot be allowed to continue its exponential rise forever. Therefore, public healthcare issues need to be kept in mind so that nanotechnology can continue to provide affordable and patient-friendly therapy accompanied by long-term effectiveness.

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Executive summary

- Major problems in coronary artery disease include restenosis after angioplasty, and detection and therapy of vulnerable plaque.
- Drug eluted stents are used to inhibit restenosis, prevent proliferation of SMC, enhance re-endothelialization and attenuate neointimal hyperplasia, but further advances are needed.
- Antirestenosis drugs can be delivered by being encapsulated in nanoparticles (NPs) including, micelles, liposomes or polymeric NPs.
- Dendrimers, gel-like NPs and gene therapy strategies have also been used to target restenosis
- Sophisticated strategies using nanotextured polymer coatings and nanotextured ceramic coatings have been applied to intra-arterial stents to prevent restenosis
- Metal NPs and optical fluorescence probes have been used for intra-arterial imaging
- Molecular imaging approaches including positron emission tomography, single photon emission computed tomography and magnetic resonance imaging have been used to detect vulnerable plaque.

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