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Nanotechnology in interventional cardiology

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Abstract

High-grade atherosclerotic stenoses are reduced to zero or minimal residual stenosis grades by a single or a series of balloon angioplasties. Currently, stents are implanted to prevent immediate vascular recoil and elution of an anti-mitotic drug from the stent struts minimizes restenosis. An unwanted side-effect of this drug-elution is delayed re-endothelialization which requires the treatment with two anti-platelet drugs in many cases for a minimum of one year to prevent acute in-stent thrombosis. Advances in stent-design and drug-elution technology, now in its fourth generation, have not abated this issue. Nanotechnology-based local drug delivery has the potential to achieve restenosis prevention while not impeding endothelial healing. Molecularly targeted drugs can be aimed to specifically bind to epitopes in the injured media and adventitia. Thus, endothelial healing may progress unhindered. To prevent restenosis, this technology may be used with bare metal or biodegradable stents. In this article novel nanoparticulate agents will be compared regarding their potential to deliver drugs to molecular targets within the vascular wall. Potential molecular targets, targeting mechanisms, drug-delivery propensities, and biocompatibility will be reviewed.

Keywords

Atherosclerosis; Nanotechnology; Drug delivery; Imaging; Restenosis

The restenosis response after cardiovascular angioplasty and stent implantation is influenced by the mechanical injury during angioplasty¹, inflammatory responses², and endothelial healing³. Hence, prevention of vascular thrombosis and restenosis are paramount factors determining the long-term success of cardiovascular stent implantation. Thrombosis and restenosis appear to be distinct pathologies with the former caused by endothelial injury and the restenosis response triggered by the acute over-stretch injury to the media and adventitia. However, a prolonged endothelial healing period as well as ongoing endothelial dysfunction may also extend the restenosis response emphasizing the benefit of early re-endothelialization.

Current drug eluting stents minimize the restenosis response at the expense of prolonged endothelial healing. As a consequence, in-stent thrombosis needs to be prevented with the use of dual anti-platelet agents for a minimum duration of one year. Nevertheless, acute stent thrombosis has been described up to and exceeding 4 years after stent placement^{4–6}. Thus, treatment with dual platelet agents is often recommended for periods far in excess of one year. While this is the currently best treatment option and thus standard of care, it leaves several areas of potential improvement. (1) Urgent surgeries typically require the cessation of dual anti-platelet agents putting the patient at risk for acute stent thrombosis and thus pose challenging clinical management issues. (2) Patients that are known to require surgeries within a few months of stent placement often do receive bare metal stents and thus do not get the benefit of local drug treatment. (3) Early and late coronary stent thrombosis occurs in

a small, yet significant number of patients up to at least 3 years following implantation of drug eluting stents⁷. (4) Small vessels or extremely tortuous vessels may preclude the use of stents altogether and drug-covered angioplasty balloons have so far mostly yielded unsatisfactory restenosis results. (5) While drug eluting stents have significantly decreased the need for revascularization, restenosis still does occur. Finally, another more principal question may be entertained: Since the atherosclerotic stenosis can be treated in a single interventional procedure, should the ideal treatment modality not also allow minimizing the duration of pharmacological follow up treatments?

In conclusion, an ideal drug/agent used to prevent restenosis after angioplasty should be antiproliferative, anti-migratory, anti-inflammatory, and pro-healing. Thus, it should specifically inhibit the restenosis response, i.e. largely inhibit SMC replication. Its duration of action should not unnecessarily exceed the time of restenosis “trigger”. Furthermore, it should be non-toxic to the adventitia and endothelium, i.e. not delay endothelial healing and return of endothelial function. Lastly, it should not have systemic effects.

Local non-targeted drug-delivery during interventional procedures

Systemic drug delivery has shown some success in decreasing restenosis following balloon angioplasty and coronary stent implantation^{8, 9}. However, in most cases systemic delivery is inefficient¹⁰ and largely produces side effects^{11, 12}. The failure of systemic pharmacotherapy is due to inadequate local tissue concentration at systemic doses of the respective biological agent that can be safely given to the patient. These considerations provide a rationale for local drug delivery.

Local drug infusion

Initially, porous balloons and microporous balloons were evaluated for this task. With these devices drugs¹³, antisense oligodeoxynucleotides¹⁴, adenoviral vectors¹⁵ and even nanoparticulate agents^{16, 17} have been successfully delivered in animal models. The efficiency of porous balloon drug delivery varies substantially between different devices¹⁸, agents delivered¹⁹ and in case of nanoparticles the size of these particles²⁰. A drawback is the observation that high delivery pressures and large volumes of perfused solutions may cause significant vascular damage and thereby may increase intimal thickening¹⁷. Concerns regarding vascular barotrauma are related to balloon type, infusion pressures, agents used, and type and condition of the vessel treated^{21, 22}. In addition, non-homogenous local as well as unavoidable systemic delivery, remain issues. All these parameters need to be evaluated for any new compound tested, which makes this technology cumbersome. Nevertheless, the local delivery of an antisense phosphorodiamidate morpholino oligomer (AVI-4126) has been successful in a small group of patients with coronary artery disease using the Infiltrator catheter²³. While the compound was shown to decrease restenosis in human coronary arteries, the balloon catheter could not be advanced into the lesion in 8 out of 44 patients and 6 coronary artery dissections occurred in the 25 patients in whom the balloon was inflated. These data highlight the technical challenges of this delivery technology. Due to such difficulties and the above mentioned concerns, these devices have not gained widespread clinical application. Another rationale to utilize targeted agents is given from the fact that even when local delivery of non-targeted agents through a porous balloon is optimized, the majority of the compounds are delivered to the vasa vasorum and periadventitial microvasculature with substantially lesser delivery to the media and intima^{24, 25}.

Compared to porous balloons, drug-coated balloons and double-balloons are less traumatic to the arterial wall. They offer the advantage of homogenous drug transfer into the vessel wall either during a low-pressure inflation of the drug-coated balloon or during an extended incubation time between two balloons that entrap the drug in the arterial segment of interest.

Local drug delivery with drug-coated balloons

Drug-coated balloons have undergone intensive investigation including evaluation in clinical trials. Paclitaxel-coated balloon (PCB) catheters have significantly decreased in-segment late luminal loss and restenosis at 6 months in a trial that enrolled 52 patients with in-stent restenosis²⁶. At 12 months, the rate of major adverse cardiac events was 4% in the coated balloon group vs. 31% in the uncoated balloon group ($P=0.01$). In the Paclitaxel-Eluting PTCA-Balloon Catheter in Coronary Artery Disease-2 In-Stent Restenosis (PEPCAD-2 ISR) trial treatment of in-stent restenosis using a balloon coated with paclitaxel was shown to be as good as or better than Taxus paclitaxel-eluting stents at treating in-stent restenosis²⁷. In the PEPCAD-3 trial, the paclitaxel-coated-balloon/stent system was compared against the sirolimus-eluting Cypher stent in 637 patients with de-novo coronary artery stenoses (Hamm CW. AHA Scientific Sessions 2009: Nov. 14, 2009). Here it did not meet non-inferiority criteria and its associated target-lesion and target-vessel-revascularization rates were double those reported with Cypher stenting. Another trial with this paclitaxel-coated balloon system was terminated prematurely when the PCB group showed higher per cent diameter stenosis (43.6% vs. 24.3%, $p=0.029$), angiographic restenosis (32.1 vs. 10.3%, $p=0.043$) and MACE (35.7% vs. 13.8%, $p=0.054$) when compared to Taxus DES after PCI of small coronary arteries²⁸. Overall, the results of drug-coated balloon trials are mixed, but encouraging nevertheless to continue further investigation.

Local drug delivery with double-balloon catheters

In an early study, local delivery of paclitaxel using a double-balloon perfusion catheter and bare metal stent implantation failed to show any difference compared to placebo in response to balloon-injury in porcine coronary arteries²⁹. However, in a more recent study including some investigators of the aforementioned study, locally-delivered paclitaxel showed marked reduction of neointimal proliferation and complete endothelialization 42 days after treatment in a porcine model³⁰. Compared to the earlier study, the amount of drug infused was similar, but a new double-balloon device and higher instillation pressures were used. Also, dual anti-platelet medication was given daily. Another compound that had been shown to inhibit vascular smooth muscle cell (VSMC) growth *in vitro*, the glucose analog 2-Deoxy-D-glucose (2-DG), has been evaluated in the prevention of restenosis using a double-balloon approach in a porcine coronary artery model³¹. 42 days after balloon overstretch-injury and local infusion through the Genie catheter, arteries exposed to this compound showed similar degrees of neointimal thickness, area stenosis, and re-endothelialization as those treated with rapamycin-eluting stents.

Limitations of the double-balloon catheter technology are the potential for local barotrauma to the vessel caused by the two balloons, regulation of the infusion pressure, and duration of incubation times. Modifications to the catheters have allowed blood to stream through a central channel in the catheter and thus prevent ischemia in the distal vessel. Nevertheless, ischemia in the occluded segment of the vessel and trauma within the two balloon-occluded segments will occur and increase with the duration of incubation. Especially with non-targeted infusates, occlusion times to enable sufficient drug delivery may reach 30 min³². These considerations provide a strong argument for the use of targeted infusates, which may decrease the incubation times to less than 2 minutes. Another limitation of the double-balloon delivery technology that may at least be partially overcome by molecularly-targeted drug-delivery is the loss of infusate through side branches. In this scenario, molecularly-targeted nanoparticles may still allow for adequate drug delivery, while non-targeted agents may run off too quickly to allow for significant retention times at the targeted tissue.

Drug eluting stents (DES)

The concept of using stents as a reservoir for drugs is appealing due to reliability of drug delivery and predictable release kinetics. Indeed, in today's clinical practice most patients receive local-drug delivery through drug-eluting stents. Important parameters that are of interest for drug delivery through stents pertain to 1) drug type, 2) drug release kinetics and physiological transport forces, 3) mechanical issues such as stent strut apposition and deployment forces, and 4) oral antiplatelet regimen post DES implantation. All these parameters exert effects on restenosis and endothelial healing. An extensive literature review regarding the differences in the restenosis suppression for currently FDA approved DES has been recently published³³. Of interest here is the observation that at least in some cases higher rates of restenosis suppression may be paralleled by prolonged endothelial dysfunction^{34, 35}. This may be related to particular drugs, but it may also be simply related to concentration gradients. Stent-based delivery of drugs leads to large concentration gradients over a few micrometers from the stent struts³⁶. In order to achieve therapeutic drug effects in the media, the immediate subendothelial layer is exposed to relatively high drug dosages, which delays endothelial healing. In general, hydrophobic drugs achieve higher mean concentrations closer to the intima when compared to hydrophilic drugs and both are affected by inhomogeneous strut placement³⁶. If stent struts are not apposed well, the restenosis response may not be inhibited and similarly to bare metal stents (BMS), the struts can become a nidus for thrombus formation. To avoid such stent strut mal-apposition, DES and BMS are often deployed at much higher pressures when compared to plain balloon angioplasty which is aimed at stenosis reduction. While this solves the anatomical problem, a sometimes neglected fact is that restenosis severity correlates with stretch-injury severity, at least in the porcine model¹. Thus, lower drug levels might achieve similar restenosis suppression, if they can be delivered with less vascular injury. This observation provides another rationale for further research into nanotechnology-based drug delivery, which might be successful without stent placement. After DES implantation, stent-thrombosis needs to be prevented with long-term dual antiplatelet therapy. Apart from clinical situations such as trauma or emergent surgeries, when some or all such therapy needs to be temporarily withdrawn, a small but significant number of patients fail to continue adhering to dual antiplatelet therapy for various reasons. These appear to be the scenarios where most of the stent thrombosis events occur^{37, 38}. Overall, the number of patients not receiving or taking dual-antiplatelet therapy according to guidelines is significant and has been reported to occur in up to 22–29% of patients in the “real-world” experience outside clinical trials^{39, 40}. This has led to the issuance of specific guidelines⁴¹. Importantly, very late stent thrombosis (> 1year) has been reported to at least 4.5 years with DES and up to 11 years with BMS, with very late stent thrombosis and reinfarction being more frequent with DES⁴. Again, these concerns provide a rationale for further research of nanotechnology-based delivery options that may achieve adequate restenosis suppression without requiring long-term dual antiplatelet therapy.

While the initial successes of DES were measured with the suppression of restenosis, endothelial healing is receiving increasing attention. As mentioned above, there often is a dichotomy between restenosis suppression and endothelial healing. In some cases the impact on endothelial healing may be so severe that a drug-eluting stent has to be withdrawn from clinical testing despite excellent results in restenosis prevention⁴². Since delayed endothelial healing is more pronounced in DES than BMS and plain angioplasty, intense research efforts are taking place to improve on this. Different drugs for delivery are being tested, modifications to polymers are being made, and even polymer-free drug-carrying stents are being tested. Polymer coatings were used early on to facilitate drug-adhesion to the stent and controlled release from the stent. The ideal polymer should be biological inert. Indeed some animal studies suggested that phosphorylcholine-coating (PC) of stents may have good

biocompatibility^{43, 44}. Yet, others showed extensive inflammatory responses and fibrocellular proliferation in response to stents coated with different polymers. As an example, in a study in porcine coronary arteries four weeks after implantation of polymer-loaded stents, the thickness of tissue response was 0.79 ± 0.22 , 1.12 ± 0.01 , 2.36 ± 0.60 , 1.24 ± 0.36 , and 1.43 ± 0.15 mm, for the biodegradable polycaprolactone (PCL), polyhydroxybutyrate valerate (PHBV), and polyorthoester (POE) and the non-biodegradable polyurethane (PUR) and silicone (SIL). Less, but still severe responses were observed for the biodegradable polyglycolic/polylactic acid (PGLA) and polyethylene oxide/polybutylene terephthalate (PEO/PBTP) (0.46 ± 0.18 and 0.61 ± 0.23 mm, respectively) and for the non-biodegradable polyethylene terephthalate (PETP) (0.46 ± 0.11 mm) coated stents⁴⁵. More novel polymers as well as antiproliferative agents and polymer-free drug-eluting stents are undergoing clinical evaluation and have been recently reviewed by S. Garg and P. Serruys⁴⁶. Exemplifying a rationale for further research into polymer-free drug delivery, polymer-free sirolimus stents compared to polymer coated sirolimus (Cypher®) stents have been recently shown to improve endothelial healing in an iliac artery injury model in New Zealand White rabbits⁴⁷. However, the suppression of neointimal thickening was less robust. Furthermore, significant differences in the rate of endothelialization of DES in current clinical use have been demonstrated in animal models⁴⁸. The question remains, if sacrificing some restenosis suppression effects in favour of improved endothelial healing will be clinically more important. Furthermore, could a technology such as molecularly targeted delivery achieve both, improved endothelial healing and reduced restenosis?

Likely more important than endothelial healing as assessed by microscopy *ex vivo* or optical coherence tomography *in vivo* may be the endothelial function. While initially not completely understood, endothelial function does not necessarily coincide with endothelial healing.

Endothelial dysfunction has been demonstrated for sirolimus-eluting stents but not for BMS for periods up to 6 months^{34, 35}. The clinical significance of these findings remains uncertain. However, it is known that endothelial dysfunction contributes to atherogenesis and is independently associated with future cardiovascular events^{49–51}. Thus, avoiding endothelial dysfunction after percutaneous coronary intervention (PCI) may be at least of hypothetical benefit.

In conclusion, areas of improvements with DES technology which also open avenues for alternate modalities such as molecularly targeted nanotechnology are improvements in endothelial healing, including early return of endothelial function and prevention of stent-thrombosis. Ideally, this should be accomplished while decreasing duration of dual-antiplatelet therapy. In addition, molecularly-targeted drug-delivery may be tested for delivery to anatomical locations that evade deliverability of stents, or in case of restenosis in vascular segments that already have two or more layers of stents.

Nanoparticle-facilitated drug-delivery during interventional procedures

Rationale for molecularly-targeted delivery

Despite two decades of optimization of mechanical delivery systems and strategies, delivery efficiency as well as retention of the respective agent at the target site, remain issues. Many agents may have failed to show beneficial effects simply because they were washed out or could not penetrate into the target site despite long incubation periods. Nanotechnology may help specifically target such previously “failed” drugs to the epitopes of interest and elicit the desired effects. The prevention of vascular complications may be a unique feature of nanotechnology in interventional cardiology. Detection of dysfunctional endothelium may be possible with nanoprobes targeted to molecular markers that are expressed on activated

endothelium. In addition to detection, such nanoprobes may simultaneously deliver agents to interrupt the activation process and prevent atherosclerotic lesion growth.

Prevention of restenosis

In addition to the drugs that are already in clinical use on drug-eluting stents, many more agents of interest have been evaluated with various results. For instance, some studies showed promising results with respect to diminishing restenosis in animal studies, but then failed to significantly reduce restenosis in humans. These include drug eluting stents with dexamethasone⁵², heparin⁵³, glycoprotein IIb/IIIa antibodies⁵⁴, and actinomycin⁵⁵.

In contrast, sirolimus showed promising short-term (28–30 days) results for the prevention of restenosis in porcine models^{56–58}, but failed to show a statistically significant difference compared to bare-metal stents 90 and 180 after implant⁵⁸. Nevertheless, clinical trials in humans have shown excellent long-term results for sirolimus-coated stents^{59, 60}. These data highlight the fact that animal studies are not necessarily translatable to the human condition. In case of the “failed” drugs, it is important to remember that these drugs were not chosen randomly but due to proven efficacy for instance in the inhibition of VSMC in *in vitro* models. Thus, the lack of efficacy in animal studies and/or human trials may not necessarily be the result of “failure” of the drug, but may be due to lack of sufficient delivery and/or retention at the target site. Here, novel nanotechnology-based delivery methods may open new avenues to test the efficacy of some of the drugs that did not show satisfactory restenosis prevention with conventional delivery methods.

Delayed endothelial healing⁶¹ and endothelial dysfunction³⁴ are additional concerns after implantation of drug-eluting stents. In the quest to find agents that may inhibit in-stent restenosis while also improving endothelial function, potential drugs as well as local gene transfers have shown promising results. Among the drug-candidates is angiotensin-(1–7). This is an endogenous, biologically active peptide of the renin-angiotensin system. It has vasodilatory, antithrombotic, and antiproliferative properties and has been shown to inhibit stent-induced neointima formation in male Wistar rat aortas⁶². Interestingly, angiotensin-(1–7) also attenuated stenting-induced impairment of endothelium-dependent relaxation to phenylephrine. Among the gene-candidates are human vascular endothelial growth factor (VEGF) gene eluting stents. Upon deployment of these stents into iliac arteries of hypercholesterolemic rabbits, restenosis was diminished at 3 three months and re-endothelialization was nearly complete after 10 days⁶³.

Targets for nanoparticle-facilitated drug-delivery

Non-specific

So called “bioadhesive” nanoparticles have been generated by applying carbopol 940 as stabilizer and bioadhesive agent to poly (lactide-co-glycolide) PLGA nanoparticles⁶⁴. These particles are spherical and measure 157–248 depending on the concentration of carbopol. The rapamycin, a highly hydrophobic drug, is entrapped during the formation process of these nanoparticles. In an *ex vivo* perfusion model using dog carotid arteries, the investigators showed that retention and uptake efficiency on the arterial wall increased with increasing concentrations of carbopol 940⁶⁴. While the “bioadhesive” approach is intriguing, it does not entail targeting to any specific epitopes, but that the particles will stick to any tissue they get in contact with. Thus, local delivery will be paramount. Another potential pitfall of this approach is the bioadhesive agent used. Carbopol 940 is a cross-linked polyacrylate polymer that is used in gels for skin and hair applications. It is hygroscopic and provides high viscosity. The toxicological properties of this material have not been clearly defined for intra-corporal use.

One novel strategy to enhance local drug delivery is to utilize magnetically induced release of a drug-payload of a nanoparticle. Here, the nanoparticles are not targeted to specific epitopes in the tissue, but magnetically attracted to the metallic stents using uniform field-induced magnetization. Recently, magnetic nanoparticles (MNPs) designed for efficient entrapment and delivery of paclitaxel have been shown to inhibit VSMC *in vitro* and to achieve higher localization rates of locally delivered MNPs to stented arteries in rats under magnetic conditions⁶⁵. Both, VSMC growth *in vitro* and in-stent restenosis *in vivo* were inhibited. Advantages of this technology may be the ability to individualize drug dosing, and repeat delivery to the target site. A disadvantage in the use of magnetic fields may be given for patients with metallic implants unless lower field strengths than used in this study, may prove efficient.

Endothelium

Intercellular cell-adhesion molecule-1 (ICAM-1) is constitutively expressed on the luminal surface of endothelial cells⁶⁶ and up-regulated in vascular inflammation^{67, 68}. Furthermore, blocking of ICAM-1 with antibodies has been shown to diminish leukocyte adhesion to endothelial cells⁶⁹, and antibody-ICAM-1 conjugates have been shown to become internalized into cells⁷⁰. Thus, ICAM is an attractive target not only for imaging but also therapeutic nanoparticle-based strategies. Indeed, catalase nanoparticles targeted to ICAM-1 have been shown to be internalized into and protect endothelial cells from oxidative stress in human umbilical endothelial cells^{71, 72}. More recently, a nanoparticle-peptide conjugate has been created by covalently linking PEGylated PLGA nanoparticles to the cLAbL peptide⁷³. These nanoparticles demonstrated rapid binding to HUVEC with up-regulated ICAM-1 and they were quickly endocytosed and trafficked to lysosomes⁷³. In an early step to develop an ICAM-1 targeted nanoparticulate agent for CT imaging of atherosclerotic plaque, anti-ICAM-1 antibodies have been covalently bound to liposomes containing iohexol and were shown to bind to the surface of activated human coronary artery endothelial cells *in vitro* with high specificity as determined with ELISA and shown in immunohistochemistry⁷⁴.

Vascular adhesion molecule-1 (VCAM-1) is another endothelial cell adhesion molecule that has been targeted for nanoparticle-based imaging. In these experiments, a phage display-derived VCAM-1-targeting peptide was attached to monocrystalline magnetic nanoparticles (VINP-28) with a diameter of 38 nm and injected into apoE^{-/-} mice⁷⁵. MRI and optical imaging showed colocalization of the VINP-28 with endothelial cells and atheromata of atorvastatin-treated apoE^{-/-} mice showed reduced VINP-28 deposition and VCAM-1 expression. Nanocarriers have also been targeted to platelet-endothelial cell adhesion molecule (PECAM-1). Interestingly, differences in the intracellular trafficking were observed with mAb62-nanocarriers trafficking to lysosomes, whereas mAb32-nanocarriers had prolonged residence in pre-lysosomal vesicles⁷⁶. These experiments enforce the concept that nanoparticles can be designed to preferentially target specific intra-cellular localizations.

Another group of investigators generated nanoparticles targeted to E- and P-selectin by covalently binding a ligand of E- and P-selectin to poly-L,D-lactic acid polymer (PLA) nanoparticles and showed adhesion of these nanoparticles to HUVEC *in vitro*⁷⁷.

A different type of nanoparticles, PAMAM dendrimers, has also been successfully targeted to E- and P-selectin by attaching anti E/P-selectin antibodies onto the PAMAM dendrimers⁷⁸. These nanoparticles increased gene delivery to endothelial cells expressing E-selectin in human saphenous vein segments *ex vivo*.

Very intriguing experiments have expanded on the antibody-facilitated ICAM-1 targeting additional evaluation of not only different antibody concentrations per particle but various

physical shapes on the efficacy of nanoparticle delivery. Interestingly, nanoparticle carrier geometry significantly influenced not only endothelial drug targeting, but also intracellular trafficking and effects. In these experiments, ICAM-1 targeted disk-shaped nanoparticles had longer systemic half-lives and more targeting specificity, while nanoparticle spheres were endocytosed much more rapidly⁷⁹. In additional experiments, it was shown that various nanoparticle-antibody densities and carrier concentrations lead to similar saturations of activated endothelial cells but not quiescent cells⁸⁰.

In the quest to discover novel peptide ligands that bind to proteins expressed on endothelial cells, *in vivo* phage display screenings in humans have identified a large collection of unique targets⁸¹. While the use of *in vivo* phage display to identify peptides interacting differently with protein targets in vascular beds of organs or tissues was first reported 15 years ago⁸², novel technologies including bacteria-free phage display, and real-time PCR have allowed the creation of large libraries of ligand-targeted phage displays that may be used not only for peptide therapy but also gene therapy⁸³. Proteomics presents an alternative way to identify membrane proteins. A novel three-dimensional separation method which outperforms the current two-dimensional methods by significantly enhancing protein identifications has recently been published⁸⁴. Using endothelial cell membranes from lung tissue, more than 500 lipid-embedded proteins were identified with this new method. Like the newer phage display methods, this novel proteomics method is likely to facilitate detection of a multitude of heretofore unknown membrane-embedded ligands for nanoparticle-based targeting.

Another interesting approach to prevent injury-mediated endothelial cell activation is the genetic modification of endothelial cells through magnetic nanoparticle-mediated transfection with therapeutic genes. Initially, viral vectors were attached to superparamagnetic nanoparticles and an external magnetic field applied to enhance the target-specific localization of the nanoparticles. With this methodology, enhanced transfection could be demonstrated *in vitro* and *in vivo*^{85, 86}. Using thermally cross-linked superparamagnetic iron oxide nanoparticles covalently conjugated with polyethylenimine linked with plasmid DNA, increased cellular uptake was shown in the presence of an external magnetic field and the expression of PAI-1 was inhibited⁸⁷. In order to increase resistance of endothelial cells to oxidative damage, catalase has been delivered successfully using magnetically guided delivery with magnetically responsive nanoparticles⁸⁸. In these experiments, the nanoparticles were formed by precipitation of calcium oleate in the presence of magnetite-based ferrofluid (controlled aggregation/precipitation) and filled with catalase and superoxide dismutase. In a magnetic field, these nanoparticles were rapidly taken up by cultured endothelial cells and provided increased resistance to oxidative stress⁸⁸.

Media – HDL / macrophages

High-density lipoproteins (HDL) naturally penetrate the endothelium because of their small size and play a pivotal role in the reverse cholesterol transport from lipid-rich atherosclerotic lesions. Since the HDL particles are naturally biocompatible and can be modified to carry imaging-payloads, they have been investigated as an attractive nanoparticulate carrier to image vulnerable atherosclerotic plaque. In earlier experiments, a reconstituted HDL (rHDL)-like particle was incorporated with a gadolinium-chelate and shown to facilitate imaging of atherosclerosis in the abdominal aorta of apoE^{-/-} mice by MRI following tail vein injection⁸⁹. These experiments, which were carried out with spherical HDL particles, were later extended using disk-shaped rHDL-like particles prepared either with a sonication process to form the particles or cholate dialysis with phospholipids conjugated with Gd-DTPA⁹⁰. As in the previous experiments, these particles accumulated in the atherosclerotic abdominal aorta with the highest signal intensity 24 hours after injection into the tail veins of apoE^{-/-} mice. Similar to detecting differences in the influence of the shape of

nanoparticles on cellular binding on uptake observed with ICAM-1 targeted particles (as reviewed above⁷⁹), the physical particle shape of the various HDL-like particles may also explain some of the differences in efficacy.

Further expanding the armamentarium of macrophage-based media-targeting, a fully-synthetic HDL/apoA-1 mimicking nanoparticle has been developed and been shown to signal-enhance atherosclerotic apoE^{-/-} mouse aortas when imaged with MRI⁹¹. To extend HDL particle based imaging of atherosclerosis to imaging modalities such as CT and optical imaging, inorganic nanocrystals either containing gold for CT or quantum dots for optical imaging, have been used to replace the natural HDL core⁹². While HDL-like particles have been useful to detect atherosclerosis *in vivo*, only a few studies have explored rHDL-like particles for the delivery of drugs to the liver⁹³, but not for suppression of atherosclerosis.

Another approach takes advantage of the fact that atheromatous plaque, especially the so called vulnerable plaque, contains a large number of macrophages⁹⁴. To exploit the migration of macrophages into such plaque, crystalline iodinated particles containing an esterified derivative of the X-ray contrast agent diatrizoic acid were dispersed with biocompatible surfactant and intravenously injected into rabbits with atheromatous plaques generated by balloon injury in the abdominal aorta⁹⁵. Enhancement of atherosclerotic plaques was most pronounced two hours after injection of these nanoparticles as imaged with CT. Furthermore, a nanoparticulate agent was developed for detection of macrophages in atherosclerotic plaque using MRI. In these experiments, Gd-DTPA-carrying immunomicelles were linked with a biotinylated monoclonal rat anti-mouse antibody to murine macrophage-scavenger receptor-A types I and II and infused into apoE^{-/-} mice⁹⁶. A strong correlation between macrophage content in atherosclerotic plaques as quantified via histology and signal-enhancement in *in vivo* MRI was observed.

Overall many interesting avenues to interrogate the vascular media for lipid deposition and potential vulnerability of plaque are available. At present, these modalities are useful for research and diagnostic purposes. It appears feasible that nanoparticles aimed at macrophages and lipid accumulation may also be used as drug carriers and thus stabilize vulnerable plaque. Given the inherent time delay to target and incorporate into macrophages and for macrophages to migrate into the lesions, this technology is unlikely to be used during interventional procedures, but it may non-invasively treat lesions, or via detection of vulnerable plaque, initiate interventional procedures.

Media – SMC

Locally delivering drugs to targets within the media is especially intriguing with regards to preventing restenosis since restenosis is largely driven by VSMC. Activation of VSMC is triggered by the angioplasty and results in increased rates of mitoses and stimulates migration. While drug-eluting stent technology has been successfully delivering drugs to this target with drug-concentration gradients decreasing with the physical distance from the drug-eluting stent, nanotechnology-based molecularly-targeted drugs can be delivered deep into the tissue fissures created by angioplasty and retained locally.

In one recent example, combining stent placement with local magnetically guided delivery of magnetic paclitaxel-containing nanoparticles has resulted in significant retention of this drug in the arterial wall and inhibition of in-stent stenosis in a rat carotid stenting model⁶⁵.

Promising with respect to practicability for local delivery during PCI procedures and successfulness of prevention of vascular restenosis are perfluorocarbon (PFC) nanoparticles which can be molecularly targeted, non-invasively imaged, and reliably function as drug-carriers⁹⁷. These nanoparticles with an average mean diameter of 250 nm, have been

successfully targeted to $\alpha_v\beta_3$ -integrin exposed in overstretch-injured carotid arteries in pigs using local infusion and epitope-specific binding has been shown with MRI and histology⁹⁸. In these experiments, the nanoparticles carried gadolinium as “imaging-payload”, which facilitated non-invasive imaging. In subsequent experiments, these PFC nanoparticles were additionally conjugated with paclitaxel as therapeutic payload and shown to inhibit overstretch-injury-induced restenosis in NZW rabbit femoral arteries (figure 2)⁹⁹. The PFC nanoparticle platform is particularly intriguing because it is very versatile with respect to targeting numerous epitopes, imaging capability with ultrasound, X-ray, MRI, or SPECT depending on the imaging payloads, and drug delivery abilities¹⁰⁰. Another advantage of the PFC nanoparticles is their biocompatibility.

Blood platelets – thrombus

Molecular imaging of thrombus is intriguing because 1) information may be obtained non-invasively, 2) without using radioactive tracers, 3) highly sensitive nanoparticle agents may detect very small thrombus in developing stages, and 4) thrombus-specific targeted thrombolytic therapy may be provided without exerting undue systemic effects.

Activated blood platelets on clots express P-selectin. A paramagnetic nanoparticle containing gadolinium diethylene-triamine-pentaacetic acid (Gd-DTPA) was mixed with an anti-human P-selectin lectin-EGF domain monoclonal antibody (PsL-EGFmAb) and incubated with platelet and blood clots respectively *in vitro* as well as intravenously infused into dogs upon left femoral vein de-endothelialization with a nylon monofilament¹⁰¹. T1-weighted imaging in a 1.5-T clinical MR imager showed significant contrast enhancement in the platelet and blood clots targeted with these nanoparticles *in vitro* and *in vivo* contrast enhancement in the dogs showed higher signal intensities in the injured femoral vein areas than the control. In another model, liposomes were decorated with RGD peptides and targeted to the GpIIb/IIIa integrin, which is up-regulated on activated platelets¹⁰². These nanoparticles were infused into the left cardiac chamber in a carotid artery restenosis model in rats and subsequently shown to be co-localized with thrombus on the injured endothelium using fluoroscopy and SEM. Alternatively to P-selectin and the GpIIb/IIIa integrin, the $\alpha_v\beta_3$ -integrin has a long track-record for successful nanoparticle-facilitated targeting of thrombus¹⁰³. In initial studies, perfluorocarbon-nanoparticles were targeted to this integrin in a canine thrombosis model and imaged with ultrasound¹⁰³. The same group recently created manganese-based nanoparticles for MR imaging and targeted them against fibrin¹⁰⁴. In the initial studies these manganese oxide nanocolloids (ManOC) bound to fibrin *in vitro* and the MR characteristics were established while *in vivo* studies are currently underway¹⁰⁴.

Conclusion

The restenosis response after cardiovascular angioplasty and stent implantation is influenced by the mechanical injury during angioplasty, inflammatory responses, and endothelial healing. Thus an ideal drug/agent used to prevent restenosis after angioplasty should be antiproliferative, anti-migratory, anti-inflammatory, and pro-healing. Drugs fulfilling some or most of these criteria typically cannot achieve meaningful local effects when systemically delivered. Even when locally delivered, non-targeted drugs often do not accumulate in quantities high enough to achieve the desired effects. Drug-eluting stents are standard-of-care but require long-term oral dual-antiplatelet therapy. An ongoing issue pertaining to restenosis-prevention and endothelial healing in the design of drug- and delivery-formulations for DES is that improvements in achieving of the one goal may be met with a worsening of the other.

Molecularly-targeted nanoparticle-facilitated delivery of drugs to selected epitopes in the angioplasty-injured vascular wall has already been shown to be feasible for several types of

nanoparticles and in various animal models of vascular injury. Individual cell-types (e.g. macrophages, VSMCs), extracellular components (e.g. collagen), or epitopes occurring on various cell-types (e.g. $\alpha_v\beta_3$ -integrin) can be specifically targeted and thus therapy tailored to the desired effects. Importantly, nanoparticle-facilitated delivery of drugs deep into the media during angioplasty has been shown to result in restenosis prevention while not delaying endothelial healing in animal models. Current research is focussed on optimizing the effects on both restenosis prevention and endothelial healing. It is anticipated that in the near future, some of these nanoparticle-drug formulations will enter clinical trials. In interventional procedures, the molecularly-targeted nanoparticles will be locally infused using double-balloon catheters, between the initial angioplasty and the subsequent stent placement.

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	Non-targeted delivery	Targeted delivery
Targeting specific epitopes	–	+
Targeting specific cells	–	+
<i>Ex vivo</i> labeling of cells	+	+
Enhancement of delivery through utilization of a magnetic field (magnetofection)	+	+
Delivery in conjunction with an interventional procedure	+	+
Systemic delivery without an interventional procedure	–	– – +
Prolonged local contact necessary for delivery	+++	– – +
Downstream effects, i.e. washout of nanoparticles	++ – +++	– – +

Table 2

Targets for nanoparticle-facilitated vascular imaging and therapy

Target cell or tissue	Target epitope or technology	References
Endothelial cells	ICAM-1	71–74, 80
	VCAM-1	75
	PECAM-1	76
	E-, P-selectin	77, 78
	Superparamagnetic nanoparticles	87
Macrophages and lipid-containing atheroma in the vascular wall	Spherical, reconstituted (r) HDL particles	89
	Disk-shaped HDL-like particles	90
	Fully synthetic nanoparticles	92, 95, 96
Smooth muscle cells in the media	Magnetic nanoparticles	65, 98, 99
	$\alpha_v\beta_3$ -integrin	98, 99
Thrombus imaging	P-selectin	101
	GpIIb/IIIa integrin	102
	Fibrin	104
Novel epitopes on various cells	Phage-display or proteomics-based technologies to screen for novel targets	81, 83, 84