Although the rate of target lesion revascularization has been reduced by the advent of the drug-eluting stent, the incidence of in-stent restenosis remains unacceptably high. Gene therapeutic strategies, including catheter-based gene delivery and gene-eluting stents, offer novel treatment methods to promote re-endothelialization, and inhibit inflammation, neointimal hyperplasia and late stent thrombosis. The translation of gene therapy into clinical application must be safe and requires an effective, site-specific delivery system as well as the ability to provide sustained transgene expression. The progress of magnetic nanotechnology and genetic engineering of human stem cells can provide such elements. In the present review, the authors discuss the evolution of antirestenosis therapy, underlying mechanisms of restenosis and the applications of gene therapy to prevent in-stent restenosis. Current gene delivery methods, including gene vectors and delivery strategies, are critically reviewed.

**Key Words:** Gene therapy; Intimal hyperplasia; Nanotechnology; Re-endothelialization; Restenosis; Stem cell
magnetic nanotechnology and genetic engineering of human stem cells have also emerged as exciting alternatives to eliminate the underlying mechanisms of ISR, suggesting their possible roles as the next generation of antirestenosis therapy.

### THERAPEUTIC GENE TARGETS

**Re-endothelialization**

Stent implantation for coronary artery disease causes a marked vascular injury, inducing de-endothelialization, which is associated with thrombus formation and abnormal responses to endothelium-dependent agonists (19). In recent years, several studies have focused on the use of vascular gene therapy targeting multiple pathways to promote re-endothelialization (20-26). The potential of vascular endothelial growth factors (VEGFs), a group of potent endothelial mitogens, to treat postangioplasty restenosis was first investigated in animal models. Several VEGF family members, including VEGF165, VEGF-2, VEGF-C and VEGF-D, have been found to inhibit neointimal thickening, reduce thrombogenicity and restore the endothelium-dependent vasomotor reactivity (27-29). Yang et al (30) developed a stent coated with bilayered poly-lactide-coglycolide (PLGA) nanoparticles (NPs) containing VEGF plasmid in the outer layer and PTX in the inner core with PTX in the inner core and found that it significantly promotes early endothelial healing while inhibiting SMC proliferation through sequential release of the VEGF gene and PTX (23).

Another interesting target is nitric oxide (NO), which is mainly synthesized by NO synthase (NOS). Most studies have focused on two main types of NOS, inducible NOS and endothelial NOS (eNOS), to reduce neointimal hyperplasia and accelerate re-endothelialization (30-33). Both catheter- and stent-based delivery techniques have verified eNOS and inducible NOS as potential therapeutic genes for accelerating re-endothelialization and reducing neointimal hyperplasia (Tables 1 and 2) (31-33). Sharif et al (31) found that eNOS gene delivery can result in significantly enhanced re-endothelialization and decreased neointimal formation using an adenovirus GES. The same laboratory reported that nonviral, liposome-based gene delivery of eNOS to the blood vessel wall in vivo results in enhanced endothelialization as well as prolonged and localized eNOS expression in the blood vessel wall in a hypercholesterolemic rabbit model (22). Cyclooxygenase-1 (COX-1) is the rate-limiting component in the synthesis of prostacyclin (PGI₂), a potent vasodilator and antithrombotic molecule. In balloon-injured, atherosclerotic porcine and hypercholesterolemic rabbit models, COX-1 gene transduction induced an early increase in the production of PGI₂, prostaglandin E₂ and prostaglandin E₁, resulting in long-lasting vasodilatation (34).

The metabolic syndrome is defined as a cluster of numerous cardiovascular risk factors that encompass dyslipidemia and hypertension. Patients with the metabolic syndrome are more prone to developing ISR (35). Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is a membrane protein that can bind and internalize oxidized low-density lipoprotein and advanced glycation end products, which further promote endothelial injury after percutaneous coronary interventions (PCI). Targeting the LOX-1 gene with gene silencer pyrrole-imidazole polyamide significantly decreased the expression of LOX-1 and promoted re-endothelialization, thus inhibiting neointimal hyperplasia after arterial injury (24). Gene transfer of PGI₂ synthase has also been shown to accelerate re-endothelialization and prevent neointimal formation in balloon-injured arteries. In a rabbit model, PGI₂ synthase gene delivery by the lipotransfection method via dispatch catheter can accelerate re-endothelialization and attenuate neointimal formation (36). Douglas et al (20) found that endothelial-specific overexpression of GTP-cyclohydrolase-1 can enhance endothelial cell function and increases NO production, thus promoting re-endothelialization after stent deployment.

**Antineointimal hyperplasia**

Following injury of coronary arteries during interventional procedures, including balloon angioplasty and/or stent placement, medial SMCs change their phenotype from contractile to synthetic, proliferative and migrative in nature, initiating the process of neointimal hyperplasia (37). Therefore, proliferation and migration of SMCs are crucial targets for the inhibition of intimal hyperplasia and restenosis. A number of studies have shown that transfer of cytotoxic antiproliferative genes, including thymidine kinase, cytosome deaminase and Fas ligand, which selectively destroy SMCs as they enter the S phase of the cell cycle, are able to significantly inhibit neointimal hyperplasia in balloon-injured vessels (38-40). In addition, the transfection of antisense oligodeoxynucleotides (ODNs) targeting DNA binding proteins and transcription factors – including CDC2 and cyclin G1, which encode for regulatory proteins that are involved in the cell cycle – have also been shown to inhibit neointimal formation (41,42). MicroRNA, a 21 to 24 nucleotide RNA molecule, inhibits their messenger RNA target by binding to complementary ‘seed’ sequences, leading to post-transcriptional repression or target messenger RNA degradation. Several microRNAs have been found to significantly reduce injury-induced neointima formation by inhibiting VSMC proliferation (43,44). Merlet et al (43) found an upregulation of mir-424/322 after vascular injury. Overexpression of mir-424/322 in injured rat carotid arteries using an adenovirus has been demonstrated to inhibit restenosis.

Apart from the antiproliferative approach, inhibition of the migration of SMCs can reduce neointimal hyperplasia. Extracellular matrix degradation has been shown to play a crucial role in SMC migration, which is mainly regulated by matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) (45). Although early studies using marimastat, a broad-spectrum MMP inhibitor, showed significantly reduced neointimal formation in cultured human saphenous vein graft segments, the potential of marimastat as an antimitastatic agent was halted at phase III due to a lack of efficacy and dose-limiting toxicity (46). The potency of TIMP-1 as a therapeutic gene for antineointimal hyperplasia has been verified in various animal models (47,48). Johnson et al (48) found that stent-based delivery of TIMP-3 adenosine inhibits neointimal formation in porcine coronary arteries (48). Downregulation of platelet-derived growth factor-β receptor expression and upregulation of plasminogen activator inhibitor-1 and light-type caldesmon, a potent cytostatic and antiangiogenic protein, using a gene targeting and adenoviral gene transfer approach, has also been found to inhibit neointima formation (49). Lompré et al (50) investigated the functional effects of adeno-associated virus (AAV) 2/5-mediated sarco/endoplasmic reticularum Ca²⁺-ATPase isoform 2a (SERCA2a) – a human gene known to inhibit VSMC proliferation – in a rat model of carotid artery balloon injury and found that AAV2/5-mediated SERCA2a gene transfection significantly decreases the neointimal hyperplasia without inducing obvious inflammation in vivo.

Activation of the tyrosine kinase family JAK and subsequent activation of signal transducer and activator of transcription (STAT)
proteins are key events in signal transduction of different cytokines and growth factors. These proteins regulate different biological processes including SMC proliferation (51). Suppressor of cytokine signalling-3 (SOCS3) protein decreases the JAK/STAT signalling by blocking the JAK tyrosine kinase activity and the activation of STAT factors, suggesting that it is a novel antineointimal hyperplasia target. Recently, our laboratory has found a significant reduction in SOCS3 expression in response to a variety of proinflammatory stimuli. Using small interfering RNA demonstrated a potent inhibitory effect on CREB-binding protein using anti-DNA antibody-immobilized stent. Inhibition of inflammatory gene expression may provide the therapeutic effect of reducing neointimal hyperplasia after balloon injury-induced NF-κB acetylation and attenuated neointimal hyperplasia (55).

Anti-inflammation

Inflammation is a protective attempt by the organism to remove the injurious stimuli. However, stent implantation, including BMS and polymer-coated DES implantation, induces acute and chronic inflammation that stimulates accumulation of inflammatory cells, such as macrophages and T-lymphocytes, and inflammatory cytokine secretion (8). Inhibition of inflammatory gene expression may have the therapeutic effect of reducing neointimal hyperplasia. Transforming growth factor-beta (TGF-β) is a crucial inflammatory cytokine that controls immunity/inflammation and cell proliferation. Delivery of the gene silencer pyrrole-imidazole polyamide, which targets the TGF-β1 promoter, can suppress neointimal hyperplasia after arterial injury by downregulating TGF-β1 expression and inhibiting restenosis (25). Monocyte chemotactic protein-1 (MCP-1) is a proinflammatory chemokine specific for monocytes and is shown to be present at increased levels after vascular injury. The peptide from the C-terminal domain of MCP-1 (ingramon) has been shown to inhibit monocyte migration and possess anti-inflammatory activity while at the same time inhibiting the postangioplasty restenosis in animal models (54). In rabbit and monkey models, the same laboratory found that the local delivery of the C-terminal domain of MCP-1 (7ND) by a GES system attenuates in-stent stenosis or injury model.

Efficient transfection of eNOS locally in the arterial lumen suppressed SMC proliferation and promoted re-endothelialization of the artery showing a significant reduction of restenosis (25). Monocyte chemoattractant protein-1 (MCP-1) is a proinflammatory cytokine specific for monocytes and is shown to be present at increased levels after vascular injury. The peptide from the C-terminal domain of MCP-1 (ingramon) has been shown to inhibit monocyte migration and possess anti-inflammatory activity while at the same time inhibiting the postangioplasty restenosis in animal models (54). In rabbit and monkey models, the same laboratory found that the local delivery of the C-terminal domain of MCP-1 (7ND) by a GES system attenuates in-stent stenosis (55). In cynomolgus monkeys, a catheter-based anti-MCP-1 gene delivery system has also been shown to attenuate in-stent neointima formation (55).

As an essential transcriptional factor for inflammation, nuclear factor-kappa B (NF-κB) has been found to play a pivotal role for restenosis after PCI. In an open-label phase 1/figlla clinical trial, Egashira et al (56) found that NF-κB decoy ODN transfection can suppress the inflammatory response and prevent restenosis. CREB-binding protein is a powerful transcriptional coactivator that regulates inflammation, cell proliferation and apoptosis in vascular endothelial and SMCs. Knockdown of CREB-binding protein using small interfering RNA demonstrated a potent inhibitory effect on balloon injury-induced NF-κB acetylation and attenuated neointimal formation in balloon-injured rat carotid artery (57). Activator protein-1 is another crucial transcription factor regulating gene expression in response to a variety of proinflammatory stimuli. Using
TABLE 2
Summary of major studies using viral vector for antirestenosis gene therapy

<table>
<thead>
<tr>
<th>Author (reference)</th>
<th>Vector(s)</th>
<th>Gene(s)</th>
<th>Model/patients</th>
<th>Main outcome(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al (44)</td>
<td>Adenovirus</td>
<td>miR-663</td>
<td>Mice carotid artery injury model</td>
<td>Adeno-miR-663 markedly suppressed the neointimal lesion formation in mice</td>
</tr>
<tr>
<td>Merlet et al (43)</td>
<td>Adenovirus</td>
<td>miR-424/322</td>
<td>Rat carotid artery injury model</td>
<td>Adeno-miR-424/322 markedly suppressed the neointimal lesion formation in rats</td>
</tr>
<tr>
<td>Chomy et al (77)</td>
<td>Adenovirus</td>
<td>Magnetic nanoparticles</td>
<td>Rat carotid artery injury model</td>
<td>Magnetically targeted adeno-virus-loaded magnetic nanoparticles represent a novel delivery system for efficient, vascular gene transfer in vivo</td>
</tr>
<tr>
<td>Wu et al (80)</td>
<td>Adenovirus</td>
<td>Dominant-negative Skp2</td>
<td>Rat carotid artery injury model</td>
<td>Knockdown of Skp2 inhibited VSMC proliferation, and the subsequent neointimal thickening in rat arteries</td>
</tr>
<tr>
<td>Fishbein et al (103)</td>
<td>PABT/PEi(PDT)/HL-tethered adenoviral vectors</td>
<td>INOS</td>
<td>Rat carotid artery stent model</td>
<td>Rat carotid stent delivery of INOS resulted in significant inhibition of restenosis in rats</td>
</tr>
<tr>
<td>Johnson et al (48)</td>
<td>Adenovirus</td>
<td>TIMP-3</td>
<td>Porcine coronary stent model</td>
<td>Adenovirus-coated TIMP-3 stent significantly inhibited the in-stent neointimal formation in pigs</td>
</tr>
<tr>
<td>Hedman et al (66)</td>
<td>Adenovirus</td>
<td>VEGF</td>
<td>Patients with coronary artery disease</td>
<td>Myocardial perfusion showed a significant improvement in the VEGF-adenovirus-treated patients after the six-month follow-up</td>
</tr>
<tr>
<td>Sinnaeve et al (104)</td>
<td>Adenovirus</td>
<td>PKG mutant</td>
<td>Porcine coronary stent model</td>
<td>Expression of a constitutively active PKG reduces neointimal formation after balloon injury in rats and reduces coronary in-stent restenosis in pigs</td>
</tr>
<tr>
<td>Liu et al (34)</td>
<td>Adenovirus</td>
<td>COX-1</td>
<td>Rabbits carotid artery balloon injury model</td>
<td>Local gene transduction of COX-1 increases blood flow in injured atherosclerotic rabbit arteries</td>
</tr>
<tr>
<td>Lompré et al (50)</td>
<td>AAV2.5</td>
<td>Sarcomendoplasmic reticulum Ca2+ ATPase isoform 2a</td>
<td>Rat carotid artery balloon injury model</td>
<td>AAV2.5 vector can be considered as a promising safe and effective vector for antirestenosis gene therapy in vivo</td>
</tr>
<tr>
<td>Sharif et al (79)</td>
<td>AAV2</td>
<td>DNase-resistant particles</td>
<td>Rabbits iliac artery stent model</td>
<td>AAV2-coated stents can be used to deliver genes to the blood vessel wall for up to 28 days in vivo</td>
</tr>
<tr>
<td>Pankajakshan et al (82)</td>
<td>AAV2/9</td>
<td>SM22α promoter</td>
<td>Swine coronary and peripheral arteries</td>
<td>AAV2/9 vector can be considered as a promising safe and effective vector for antirestenosis gene therapy in vivo</td>
</tr>
<tr>
<td>Ramirez Correa et al (78)</td>
<td>Recombinant AAV</td>
<td>TIMP-1</td>
<td>Rat carotid injury arteries</td>
<td>Local AAV-TIMP-1 gene transfer represent an efficient strategy to prevent restenosis in rats</td>
</tr>
<tr>
<td>Squadrillo et al (105)</td>
<td>Recombinant AAV</td>
<td>NF-κB inhibitory protein IxBα</td>
<td>Mice carotid artery injury model</td>
<td>Recombinant AAV-mediated gene transfer of IxBα inhibited the restenosis in mice</td>
</tr>
<tr>
<td>Yang et al (57)</td>
<td>Lentivirus</td>
<td>CBP</td>
<td>Rat carotid injury arteries</td>
<td>Lentivirus-mediated CBP silencing may represent a novel therapeutic approach for the prevention of restenosis after vascular interventions in rats</td>
</tr>
<tr>
<td>Bonta et al (76)</td>
<td>Lentivirus</td>
<td>Nur1</td>
<td>Rat carotid injury arteries</td>
<td>Lentivirus-mediated nuclear receptor Nur1 is an attractive novel target for (local) intervention in restenosis in rats</td>
</tr>
</tbody>
</table>

AAV Adeno-associated virus; CBP CREB-binding protein; COX Cyclooxygenase; iNOS Inducible nitric oxide synthase; Nur1 Nuclear receptor-related 1; PKG Protein kinase G; TIMP Tissue inhibitor of metalloproteinase; VEGF Vascular endothelial growth factor; VSMC Vascular smooth muscle cell

decoy ODN transfection against the activator protein-1 binding site has recently been shown to effectively increase re-endothelialization and inhibit neointimal proliferation (21).

Antithrombosis
Stent implantation-induced thrombosis is a classical example of device-induced, platelet-mediated arterial thrombosis (58). In a porcine model of coronary restenosis, anti-CD34 antibody-coated sirolimus-eluting stents were associated with greater endothelialization, thus inhibiting platelet activation. However, a clear clinical benefit of stents coated with this technology has not been shown (58,59). ADP, a key agonist required to trigger platelet aggregation, has received much attention as a potential antithrombotic target. Takemoto et al (60) found that transfection of the human p3-NTDase gene, encoding an enzyme that rapidly hydrolyzes ATP and ADP to AMP via cationic gelatin-coated stents, inhibits subacute in-stent thrombosis and suppresses neointimal hyperplasia and inflammation without antiplatelet drugs. More established antithrombotic genes, including PGI2 synthase, COX-1 and tissue factor pathway inhibitor, have been investigated in recent years for their potential in postangioplasty intervention, and have demonstrated successful suppression of thrombosis and/or restenosis in various animal models (34,36,58,61).

GENE DELIVERY SYSTEMS
Gene vectors
Viral vector and nonviral vector are the classic gene delivery vectors, both of which have the ability to introduce therapeutic genes into vascular tissue (6). For greater safety and less immunogenicity, nonviral vectors have held more promise for clinical application than viral vectors. However, the complex process of migration through the vascular endothelium and the extracellular matrix makes nonviral vectors inefficient in the areas of transfection and rapid intracellular degradation (9), which hinders the application of vectors of this kind. Several forms of nonviral vectors, including naked plasmid DNA, DNA-containing cationic liposomes, DNA-polycation complexes, DNA-phosphorylcholine polymer complexes, DNA-PLGA NPs and ODN, have been investigated as potential gene vectors for antirestenosis therapy (33,62-69) (Table 1). Chemical formulations, such as polyplexes, inhibit the endogenous DNAases, thereby protecting plasmid DNA from degradation in the extracellular matrix (70). Furthermore, dodecylated chitosan-plasmid DNA complexes formed stable,
positively charged nanospheres with small vector size and increased EGFP-C1 gene expression in stents but not in adjacent arterial segments or distal organs, suggesting an interesting method for sustained and site-specific nonviral vector transfection (71). In addition, novel dendrimer-based poly (L-glutamic acid) derivatives have also been found to be an efficient and biocompatible gene delivery vector to inhibit restenosis in balloon-injured rabbit carotid arteries (72). Furthermore, recent studies have shown that small vector sizes in nanoscale-range particles (ie, NPs) have many advantages in DNA transfection including increased cellular uptake, decreased inflammatory response and increased cellular incorporation. This suggests NPs as a novel platform for nonviral gene vectors (73,74).

Compared with nonviral vectors, viral vectors have evolutionary advantages in their extra- and intracellular interactions. However, viral vectors can be subjected to a host of immune responses that not only negate the vector efficacy but also result in inflammatory responses in vivo (75). Several forms of viral vectors, including adeno-, AAV and lentivirus, have been applied in preclinical studies and clinical trials (Table 2) (34,43,44,50,57,76-80). Adenoviruses are commonly used viral vectors because they have many features that make them well suited for gene therapy. As shown in Table 2, adenovirus-coated genes, including micro-RNA, TMP-3 and VEGF, can have significant inhibitory effects on in-stent neointimal formation in multiple artery stent/injury restenosis animal models (34,43,44,77,80). AAV vectors have emerged as a versatile vehicle for gene delivery due to their efficient infection of dividing and nondividing cells in the presence of a helper virus (50,79). Compared with native AAVs, vascular cell-specific peptide-modified AAVs have been shown to target gene delivery to vascular tissues, such as vascular endothelial cells (ECs), in vivo (81). Our group has successfully transfection a novel recombinant AAV-2/9 vector with SM22α promoter to the medial layer SMCs of swine coronary and peripheral arteries (82). It warrants mention that AAV-2/9 viral transduction in our study has no significant effect on serum amylase, fibrinogen and C-reactive protein levels, suggesting that AAV-2/9 is a safe and effective gene therapeutic vector for antirestenosis (82). Lentiviral vectors also provide a promising strategy for the treatment of cardiovascular diseases due to their durable gene transfer and their ability to govern efficiently. Several groups have verified that lentivirus-mediated gene transfection may represent a novel therapeutic approach for the prevention of restenosis after vascular interventions (57,76) (Table 2).

Vector delivery strategies
Percutaneous catheter-based delivery: Percutaneous catheter-based gene delivery, currently the most frequently used strategy for antirestenosis therapy, has several major theoretical advantages: concomitant performance with other percutaneous procedures, such as combining gene therapy with mesenchymal stem cell therapy; utilization of smaller quantities to reduce potentially harmful effects of systemic delivery (83); and easy preparation, characterization and reproducibility. However, several major limitations have also limited the application of this strategy. First, most catheters require prolonged total occlusion of the target vessel for effective vector delivery, which may increase the risk for myocardial ischemia. Second, there is lack of efficiency in site-specific gene delivery, although current vector delivery systems have focused on modified balloon catheters that can trap a fluid within a short segment of vasculature (84). Finally, the delivered viral or nonviral vectors inevitably disperse from side branches of the coronary vasculature, which carries the potential risk for distal spread.

Another promising method of transluminal gene delivery involves using vascular tissue-specific targeted systemic treatments. White et al (81) isolated human venous EC-targeting peptides and genetically incorporated them into AAV capsids. Intravenous infusion of engineered AAVs into mice caused reduced vector accumulation in the liver while enhancing uptake of viruses in the vena cava (81). Deglau et al (85) investigated a site-specific delivery system. First, they injured rabbit femoral arteries using biotin molecular-loaded balloons, then intravenously administered avidin-coated microspheres, which has a high affinity for biotin. They found that these microspheres attached to the biotin on the arterial wall, suggesting the potential to locally deliver a systemically injected antirestenotic agent (85). In addition, genetic engineering of human stem cells with VEGF holds great potential as a platform for cell-based therapy to promote vascularization and tissue regeneration (86). Nonviral, biodegradable polymeric NPs were recently developed to deliver genes to human MSCs (87), and their surface can be easily manipulated with the addition of special ligands. This may be responsible for enhancing vascular injury site-specific NP permeability, suggesting that stem cell-based delivery of gene-loaded NPs offers an interesting option to overcome such deficiency in transluminal gene delivery.

Stent-based delivery: As with DES, GES use stents as permanent scaffolds to deliver biologically active agents locally for a prolonged duration to the site of vascular disease (65). Dichek et al (88) seeded stents with genetically engineered endothelial cells using retroviral-mediated gene transfer – the first progress in the field of GES therapy to prevent ISR. In a pig coronary angioplasty model, Klugherz et al (89) reported the first successful transfection in vivo using a DNA controlled-release stent and a collagen-coated stent with monoclonal antibodies covalently bonded to adenovirus (90). Much later, Walter et al (65) improved the efficiency of local delivery of naked plasmid DNA encoding for human VEGF-2 via GES in hypercholesterolemic rabbits. Swanson et al (91) also observed the effect of VEGF-coated stents on restenosis in hypercholesterolemic rabbits. Currently, stent backbones designed for gene loading include biodegradable, bioabsorbable, nanoporous metal stents coated with an outer layer of polymer, which may be bioabsorbable or nonbioabsorbable material, and can be loaded with therapeutic genes, thus providing more controlled and sustained gene delivery and allowing for more optimal gene-tissue interactions (26). Non-biodegradable polymers (BP) are currently the most frequently used method of coating the stents. However, considering that stents, similar to any other foreign material inside the human body, can cause infection and inflammation, BPs including poly-L-lactide, polylactic-polyglycolic acid, polylactide-cocaprolactone and PLGA, offer greater advantages. Bioresorbable stents, often composed of poly lactides such as poly-L-lactide or poly-lactide-cocaprolactone, are typically completely metabolized in approximately 12 to 18 months (92). In a porcine model, Lincoff et al (26) have found that stents composed of high-molecular-weight poly-L-lactide produced minimal inflammation and durable results compared with uncoated stents. Although BP-DES have yet to receive approval in the USA, they are widely used worldwide, including in Asia and Europe. In a five-year follow-up study, Kuramitsu et al (93) investigated the long-term coronary arterial restenosis of BP-bisulphur-eluting stents (BES) compared with durable polymer sirolimus-eluting stents and BMS, and found that BPE-BES shows a favourable coronary arterial response compared with sirolimus-eluting stents (93). However, in a network meta-analysis that included 113 trials involving 90,584 patients performed to compare the safety and efficacy of BPs, DES, BMS and durable-polymer DES in patients undergoing coronary revascularization (94), BP-BES was found to be associated with a higher risk for definite or probable ST compared with cobalt-chromium everolimus-eluting stents. These findings suggest the benefits of biodegradable polymer coating over a nonbiodegradable one such as durable polymer (26). However, additional studies are warranted to confirm these conclusions.

Compared with a catheter-based delivery strategy, GES represents a more appealing method for gene delivery to ath erosclerotic coronary vessels. However, there are a number of technical and biological challenges with a stent-based delivery strategy. First, the limited surface area of the stent does not always guarantee the abundant loading of vectors, which is necessary for sustained transgene expression. Second, there are difficulties with regard to preservation of the mechanical properties of stents after coating the material. Third, the interaction between the biological coating material and the tissue limits the cellular uptake and intracellular stability of the gene construct, as well as...
the efficient transcription and translation of the encoded protein. In addition, larger-sized particles loaded in GES may be taken up by macrophages rather than the target SMCs or ECs (6).

**Magnetically guided delivery:** Magnetic guidance is a physical targeting strategy with the potential to improve the safety and efficacy of a variety of therapeutic vectors (95). Magnetically guided delivery systems loaded with drug and cells to stented blood vessels has recently been proposed as a potential therapeutic strategy for in-stent restenosis (96,97). Magnetically targeted delivery systems are essentially composed of two primary components: magnetic particle-loaded therapeutic vectors and a magnetic field source generating a force attracting the vectors (Figure 2). Poljak et al (97) were the first to demonstrate high-magnetic-field gradient targeting of magnetic NP (MNP)-loaded endothelial cells to the surfaces of steel stents. At first, the investigators preloaded bovine aortic endothelial cells with biodegradable polymeric MNPs, thereby rendering the cells magnetically responsive. Then, the cells were injected via a catheter to the site advanced beyond the stent to the aortic arch. Under an external homogeneous magnetic field, the MNP-loaded bovine aortic endothelial cells were successfully targeted to the stent wires (97). Recently, Chorny et al (77) reported a novel site-specific gene delivery to stented arteries using magnetically guided delivery. In their study, zinc oleate-based MNPs were loaded with replication-deficient adenovirus, which is limited in its clinical applicability by rapid inactivation. Under magnetic conditions, adenovirus-loaded MNPs were effectively transduced into cultured ECs and SMCs. In addition, localized arterial gene expression in the magnetically guided MNP delivery group were significantly stronger than in control groups in a rat stent model, confirming the feasibility of using adenovirus-loaded MNPs to achieve site-specific transduction in stented blood vessels (77). Considering that recombinant vectors, whether viral or nonviral, must migrate through various biological barriers in one or more compartments to subsequently transfect target cells such as SMCs, an MNPs-based delivery system under a magnetic field may be seen as an exciting alternative for improving targeted antiproliferation gene release under catheter-based or stent-based delivery strategies (Figure 2).

**CONCLUSION**

The design of new antineointimal hyperplasia and re-endothelialization systems for the treatment of ISR was the major purpose of developing next-generation PCI technologies, which more safely and effectively prevent the progress of atherosclerosis. Vascular gene therapy offers the potential to overcome the limitations of pharmacological interventions such as the long-term use of dual antiplatelet therapy and the side effects of antiproliferation drugs loaded in DES. Many candidate genes have the potential to prevent restenosis in various artery stent/injury animal models. However, to date, few clinical studies have demonstrated similar success rates, which may be related to the species-specific differences between human and animal models of the cardiovascular system and host-immune response. Swine coronary restenosis highly resembles restenosis in humans; thus, the swine is widely regarded as an accurate model for mimicking the proliferative component of human restenosis (98). There is a growing understanding that the physiological barriers to efficient, targeted gene transfer by gene vectors must be taken into consideration during the design of optimized therapeutic strategies. At present, viral vectors, including AAV and lentivirus, have the most potential to maximize transduction efficiency, although the toxicity, inflammation and interaction with tissue components remains to be fully evaluated. A GES-based delivery strategy is superior with regard to site-specific and sustained transgene expression. However, the stent design and material technology must be improved. The novel MNP formulation designed for magnetically guided adenoviral gene transfer to cells of the blood vessel wall addresses several prerequisites for a safe and effective gene transfection, especially targeting antiproliferation genes to SMCs. Genetic engineering of human mesenchymal stem cells using biodegradable polymeric NPs has recently been shown to enhance angiogenesis, offering an interesting option for enhancing endothelialization. These results suggest that gene-loaded NP systems and mesenchymal stem cells may be a next step for antirestenosis therapy, although the exact effect remains to be elucidated in preclinical studies and clinical trials.

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