Chapter 15

Use of Biomaterials and Biomolecules for the Prevention of Restenosis

Nelisa Türkoğlu Laçin, Kadriye Kızılbey and Banu Mansuroğlu

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/61081

Abstract

Coronary balloon angioplasty and coronary stenting are the procedures used in healing coronary artery disease. However, injury of arteries during angioplasty and stenting causes cell stimulations in tissue. Cell movement and thrombosis lead to re-narrowing of widened vessel called restenosis. Several new types of carriers and technology have been developed to suppress and/or prevent restenosis via prevention of migration/proliferation of smooth muscle cells (SMCs). The conventional approaches are not fully effective for inhibiting restenosis. In order to eliminate such problems, stent-based delivery methods are developed to replace traditional vascular approaches. A series of materials have been improved for controlled delivery/release of genes, miRNAs, peptide structures, siRNAs, miRNAs, and antisense molecules to the target tissue. Agents to be delivered are either attached to the materials or entrapped in polymeric structure. In particular, biodegradable polymers have held great interests in drug delivery for targeting or prolonging implantable drug release. This chapter summarizes the molecular mechanisms of in-stent restenosis, the role of SMCs and endothelial cells in restenosis, and recent researches about the polymeric materials featured in drug/gene carrier systems, nanovehicles, and stent coating materials to prevent restenosis.

Keywords: Restenosis, smooth muscle cells, biomolecules, drug delivery

1. Introduction

Atherosclerosis is a disease in which a plaque builds up inside your arteries. The plaque builds up of fat, cholesterol, calcium, and other substances found in the blood. Over time, plaque hardens and narrows your arteries [1]. Arteries are blood vessels that carry oxygen-rich blood to organs in the body, and this plaque limits the flow of oxygen-rich blood to organs and other parts of body. Thus, atherosclerosis can lead to serious problems, including heart attack, stroke, or even death. Percutaneous transluminal coronary angioplasty (PTCA) is a technique used to widen the narrowing in a coronary artery without surgery.

2. What happens during angioplasty?

At the beginning, the doctor moves a guiding catheter into the artery with the blockage. Once the guiding catheter is in right place, a guide wire is moved across the blockage site and then a balloon catheter is moved to the blockage site. The balloon is inflated for a few seconds to compress the blockage against the artery wall and then the balloon is deflated. This proceeding can be repeated for a few times. Each time the balloon is pumped, the plaque widens a little more and enables the blood to flow through. If it is needed, a stent is placed within the coronary artery to keep the vessel open. Following this, the catheter is removed and the procedure is completed, as seen in Figure 1. As a result, the narrowed artery is enlarged by PTCA. PTCA is sometimes called coronary angioplasty. Coronary angioplasty has become increasingly popular as a result of its low morbidity and mortality and reduced hospital stay in comparison with surgery. Coronary angioplasty is generally effective and safe, but restenosis is frequent, occurring in about 30-40% of cases [2]. Restenosis limits the long-term beneficial effects of PTCA and related procedures. PTCA may be defined as the initial gain in artery lumen size, and restenosis can be defined as the loss of gain. Prevention of restenosis after successful PTCA remains one of the most challenging issues in the obstructive treatment of coronary artery disease [3].

3. What causes restenosis?

As we mentioned above, stent placement is another option that is applied during angioplasty. Stent is a metallic scaffold that keeps the narrowed coronary artery portion open. Besides, as a metallic scaffold, the body may also perceive as alien. In fact, the trauma created by angioplasty and stenting in tissue is more effective on restenosis. The trauma created by angioplasty and stenting leads cell stimulations in tissue, triggers cells in that region, and causes cell proliferation, migration, and thrombosis. Finally, cell movement and thrombosis lead to the renarrowing of the vessel.

Restenosis following balloon dilation of the vascular endothelium is thought to occur in three steps:

http://dx.doi.org/10.5772/61081

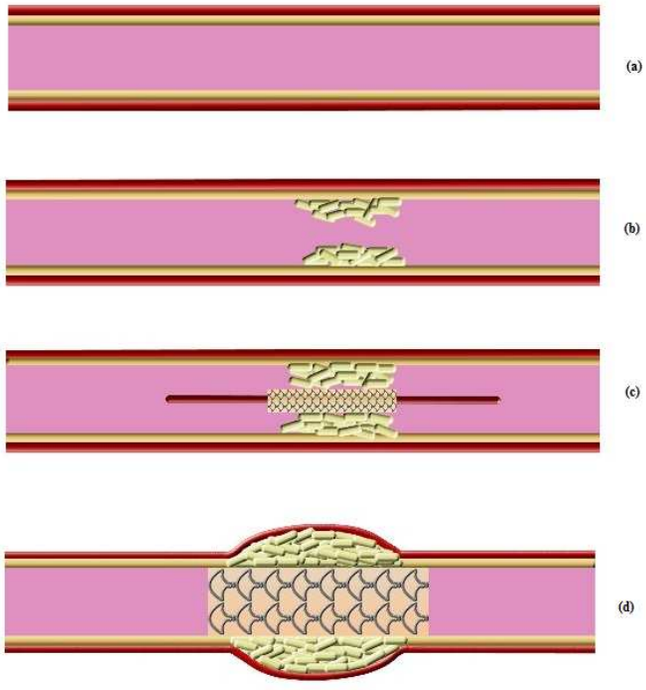

Figure 1. Balloon angioplasty and stent placement: (a) artery, (b) plaque formation, (c) balloon catheter at the blockage site, (d) balloon inflation and stent placement.

1. Elastic recoil, which tends to occur within the first 24 h of the procedure
2. Thrombus formation, which occurs within the first 2 weeks
3. Neointimal hyperplasia involving smooth muscle cell (SMC) activation and synthesis of extracellular matrix, which occurs over the course of the first 3 months [4]

Over 1.5 million percutaneous coronary revascularization procedures are performed annually worldwide, most being intracoronary stenting. Clinically significant restenosis continues to occur in ◎14% of elderly patients within the first year undergoing PTCA. Therefore, there are great efforts for the prevention of restenosis [3]. For the prevention of restenosis, infusing a drug in solution at the site of injured artery is a simple approach and is not successful because of the rapid washout of infused drug from the arterial tissue. Almost 90% of infused drug is lost within 30 min with almost complete loss occurs in less than 24 h [5].

4. Drug-Eluting Stent (DES)

Early difficulties with coronary stents included a risk of early thrombosis (clotting) resulting in occlusion of the stent. Coating stainless steel stents with some other materials such as platinum or gold were evaluated. However, this approach by itself did not eliminate the problem. Then researchers coated stent surface with biocompatible polymers; moreover, the idea of using these polymers as drug reservoir is generated. Scientists developed drug-eluting stents and used the devices themselves as a tool for delivering medication directly to the

arterial wall. The medication is entrapped in polymer layers or loaded into polymeric nanoparticles, and nanoparticles are embedded in polymeric layers. A drug-eluting stent consists of three main parts.

The first part is the metallic scaffold. The metallic scaffold may be constituted by using different types of metallic materials such as stainless steel, nitinol, cobalt, chromium, platinum, gold, magnesium alloy, etc.

The second part is the drug-eluting polymer-coated inner surface of the scaffold. The polymer-coated inner surface of the scaffold is generally used as a drug carrier, which holds and elutes the drug in a controlled manner. The polymers used for DES is generally biodegradable polymers like polylactic acid (PLA), polyglycolic acid (PLGA), and polycaprolactone (PCL). Besides, nonbiodegradable polymers like polybutylmethacrylate (PBMA), polymethylmethacrylate (PMMA), phosphorylcholine, and polyethylene terphthalate (PET) are also evaluated. The third part is the medication that is released from stent directly to arterial wall. Drugs used in DES are immunosuppressive and antiproliferative drugs like sirolimus, everolimus, zotarolimus, paclitaxel, etc., to inhibit neointimal growth, which would cause restenosis [2].

Although the drug-eluting stents significantly reduced the rate of restenosis, it did not completely eliminate restenosis, especially in complex lesions. Additionally, delayed endothelialization after drug-eluting stent implantation is considered to be the cause of late thrombosis. Therefore, scientists have suggested that gene transfer can be an option to address these problems by inhibiting proliferation of vascular smooth muscle cells (VSMCs) and by promoting endothelialization with some genes [6]. Then scientists used stents as a tool to deliver growth factors, plasmids, and antisense oligonucleotides directly to arterial wall. Several studies have been carried out for the delivery and controlled release of genes encoding antiproliferative proteins, miRNAs, peptide structures, and siRNAs to the target tissues through different polymeric materials.

5. Gene therapy for the prevention of restenosis

Gene therapy is the use of genes as a means to achieve high levels of the therapeutic gene product to treat acquired cardiovascular diseases. It can be used as a gene replacement strategy to enhance normal protein function to correct genetic defects. Also, it can be used for local gene transfer to provide a means of delivering a high concentration of therapeutic proteins at the targeted tissue. The vectors used for gene delivery can be classified into two categories, nonviral and recombinant viral vectors. Given the focus of this chapter on gene delivery approaches, we will just briefly discuss the nonviral (polycationic) vector choices. A delivery vehicle of either viral or nonviral origin is essential to carry the foreign gene into a cell. The each of the vector choices has unique advantages and disadvantages.

Viral vectors take advantage of the easy integration of the target gene into the host and long-term expression of gene. Immunogenicity is the major problem of using viral vectors in clinical studies. Attention has turned therefore to nonviral vectors, which possess many advantages

http://dx.doi.org/10.5772/61081

over viruses in terms of safety and ease of use, and many clinical studies have now been performed using nonviral technology [7]. Although nonviral vectors are less efficient at introducing and maintaining foreign gene expression compared to viral vectors, they have the profound advantage of being nonpathogenic and nonimmunogenic [8]. Plasmid DNA is the simplest gene delivery vector. In cell transfection, the minimum amount of negatively charged naked plasmid can go by the cell membrane. Therefore, it is necessary to carry genetic materials to target cell by a vector, which are commonly liposomes or polycationic materials.

In nonviral gene therapy, the negatively charged DNA is conjugated with a positively charged cationic polymer. Nevertheless, the conjugate prepared has to be positively charged. By this way, pDNA is wrapped in a protective envelope to be delivered. Once the conjugate is inside the cell, pDNA expresses the targeted proteins to cure the target disease as seen in Figure 2.

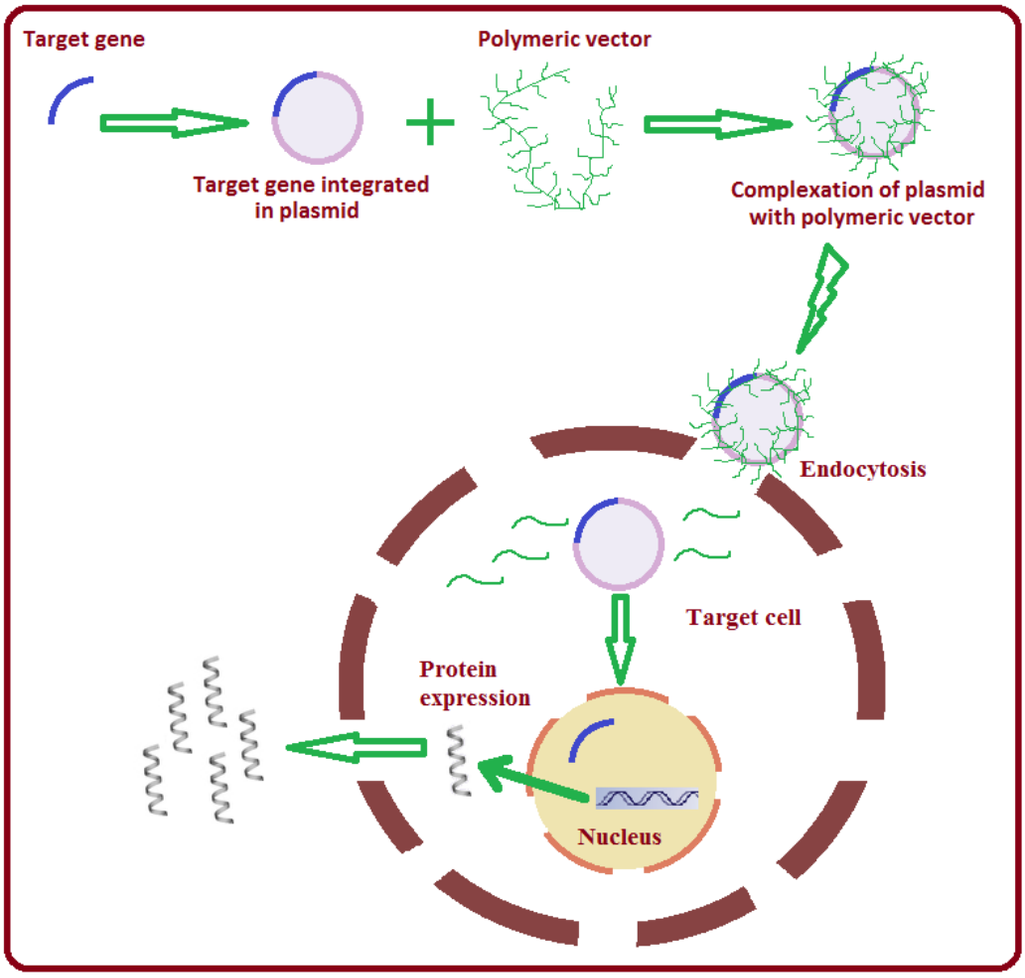

Figure 2. Polycationic gene transfer.

Human gene therapy for the prevention of restenosis is expected to provide important advances in therapeutic restenosis management. If applied in humans, it will be possible to provide long-term beneficial therapeutic effects. However, some key issues, including vector

safety and delivery mechanisms, still have to be resolved before percutaneous gene therapy can be widely applied in clinic. With the aim of inhibition of restenosis, several new types of carriers and technology have been developed, and a great number of gene therapy methods have been studied.

Vascular gene transfer is used to overexpress therapeutically important proteins and correct genetic defects. Promising therapeutic effects have been obtained in animal models of restenosis via transfer of genes, such as encoding vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), nitric-oxide synthase, thymidine kinase, tissue inhibitor of metalloproteinases, etc. [9]. In vascular gene therapy, it is required to combine a therapeutic gene or a therapeutic gene product with an appropriate vector. These complexes are delivered to target cells from a device.

6. Catheter-mediated local gene transfer

Catheter delivery system is one of the devices used in vascular gene therapy. Several balloon catheters (porous and microporous catheters, hydrogel catheters, dispatch catheters, and infiltrator catheters) have been used for gene-based delivery. Rolland et al. [10] have investigated hydrogel-coated catheters for the delivery of interested drug and gene. In recent years, Saurer et al. [11] have designed ultrathin multilayered polyelectrolyte films fabricated on embolectomy catheter balloons by alternately adsorbing layers of a hydrolytically degradable poly (β-amino ester) for the localized delivery of plasmid DNA to vascular tissue. Although catheters seem to be a simple tool for gene delivery, several factors limit its efficiency. Most of the catheters cause localized vascular injury with increased inflammatory response and neointimal proliferation. Additionally, in direct injection method and catheter-based gene delivery, transgene expression is limited within the injection site and homogenous expression is not achieved. In point of fact, in stent-based gene delivery approach, a homogenous transgene expression is achieved in comparison to catheter-based gene delivery methods.

7. Gene eluting stents

Stents represent an attractive alternative for targeted gene delivery, thanks to their permanent scaffolding structure. Polymer-coated stents are used as delivery devices for the elongated time release of small molecules. The greatest challenge with this delivery system lies in achieving a compatible relationship between the stent, coating matrix, and vessel wall. As a result of the long residence times of coatings on the stents, attention has been focused on using them as reservoirs for prolonged local drug administration. While there is much known about stent coatings for drug elution, less is known about the use of these substances for gene elution [12]. The polylactic-polyglycolic acid copolymer (PLGA) is an FDA-approved, biodegradable, and biocompatible polymer and is widely used in various drug release applications, as graft materials in tissue engineering studies.

http://dx.doi.org/10.5772/61081

Although the emergence of drug-eluting stents significantly reduced the rate of restenosis after the interventions, it is not completely eliminated especially in complex lesions. Beside, delayed endothelialization after drug-eluting stent implantation is reported and considered to be the cause of late thrombosis, which is a critical complication. Gene transfer can be an option to address these problems by inhibiting VSMCs proliferation, and with some genes, promoting endothelialization [6].

Klugherz et al. [13] have developed stents coated with polylactic-polyglycolic acid copolymer (PLGA), and they incorporated green fluorescent protein (GFP) plasmid DNA via emulsion coating. They reported the first successful in vivo transfection using a DNA controlled-release stent. GFP-encoding plasmid was efficiently expressed in rat aortic SMCs with 1% percent efficiency. In later years, the same group developed an intravascular stent with a denatured collagen-polylactic-polyglycolic acid for controlled release of GFP-encoding plasmid. Target protein expression was determined with 10.8% efficiency. The level of expression was significantly higher than previous study. They have concluded that denatured collagen incorporated into plasmid DNA-stent coating formulation increased the target protein expression via integrin-related mechanisms and associated changes in the arterial smooth muscle cell actin cytoskeleton [14]. In another study, Takahashi et al. have developed metallic stent-coated polyurethane emulsion containing plasmid DNA. They have evaluated in vivo transgene expression levels, and they have reported that transgene expression has occurred only in vessel segments in contact with the stent. Moreover, analysis of the GFP expression pattern revealed a high frequency of marker protein-positive cells occurring at or near the luminal surface. They have concluded that polymer-coated stents provide a new capability for transgene delivery to immune cells that are believed to contribute to the development of in-stent restenosis [15].

Walter et al. [16] have evaluated the delivery of human vascular endothelial growth factor (hVEGF-2)-encoding plasmid delivery from a gene-eluting stent. They did not use a vehicle to encapsulate the plasmid DNA, encoding for could achieve similar reductions in neointima formation while accelerating, rather than inhibiting, re-endothelialization. They have found that the lumen cross-sectional area (4.2 ± 0.4 versus 2.27 ± 0.3 mm2, $P < 0.001$) was significantly greater and the percentage of cross-sectional narrowing was significantly lower (23.4 ± 6 versus 51.2 ± 10, $P < 0.001$) in VEGF stents compared with control stents implanted in hypercholesterolemic rabbits [16]. In another study, Walsh et al. developed metallic stents coated with a polyurethane emulsion containing plasmid DNA (plasmid-encoded marker genes, b-galactosidase, luciferase, and GFP), which were implanted by Takahashi et al. [15] in rabbit iliac arteries to evaluate transgene delivery. They have observed transgene expressions only in vessel segments in contact with the stent, and they have also emphasized that the extent of transgene expression was dependent upon the quantity of DNA loaded onto the stent [15].

Nitric oxide (NO) is an important regulator of vascular cellular proliferation. NO promotes EC growth and inhibits proliferation of SMCs in the vessel. Additionally, NO reduces platelet adhesion and aggregation. ECs produce NO via nitric oxide synthase (NOS). Accordingly, Bohl Masters et al. [17] have evaluated the effects of localized delivery of NO from hydrogels covalently modified with S-nitrosocysteine (Cys-NO) on neoinitma formation in a rat balloon

injury model. They have reported that localized the delivery of NO from hydrogels inhibited neointima formation by approximately 75% at 14 days. Recently, Sharif et al. [18] have studied therapeutic gene delivery from a stent. They have developed lipoplexes composed of lipofectin and therapeutic eNOS gene. They have coated lipoplexes directly onto the surface of stents and have demonstrated efficient gene delivery for 28 days via liposome-mediated gene delivery.

In another study, Zhu et al. [19] have developed stent-coated dodecylated chitosan-plasmid DNA nanoparticles (DCDNPs) and used them as scaffolds for localized and prolonged delivery of reporter genes into the diseased blood vessel wall. As prepared DCDNPs were spray coated on stents, and a thin layer of dense DCDNPs was successfully distributed onto the metal struts of the endovascular stents. Both in vitro and in vivo expression levels of plasmid DNA-encoding GFP were evaluated. In cell culture, DCDNP stents containing plasmid EGFP-C1 exhibited high level of GFP expression in cells grown on the stent surface and along the adjacent area. In animal studies, reporter gene activity was observed in the region of the artery in contact with the DCDNP stents, but not in adjacent arterial segments or distal organs. Thus, they have concluded that the DCDNP stent provides a very promising strategy for cardiovascular gene therapy [19].

In recent years, Paul et al. [20] have developed a really functional nanobiohybrid hydrogel-based endovascular stent device. The hydrogel was comprised of fibrin matrices, assembled layer by layer on stent surface with alternate layers carrying endosomolytic Tat peptide/DNA nanoparticles or nanoparticles hybridized to polyacrylic acid wrapped single-walled carbon nanotubes. In vitro studies have demonstrated that CNTs incorporated in the hydrogel layers play a major role in tuning the bioactivity of the stent. In addition, the developed stent formulation can significantly reduce the loss of therapeutics while traversing through the vessel and during deployment. In addition to all these, they have demonstrated that the hydrogel-based scaffold carrying therapeutic gene significantly enhances the re-endothelialization of injured artery via in vivo experiments compared to controls. In conclusion, they have declared that this new technology is going to be very useful for controlled delivery of multiple biotherapeutics from stent and other biomedical devices [21].

Since the long-term clinical studies of DES have reported high incidence of late thrombosis, Yang et al. [22] have developed a drug and a gene containing system. They have coated the stent with bilayered PLGA nanoparticles containing VEGF plasmid in the outer layer and paclitaxel in the inner core. They have suggested that while re-endothelialization is going to promote by early release of VEGF gene, slow release of Paclitaxel is going to suppress smooth muscle cell proliferation. They have demonstrated that VEGF/Paclitaxel containing NP-coated stents showed complete re-endothelialization and significantly suppressed in-stent restenosis after 1 month compared to commercial DES [22].

Evidence about restenosis suggests that vascular injury during stent placement and angioplasty procedure activates medial VSMCs, changing them from a quiescent to a proliferative phenotype, and leads them to migrate from the media into the intima. Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases. They digest extracellular matrix components, and they play a major role in the formation of restenosis. It is well known that following

angioplasty, MMPs are secreted increasingly. MMPs are secreted as zymogens. In normal physiological vascular remodeling, the activity of MMPs is tightly regulated at the transcription level, the activation of their pro-form or zymogens, the interaction with specific ECM components, and the inhibition by endogenous inhibitors. Tissue inhibitor of matrix metalloproteinases (TIMPs) are the inhibitors of MMPs. Many MMPs and TIMPs are regulated at the level of transcription by a variety of growth factors, cytokines, and chemokines [23]. The interruption of MMPs activity by tissue inhibitor metalloproteinase infection has been shown to limit SMC proliferation and migration through various models by researchers [24]. Local gene transfer of tissue inhibitor of metalloproteinase-2 (TIMP-2) has been studied on a mouse model. TIMP-2 recombinant adenoviruses overexpressing human TIMP-2 gene have been transferred to SMCs, and the findings demonstrated significant decrease in vein graft diameter [25]. Thus, VSMCs seem to be the most promising cell type to be targeted for inhibition of restenosis. Recently, Laçin et al. [26] have used PEGylated nanoparticles poly(St/PEG-EEM/DMAPM) monosized nanoparticles with significantly high cationic charge for the transfection of TIMP-2-encoding plasmid to SMCs. Increased TIMP-2 protein expression in SMCs according to nontransfected SMCs confirmed the successful delivery and expression of the tissue inhibitor of matrix metalloproteinase-2 (TIMP-2) gene via a nonviral transfection gene therapy approach. This PEG-lated monosized, nontoxic, and highly positively charged nanoparticle poly(St/PEG-EEM/DMAPM) was successfully used in SMCs transfection studies.

Polyethylene glycol (PEG) is the polymer of choice for nonviral vector systems because it possesses several favorable properties such as the lack of immunogenicity, antigenicity, and toxicity and a high solubility in water and in many organic solvents. The cytotoxicity of the polycationic carriers used in gene therapy is an important consideration, especially when polycations with high positive charge were used. Thus, to overcome the cationic polymeric vector-induced toxicity, many researchers encapsulate genetic materials or drugs into a PEG shielded cationic liposomal bilayer [27]. PEG is also approved by the FDA for human use. PEGylation of a drug or a material helps to reduce its excretion by the kidneys and avoids its degradation by proteolytic enzyme. Additionally, PEGylation prevents molecule from reticuloendothelial (RES) clearance by enhancing the water solubility of the molecule and to reduce its immunogenicity and antigenicity [28-30].

Cardiovascular gene therapy is the third most popular application for gene therapy. Although preclinical studies of gene therapy studies for restenosis have shown promising results for the potential application of the gene delivery methods in cardiovascular disease, numerous cardiovascular gene therapy clinical trials have not demonstrated substantially positive results for effective gene transfer. A major disappointing feature of the trials is that while preclinical and uncontrolled phase-I gene therapy trials have been continued in a positive matter, none of the randomized controlled phase-II/III cardiovascular gene therapy trials have shown clinically relevant positive effects [31]. Low gene transfer efficiencies were observed with most of trials. A sophisticated efficient delivery method for cardiovascular applications is still not existing, and only low gene expression levels could be detected in target tissues [31]. Recently, several delivery approaches have been designed for the treatment of restenosis, but a number of challenging obstacles must be solved. For example, for different types of biomolecules

(miRNA, siRNA, plasmid, peptide, etc.), different types of materials and different types of vector systems are used. Therefore, it is important to develop unique gene delivery systems that have enhanced transgene efficacy, are safe, and are clinically reliable.

8. Prevention of in-stent restenosis via other biomolecules and peptides

After coronary artery angioplasty (PCI, heart stent surgery), several biomolecules participate in formation of cellular response. Leucocytes and thrombosites discharge cytokines and growth factors inside the blood vessel, adventitia, and encompassing tissue after blood vessel damage. It is well known that tumor necrosis factor α (TNF-α), platelet-derived growth factor (PDGF), and transforming growth factor β (TGF-β) modulate cellular behaviors. Following the activation and proliferation of smooth muscle cell by fibroblasts, significant cumulation and response of extracellular matrix (ECM) in the vessel wall occur. Due to the responsibilities in cellular interactions, ECM, the active component of the vessel wall, is known as a considerable player in vascular diseases. The ECM consists of a diversity of molecules, including collagen, elastin, glycoproteins, and proteoglycans.

Type III collagen is the most abundant matrix protein in a muscular coronary artery. MMPs move through and interact with the C-terminus of the collagen molecule. Several MMPs attend in the collagen degradation mechanism. Interstitial collagenases (MMP1, MMP8, and MMP13) are the most prevalent MMPs that cleave fibrillar collagens, while gelatinases are active against nonfibrillar collagen components of the ECM.

While some of the cytokines and growth factors such as uPA, MT-MMPs, IL-1, PDGF, and TNF-α arrange MMP activation, TGF-β, heparin, steroids, and tissue inhibitor of metalloproteinases (TIMP 1-4) inhibit MMP activity.

Besides, the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) are also another biomolecule group related with atherosclerosis and possibly restenosis. Protease family ADAMTS enzymes regulate ECM transformation by reducing versican (VCAN—a large extracellular matrix proteoglycan) and procollagen-type matrix components. The degradation of versican by ADAMTS-1 catalyses the migration of SMCs and intimal hyperplasia. Also, ADAMTS-7 enables SMC migration and intimal thickening by degradation of cartilage oligomeric matrix protein (COMP). ADAMTS [2, 3, and 14] involve in the removal of N-terminal peptides from procollagen to form mature collagen. Due to their substrate specificity, ADAMTS enzymes are considered as attractive pharmaceutical targets.

Devices having biomimetic surfaces coated with sequences of extracellular matrix proteins, peptides, and enzymes could accelerate endothelial regeneration and prevent from both the thrombic and proliferative effect after stent implantation. In around 25% of patients, th e development of scar tissue underneath the covering of the course may be thick to the point that it can block the bloodstream and produce a vital blockage. At the point when a stent is set in a vein, new tissue becomes inside the stent, covering the struts of the stent. At first, this new tissue comprises healthy cells to cover the blood vessel endothelium. This is a great impact in

light of the fact that the improvement of typical covering over the stent permits blood to stream easily over the stented territory without coagulating. Later, scar tissue may structure underneath the new healthy coating [32].

Due to the mechanisms of restenosis after angioplasty operation, it is known that TGF-β increases. Yamamoto et al. [33] studied on ribozymes to inhibit TGF-β by cleaving the targeted gene. TGF-β gene demonstrates 100% homology among the human, rodent, and mouse species. They built ribozyme oligonucleotides targeted to the sequence of the TGF-β gene and used it in a rat balloon injury model. Ribozyme inhibits TGF-β mRNA in cultured VSMCs, and using ribozyme oligonucleotides, TGF-β was inhibited, resulting in a significant reduction in neointimal formation in a rat balloon injury model. They also modified ribozyme oligonucleotides containing phosphorothioate DNA and RNA targeted to the TGF-β gene. TGF-β expression was decreased with modified ribozyme oligonucleotides. It was shown that the selective blockade of TGF-β resulted in the inhibition of neointimal formation and reduction in collagen synthesis. It was assumed that the modification of ribozyme oligonucleotide pharmacokinetics would create potential therapeutic strategy for the treatment of cardiovascular disease related to high TGF-β.

Merrilees and colleagues [34] mentioned the importance of viscoelastic properties of vessel wall. They concentrated on arterial matrix proteoglycans, which are related to increasing tissue volume and atherogenicity. One of the basic stimulants of proteoglycans is transforming growth factor β1 (TGF-β1). The aim of the researchers was to investigate the effects of diminishing TGF-β_1 and proteoglycan synthesis in vivo. They used rabbit with balloon catheter damage treated with a TGF-β1 antisense phosphorothioate oligonucleotide connected in a pluronic gel to the adventitia. Statistical information showed that intimal thickening and proteoglycan synthesis were inhibited with the inhibition of TGF-$\beta_1$antisense. These data affirm a part for TGF-β1 in creating neointima and exhibit a particular impact on the combination, appropriation, and gathering of proteoglycan matrix.

There has been extraordinary enthusiasm for the way of stents themselves and the methods used to embed them as boosts for in-stent restenosis. Stent design, arrangement, length, and measures of coronary stream have gotten significant consideration. Additionally, there has been incredible enthusiasm for the stents coated with gradually eluting antirestenotic specialists. The use of drug-eluting stents limits neointima hyperplasia. Pyrrole-imidazole (PI) polyamide targeting TGF-β1 is one of the candidate agents for the drug-eluting stents. In one study, the effects of PI polyamide targeting the TGF-β1 promoter in rat after balloon injury were studied. PI polyamide was designed to connect with the TGF-β1 promoter and carried out for 10 min after inducing balloon injury. Neointimal thickening and re-endothelialization were analyzed [35]. TGF-β1 was significantly decreased with PI polyamide, targeting the expression of TGF-β1 mRNA. Fibronectin and collagen were also affected after targeting. It was understood from the research that synthetic PI polyamide has potential to extinguish neointimal hyperplasia after arterial injury. It was assumed from the article that PI polyamide targeting TGF-β1 could be coated on the stent for the prevention of in-stent restenosis as next-generation drug-eluting stents [35]. Besides, the long-term benefit and safety of coated active stents are crucial research field and should be examined extensively in further studies. The

application of prohealing substances and antirestenosis drugs together as coated on stent represents a diversified approach to reduce restenosis without an increased risk for stent thrombosis [36].

The prevention of new techniques of in-stent restenosis such as peptide-loaded stents to inhibit the biological reactions of the vessel wall gains greater importance in novel studies.

According to the previous studies in the 90s, it was found that fibrin-coated stents lessened thrombogenicity. Baker et al. [37] loaded RGD peptide into fibrin-coated stents due to the inhibition effect of RGD peptide on interaction between fibrinogen with platelets. They have used those stents in an atherosclerotic rabbit model. Four weeks after stent implantation, myointimal hyperplasia in coated and uncoated stent groups were measured and it was seen from the analysis that the extension of myointimal hyperplasia in coated stent group was lower than in the uncoated stent group. Vessel cross-sectional areas of coated stents also were lesser than the uncoated stents. As a result, it was thought that RGD-loaded fibrin-coated stents have prevented vascular complications after stent implantation.

Hong et al. [38] have estimated the advantage and controlling of angiopeptin in a porcine coronary in-stent restenosis model. They have used forty pigs arranged in four groups in the experiments. Out of the control group, the other three groups were treated respectively with one-time treatment (200 μg angiopeptin) at the site of stent placement, continuous angiopeptin over a 1-week period via a subcutaneous osmotic pump (200 μg/kg total dose), and combination of both locally and systematically. In conclusion, this study has demonstrated that the group applied with continuous subcutaneous treatment with angiopeptin after stent implantation significantly has reduced in-stent restenosis by inhibiting neointimal hyperplasia.

In 1999, a synthetic octapeptide, angiopeptin, was used to inhibit tissue response against growth factor, insulin-like growth factor, and interleukin-1-mediated endothelial cell adhesion.

Wiktor brand stents were coated with polyorganophosphazene. Researchers loaded angiopeptin into that biodegradable polymer and implanted the stent in porcine coronary arteries. The group has indicated that angiopeptin increased lumen diameter and morphometric lumen area in significantly as a percentage [39].

We can observe several studies in stent implantation area about local biomolecule delivery made since 1999. Coating of stent is necessary for carrying, prolongation, and elution of the drug through the targeted area effectively and without any loss arising from catheter. Studies on physical strengths of polymers coated on stents and eliciting inflammatory reactions occurring after operation are still ongoing. A portion of the presently accessible gadgets, coatings, and stents are drawing near to making this point an achievable reality. Stent thrombosis remains an important problem after the implantation of different stent types. Coating of stents impacts thrombogenicity. Simple chemical coating lessens platelet adhesion, fibrinogen binding, and effectual against in-stent restenosis in clinical trials. Fuchs et al. [40] were also interested in solving this problem about thrombosis with vasoactive agents. They studied on in vitro and in vivo effects of C-type natriuretic peptide (CNP) that has dual effects on different cell types in a porcine restenotic model. Although gene transfer of CNP in cultures

http://dx.doi.org/10.5772/61081

of porcine vascular cells had achieved 30% reduction of growth of SMCs, the suppression of endothelial growth using CNP had failed. Usage of the CNP gene could be a solution for compress formation of restenosis while preventing late thrombosis [40].

Recent evidence point out endocrine activities are mediated by growth hormone. Shu and colleagues made studies on Ghrelin, a 28-amino acid peptide, which had been isolated from both human and rat stomach that was mediated by growth hormone secretagogue receptor. Ghrelin is expressed in stomach tissue and has several important physiological effects in secretion of growth hormone, inflammation, cell proliferation, differentiation, and apoptosis. Besides, it has wide role on cardiovascular system, such as increasing myocardial contractility, improving cardiac function, inhibiting ventricular remodeling, and attenuating cardiac ischemia-reperfusion injury. Novel studies indicated inhibition of ghrelin on vascular inflammation and proliferation of VSMCs. It also repairs endothelial cells, promotes vascular endothelial function, inhibits platelet aggregation, and exerts antithrombotic effects. Volante et al. [41] had found its protective effect on vascular endothelial function by increasing endothelial nitric oxide synthase (eNOS) expression and improving endothelial function.

Another research group has also stated that ghrelin has prevention against platelet aggregation, MCP-1 expression, and exerts antithrombotic effects. Consequently, ghrelin is considered as therapeutic candidate for the prevention and treatment of ISR [42].

In the 2000s, subjects on expanded polytetrafluoroethylene-covered stent-graft have been carried out. Hamm et al. [43] have used 15-amino acid peptide (P-15), which had cell adhesion property in supporting the endothelization on inner surface after implantation. The recovery of a utilitarian endothelium over the surfaces of the embedded gadgets may restrain both the thrombotic and proliferative reaction after gadget implantation. It was discovered from studies that matrix proteins such as collagen and laminin could improve and increase endothelial regeneration. Starting from this idea, P-15 synthetic peptide, which had cell binding cell of collagen [44], has been tried in in vitro studies with endothelial cells [45]. It was shown that cell migration and adhesion had increased on P-15-coated surface. According to those experiments, P-15 peptide-coated stents had been used in clinical applications. P-15 peptide-coated stents had demonstrated that similar healing with uncoated stents had provided high luminal support and protected from distal emboli. Based on the results from this preparatory, it was figured out that a peptide-treated stent is an alluring methodology for the treatment of stenosed saphenous vein grafts [43].

A different approach with angiotensin-[1-7], an endogenous, biologically active peptide, has come from Langeveld et al. [46]. Angiotensin-[1-7] is a part of the renin-angiotensin system, which has vasodilatory, antithrombotic, and antiproliferative properties. The effects of angiotensin-[1-7] infusion on neointimal formation after stent placement in male Wistar rats have been investigated in this study. Other than the control group, angiotensin-[1-7] [24 g/kg per hour) had been given to rats that underwent stent implantation in the abdominal aorta or sham surgery by placing an osmotic minipump. The endothelial function has been measured in isolated thoracic aortic rings after 4 weeks by histomorphometric and histological analyses. Researchers have found out that angiotensin-[1-7]-treated group has exhibited a significant decrease in neointimal thickness, neointimal area, and percentage stenosis compared with the

control group [46]. Results have showed that angiotensin-[1-7] treatment has reduced neointimal formation after stent implantation in rats. This consequence has supported the idea of Ang-[1-7] could be an alternative to the presently available aggressive antiproliferative drug-loaded stents [46].

Yu et al. [47] were interested in calcineurin/nuclear factor of activated T cells (NFAT) axis. It plays an important role in VSMCs that inhibits NFAT. In earlier studies, the main epitope site on NFAT for calcineurin was discovered. The optimization of this site had induced to the exploration of synthetic peptide VIVIT. Yu et al. [47] have used VIVIT to examine the inhibition NFAT activation and NFAT-mediated proliferation and inflammation in RAW 264.7 macrophages, Ea.Hy.926 endothelial cells and VSMCs, and blocked ionomycin-elicited nuclear import of NFAT. It was also found that VIVIT suppressed platelet-derived growth factor-BB (PDGF-BB) and thrombin induced VSMC proliferation. According to the data, it was reported that NFAT is a regulator of PDGF-BB induced vSMC proliferation. This study stents coated with VIVIT could be a candidate to more specific approaches in the antirestenosis therapy.

In parallel with the ongoing experiments, integrin-binding cyclic Arg-Gly-Asp peptide (cRGD)-loaded stents were used to bound coronary neointima formation and to increase endothelialization by attracting endothelial progenitor cells. It has been stated again that stent coating with cRGD may be useful for reducing in-stent restenosis by accelerating endothelialization [48]. Another study was about RGD-modified liposomes targeted to integrin GPIIbeIIIa on activated platelets [49]. RGD-conjugated liposomes have also been tested in vivo in a rat carotid injury model. As seen from the experiments, cyclic RGD liposomes have binded activated platelets significantly higher compared to linear RGD liposomes. Huang et al. [49] have found an approach on optimization of platelet-targeting ability of ligand-modified liposomes. It has been thought to be a solution for sensitive and selective delivery of therapeutic agents in cardiovascular diseases such as atherosclerosis, thrombosis, and restenosis where activated platelets play significant role in disease development, progression, and outcome.

In-stent restenosis is a pathobiologic methodology, histologically different from restenosis after balloon angioplasty and embodied generally of neointima arrangement. Since percutaneous coronary mediation progressively includes the utilization of stents, in-stent restenosis is moreover getting to be correspondingly more regular. Novel applicable and therapeutic approaches in humans for re-endothelialization are about coating of stents with some substances to give acceleration for the formation of endothelial coverage safely. It was indicated in a porcine model study that cRGD-coated stents expedite endothelialization [50].

In a novel study, it has been focused on the binding ratio of integrin receptor to subendothelial matrix proteins. When integrin binds to arginine-glycine-aspartic acid (RGD) peptide, it imitates naturally occurring adherent interactions. The surface modification of stents with RGD peptide also contributes selectivity for integrin alpha V beta 3, which stimulates endothelialization after stent implantation. Joner and colleagues [51] studied on the availability of RGD peptide-loaded titanium-oxide nitinol stents. Functionality of the engrafted RGD peptide has been examined by in vitro endothelial bioassays, and a subsequent 7-day in vivo endothelialization has been studied by using cRGD-coated self-expanding nitinol stents in rabbits.

Significant increase in endothelial coverage with cRGD stent implants has been stated. This study has represented as an innovative strategy to improve endothelialization and to catalyze vascular healing after stent implantation [51].

Besides, Kramer et al. [52] insisted on interventional cardiology was revolutionized by stent implantation. Stents were developed with antiplatelet therapy and new materials. They have defended the importance of oral drug usage with newly developed stents together. Angiotensin II (Ang II) is an important vasoactive peptide associated with in-stent restenosis, which is produced locally from vessel wall. Due to the Ang II AT_1 receptors' effects on relationship of Ang II with growth and inflammatory signals, A T1-receptor blocking drugs are widely used to treat hypertension and heart failure [53]. Experimental clinical trials has estimated the effect of AT_1-receptor blockers on ISR but no significant result was obtained from patients who were treated with drugs [52].

Different treatment about the movement, growth, and adhesion of endothelial cells has been tried to improve the re-endothelization of stents. Yin and colleagues [54] had synthesized muscle adhesive polypeptide mimics including dihydroxyphenylalanine and l-lysine (MAPDL). They had attached MAPDL on ethylene vinyl acetate (EVA)-coated stent with different molecular weight PEG spacers to find out optimum cell bioactivity. According to in vitro analysis, endothelial cells layer formation had significantly increased on the MAPDL-EVA-coated stents in contrast with the control bare stent. In this manner, it was demonstrated that MAPDL-coated EVA surface had decrease platelet adhesion and appeared to be promising solution for re-endothelialization of intravascular stent devices.

As is seen from the experiments, metal-based stents are mostly preferred for coronary artery disease. The recovery of endothelium around the lesion site can be achieved by coating stents with bioactive molecules. Due to restrictions in availability of proper bioactive signals that would selectively stimulate growth of endothelium and immobilization of such signaling molecules on the metal surface, Ceylan et al. had developed self-assembly, pH-dependent, Dopa-conjugated peptide amphiphile and REDV-conjugated peptide amphiphile nanofibers. Those nanofibers had mimicking property of native endothelium extracellular matrix and had been easily immobilized on stainless steel surface. In vitro experiments had showed that peptide nanofiber-coated stainless steel surface had increased adhesion of vascular endothelial cells as against uncoated surface. Besides, it had decreased viability, proliferation of vascular endothelial cells, and platelet attachment to the peptide. It was suggested in this study that this bioactive stent design has provided a futuristic approach for clinical use in prolonged cardiovascular treatments [55].

9. The role of small RNAs in-stent restenosis

Recent progress in molecular biology has resulted in development of numerous effective gene therapy methods via transferring RNA molecules for the treatment of variety diseases. There are also many studies with the purpose of prevention and treatment of vascular neointima proliferation after balloon angioplasty and stent implantation, using RNA molecules [56-58].

Recent studies suggest that microribonucleic acid-based (miRNA) are important gene regulators and seems to be suitable for the treatment of various cardiovascular diseases [56, 59]. Delivery and controlled release of miRNA through different polymeric materials to target tissues is one of the nucleic acid-based therapy approaches.

Discovered little more than decade ago, non-protein-coding RNAs are single-stranded endogenous RNAs, approximately 25 nucleotides long and they are called as miRNAs [60, 61]. They regulate gene expression negatively at the posttranscriptional level by binding to specific mRNA target, leading either to degradation or to translational target protein repression, rarely they can promote gene expression [56, 59, 62-64]. Small interfering RNAs (siRNA) are short, double-stranded RNAs (20-25 nucleotides) that induce the degradation of target mRNA and inhibits the production of the target protein, and the procedure is called RNA silencing. Unfortunately, clinical applications of RNA interference-based therapeutics such as siRNAs and miRNAs have been limited mainly due to low intracellular delivery efficiency in vitro and in vivo. However, RNA molecules promising therapeutic potential, safe, and efficient delivery methods have to be developed for targeted controlled release. Over the last decade, there has been great effort to develop effective nonviral delivery systems for the transfection of siRNA and miRNA [60]. As it is well known, RNAs are short double-stranded molecules. Due to this reason, they have more rigid structures and inappropriate distribution, making them difficult to form stable and compact particles using a wide range of cationic condensing reagents, such as polylipids, polypeptides, and polyamines, via simple electrostatic interactions. Thus, to achieve maximum target gene silencing, improved gene carrier systems have to be prepared. Therefore, attention has become focused on development of nonviral gene delivery vectors to carry small RNA molecules to target cells [65].

Several experimental and clinical data showed that miRNAs are associated with restenosis or renarrowing of the arteries which primarily results from the proliferation and migration of VSMCs into the intima after stent implantation [66, 67]. Recent evidence by several groups has decelerated that miRNAs have an important role in prevention of atherosclerosis and restenosis [62, 63, 67-71]. In fact, knockdown of miR-21, miR-221, and miR-222 and overexpression of miR145 were found to be intimately relevant to neointimal formation after vessel injury [57, 58, 62, 71-73]. miR-21 is encoded by a single gene and autonomously transcribed from a conserved promotor that is located within the intron of the overlapping protein coding gene [73]. The oncogenic activity of miR-21 has been identified by several groups [74-76]. Besides, it has been found to play important role in proliferation of VSMCs, cardiac cell growth, and death and cardiac fibroblast functions [73]. Indeed, both basic and clinical studies have demonstrated that the overexpression of miR-21 in human reduces cardiac fibrosis and prevents vascular neointima proliferation after balloon angioplasty and stent implantation [72, 77].

Similarly, Liu et al. reported that both of miR-221 and miR-222 were recognized in rat carotid arteries after angioplasty, in which their expression was upregulated and localization in VSMCs at the injured regions of vascular walls [78]. Moreover, it was shown that the overexpression of miR-221 and miR-222 decreased VSMC proliferation in vitro. Also, the knockdown of miR-221 and miR-222 in rat carotid arteries suppressed VSMC proliferation in vivo and

http://dx.doi.org/10.5772/61081

neointimal lesion formation after angioplasty [78]. However, among miRNAs, miR-145 is the most abundant type in vascular walls [58]. In addition to these, especially both miR-143 and miR-145 are significantly expressed in vascular endothelial cells (VECs), which is able of controlling vascular neointimal lesion formation [57].

siRNAs mediate specific gene silencing through a highly regulated enzyme-mediated process. Nowadays, siRNAs are established as the most important biological strategy for gene silencing that includes the degradation of target mRNA and block production of the related protein [79, 80]. Yanming et al. [81] have found that siRNAs reduce neointima formation significantly as reflected by a decreased intima/media area ratio in carotid artery sections after surgical mechanical injury of the rat carotid artery. Wang et al. [59] reported that c-myc siRNA, when given immediately after the surgery, is an effective approach for the prevention of vein graft restenosis.

Usage of nanoparticle eluting stent technologies is an important approach. Walter and colleagues [16] have developed nanoparticles containing plasmid DNA-encoding sequence hVEGF-2 and explored the ability of delivery of target sequence by NP through the stent. An alternative and novel treatment strategy, acceleration of re-endothelialization via VEGF-2 gene-eluting stents, is achieved through endothelial cell proliferation by serving to activate endothelial cell proliferation pathways [16, 82].

Strategies for enhancing gene delivery and gene transfer through stents typically involve the complexations of siRNA/miRNA molecule with cationic polymers, which can be loaded on the stent surface [82, 83].

Although there are several in vitro gene therapy studies for the prevention of restenosis, a few studies with the use of miRNA/siRNA-based therapy for the treatment of cardiovascular diseases have been carried out in humans. Although in vivo studies of miRNA-based agents, conjugated to biodegradable polymers or encapsulated in nanoparticles, were promising, to date there have been a few studies consisting of miRNA-vehicle complexes to a polymer-coated stent that allow delivery of the miRNA for achieve endothelial cell proliferation by serving to activate endothelial cell proliferation pathways [62, 82].

Patil and Panyam [84] have developed nanoparticles using the biodegradable polymer, poly(d,l-lactide-co-glycolide) (PLGA), for siRNA delivery. Additionally, they have incorporated in the PLGA matrix, a cationic polymer, polyethylenimine (PEI), to improve siRNA encapsulation in PLGA nanoparticles. The effectiveness of siRNA-loaded PLGA-PEI nanoparticles was investigated in vitro. They have reported that PEI in PLGA nanoparticle matrix has increased siRNA encapsulation by about 2-fold and also improved the siRNA release profile. Moreover, they have observed higher cellular uptake and cytosolic delivery with the encapsulated siRNA.

In order to avoid such blockages, at the site of angioplasty or stent placement, the suppression of SMCs near the implanted stent, etc., has developed a new delivery technique for Akt1 (Akt1 is a protein that plays a key role in cellular proliferation) siRNA nanoparticles to release from a hyaluronic acid (HA)-coated stent surface. For this purpose, they have used disulfide cross-linked low molecular polyethyleneimine (PEI) (ssPEI) as a gene delivery carrier. Disulfide

bonds are stable in an oxidative extracellular environment but degrade rapidly in reductive intracellular environments. They have immobilized Akt1 siRNA/ssPEI nanoparticles (ASNs) on the HA-coated stent surface. They have reported that the Akt1 released from the stent suppressed the growth of the smooth muscle at the peri-stent implantation area in the balloon-injured external iliac artery in rabbits [85].

Encouragingly, the current developments in the understanding of RNAs have reveal both miRNAs and siRNAs as a potential targets for the development of new diagnostic and therapeutic strategies for the prevention of restenosis [56, 62, 63]. Therefore, attention has become focused on the development of chemically modified RNAs to cure or prevent in-stent restenosis.

10. Conclusion

As conclusion, platelets are the main reason for the formation of thrombus. After stent implantation, platelets are activated and stimulate SMCs migration. In-stent restenosis occurs by the proliferation of SMCs to the injury site. In future studies, the blood flow can be improved, and no platelets are aggregated by coating and biomolecule loading instead of loading to the stent surface.

Author details

Nelisa Türkoğlu Laçin[1*], Kadriye Kızılbey[1] and Banu Mansuroğlu[2]

*Address all correspondence to: melisalacin@yahoo.com

1 Science and Technology Application and Research Center, Yıldız Technical University, Istanbul, Turkey

2 Department of Molecular Biology and Genetics, Yıldız Technical University, Istanbul, Turkey

References

[1] Axel DI, Kunert W, Göggelmann C, Oberhoff M, Herdeg C, Küttner A, et al. Paclitaxel inhibits arterial smooth muscle cell proliferation and migration in vitro and in vivo using local drug delivery. Circulation. 1997;96(2):636-45.

[2] Bharti ML. Recent advancement in development of drug eluting stents delivery system. International Journal of Therapeutic Applications, 2012; 4: 1-9.

[3] Bennett MR. In-stent stenosis: pathology and implications for the development of drug eluting stents. Heart. 2003;89(2):218-24.

[4] Pietersma A, Kofflard M, de Wit LEA, Stijnen T, Koster JF, Serruys PW, et al. Late lumen loss after coronary angioplasty is associated with the activation status of circulating phagocytes before treatment. Circulation. 1995;91(5):1320-5.

[5] Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Advanced drug delivery reviews. 2003;55(3):329-47.

[6] Ishikawa K, Aguero J, Naim C, Fish K, Hajjar RJ. Percutaneous approaches for efficient cardiac gene delivery. Journal of Cardiovascular Translational Research. 2013;6(4):649-59.

[7] Williams PD, Kingston PA. Plasmid-mediated gene therapy for cardiovascular disease. Cardiovascular Research. 2011;91(4):565-76.

[8] Thomas M, Klibanov A. Non-viral gene therapy: polycation-mediated DNA delivery. Applied Microbiology and Biotechnology. 2003;62(1):27-34.

[9] Ylä-Herttuala S, Martin JF. Cardiovascular gene therapy. Lancet. 2000;355(9199): 213-22.

[10] Rolland PH, Mekkaoui C, Palassi M, Friggi A, Moulin G, Piquet P, et al. Efficacy of local molsidomine delivery from a hydrogel-coated angioplasty balloon catheter in the atherosclerotic porcine model. Cardiovascular and Interventional Radiology. 2003;26(1):65-72.

[11] Saurer EM, Yamanouchi D, Liu B, Lynn DM. Delivery of plasmid DNA to vascular tissue< i> in vivo</i> using catheter balloons coated with polyelectrolyte multilayers. Biomaterials. 2011;32(2):610-8.

[12] Sharif F, Daly K, Crowley J, O'Brien T. Current status of catheter-and stent-based gene therapy. Cardiovascular research. 2004;64(2):208-16.

[13] Klugherz BD, Jones PL, Cui X, Chen W, Meneveau NF, DeFelice S, et al. Gene delivery from a DNA controlled-release stent in porcine coronary arteries. Nature biotechnology. 2000;18(11):1181-4.

[14] Perlstein I, Connolly J, Cui X, Song C, Li Q, Jones P, et al. DNA delivery from an intravascular stent with a denatured collagen-polylactic-polyglycolic acid-controlled release coating: mechanisms of enhanced transfection. Gene Therapy. 2003;10(17): 1420-8.

[15] Takahashi A, Palmer-Opolski M, Smith RC, Walsh K. Transgene delivery of plasmid DNA to smooth muscle cells and macrophages from a biostable polymer-coated stent. Gene Therapy. 2003;10(17):1471-8.

[16] Walter DH, Cejna M, Diaz-Sandoval L, Willis S, Kirkwood L, Stratford PW, et al. Local gene transfer of phVEGF-2 plasmid by gene-eluting stents an alternative strategy for inhibition of restenosis. Circulation. 2004;110(1):36-45.

[17] Bohl Masters KS, Lipke EA, Rice EE, Liel MS, Myler HA, Zygourakis C, et al. Nitric oxide-generating hydrogels inhibit neointima formation. Journal of Biomaterials Science, Polymer Edition. 2005;16(5):659-72.

[18] Sharif F, Hynes SO, McCullagh K, Ganley S, Greiser U, McHugh P, et al. Gene-eluting stents: non-viral, liposome-based gene delivery of eNOS to the blood vessel wall in vivo results in enhanced endothelialization but does not reduce restenosis in a hypercholesterolemic model. Gene Therapy. 2011;19(3):321-8.

[19] Zhu D, Jin X, Leng X, Wang H, Bao J, Liu W, et al. Local gene delivery via endovascular stents coated with dodecylated chitosan-plasmid DNA nanoparticles. International Journal of Nanomedicine. 2010;5:1095.

[20] Paul A, Shao W, Shum-Tim D, Prakash S. The attenuation of restenosis following arterial gene transfer using carbon nanotube coated stent incorporating TAT/DNA Ang1+ Vegf nanoparticles. Biomaterials. 2012;33(30):7655-64.

[21] Paul A, Shao W, Shum-Tim D, Prakash S. The attenuation of restenosis following arterial gene transfer using carbon nanotube coated stent incorporating TAT/DNA< sub> Ang1+ Vegf</sub> nanoparticles. Biomaterials. 2012;33(30):7655-64.

[22] Yang J, Zeng Y, Zhang C, Chen Y-X, Yang Z, Li Y, et al. The prevention of restenosis in vivo with a VEGF gene and paclitaxel co-eluting stent. Biomaterials. 2013;34(6): 1635-43.

[23] Yan C, Boyd DD. Regulation of matrix metalloproteinase gene expression. Journal of Cellular Physiology. 2007;211(1):19-26.

[24] George SJ, Lloyd CT, Angelini GD, Newby AC, Baker AH. Inhibition of late vein graft neointima formation in human and porcine models by adenovirus-mediated overexpression of tissue inhibitor of metalloproteinase-3. Circulation. 2000;101(3): 296-304.

[25] Hu Y, Baker AH, Zou Y, Newby AC, Xu Q. Local gene transfer of tissue inhibitor of metalloproteinase-2 influences vein graft remodeling in a mouse model. Arteriosclerosis, Thrombosis, and Vascular Biology. 2001;21(8):1275-80.

[26] Laçin N, Utkan G, Kutsal T, Dedeoğlu BG, Yuluğ IG, Pişkin E. TIMP-2 gene transfer by positively charged PEG-lated monosized polycationic carrier to smooth muscle cells. Journal of Nanoparticle Research. 2012;14(2):1-9.

[27] Güven GU, Laçin NT, Pişkin E. Monosize polycationic nanoparticles as non-viral vectors for gene transfer to HeLa cells. Journal of Tissue Engineering and Regenerative Medicine. 2008;2(2-3):155-63.

[28] Fant K, Esbjörner EK, Jenkins A, Grossel MC, Lincoln P, Nordén B. Effects of PEGylation and acetylation of PAMAM dendrimers on DNA binding, cytotoxicity and in vitro transfection efficiency. Molecular Pharmaceutics. 2010;7(5):1734-46.

[29] Beyerle A, Merkel O, Stoeger T, Kissel T. PEGylation affects cytotoxicity and cell-compatibility of poly (ethylene imine) for lung application: structure-function relationships. Toxicology and Applied Pharmacology. 2010;242(2):146-54.

[30] Alconcel SN, Baas AS, Maynard HD. FDA-approved poly (ethylene glycol)-protein conjugate drugs. Polymer Chemistry. 2011;2(7):1442-8.

[31] Abdel-Latif A, Bolli R, Tleyjeh IM, Montori VM, Perin EC, Hornung CA, et al. Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. Archives of Internal Medicine. 2007;167(10):989-97.

[32] Dangas G, Kuepper F. Restenosis: repeat narrowing of a coronary artery prevention and treatment. Circulation. 2002;105(22):2586-7.

[33] Yamamoto K, Morishita R, Tomita N, Shimozato T, Nakagami H, Kikuchi A, et al. Ribozyme oligonucleotides against transforming growth factor-beta inhibited neointimal formation after vascular injury in rat model—potential application of ribozyme strategy to treat cardiovascular disease. Circulation. 2000;102(11):1308-14.

[34] Merrilees M, Beaumont B, Scott L, Hermanutz V, Fennessy P. Effect of TGF-β1 antisense S-oligonucleotide on synthesis and accumulation of matrix proteoglycans in balloon catheter-injured neointima of rabbit carotid arteries. Journal of Vascular Research. 2000;37(1):50-60.

[35] Yao EH, Fukuda N, Ueno T, Matsuda H, Nagase H, Matsumoto Y, et al. A pyrrole-imidazole polyamide targeting transforming growth factor-beta1 inhibits restenosis and preserves endothelialization in the injured artery. Cardiovascular Research. 2009;81(4):797-804.

[36] Luscher TF, Steffel J, Eberli FR, Joner M, Nakazawa G, Tanner FC, et al. Drug-eluting stent and coronary thrombosis - Biological mechanisms and clinical implications. Circulation. 2007;115(8):1051-8.

[37] Baker JE, Nikolaychik V, Zulich A, Komorowski R, Kipshidze N. Fibrin coated stents as a depot to deliver RGD peptide inhibit vascular reaction in atherosclerotic rabbit model. Journal of the American College of Cardiology. 1996;27(2):197.

[38] Hong MK, Kent KM, Mehran R, Mintz GS, Tio FO, Foegh M, et al. Continuous subcutaneous angiopeptin treatment significantly reduces neointimal hyperplasia in a porcine coronary in-stent restenosis model. Circulation. 1997;95(2):449-54.

[39] Gunn J, Cumberland D. Stent coatings and local drug delivery. State of the art. European Heart Journal. 1999;20(23):1693-700.

[40] Fuchs AT, Kuehnl A, Pelisek J, Rolland PH, Mekkaoui C, Netz H, et al. Inhibition of restenosis formation without compromising reendothelialization as a potential solution to thrombosis following angioplasty? Endothelium. 2008;15(1-2):85-92.

[41] Volante M, Allia E, Gugliotta P, Funaro A, Broglio F, Deghenghi R, et al. Expression of ghrelin and of the GH secretagogue receptor by pancreatic islet cells and related endocrine tumors. Journal of Clinical Endocrinology and Metabolism. 2002;87(3): 1300-8.

[42] Shu ZW, Yu M, Chen XJ, Tan XR. Ghrelin could be a candidate for the prevention of in-stent restenosis. Cardiovascular Drugs and Therapy. 2013;27(4):309-14.

[43] Hamm CW, Schaachinger V, Munzel T, Maikowski C, Bonzel T, Koster R, et al. Peptide-treated stent graft for the treatment of saphenous vein graft lesions: first clinical results. Journal of Invasive Cardiology. 2003;15(10):557-60.

[44] Bhatnagar RS, Qian JJ, Wedrychowska A, Sadeghi M, Wu YM, Smith N. Design of biomimetic habitats for tissue engineering with P-15, a synthetic peptide analogue of collagen. Tissue Engineering. 1999;5(1):53-65.

[45] Jamshidi P, Mahmoody K, Erne P. Covered stents: a review. International Journal of Cardiology. 2008;130(3):310-8.

[46] Langeveld B, van Gilst WH, Tio RA, Zijlstra F, Roks AJ. Angiotensin-(1-7) attenuates neointimal formation after stent implantation in the rat. Hypertension. 2005;45(1): 138-41.

[47] Yu H, Sliedregt-Bol K, Overkleeft H, van der Marel GA, van Berkel TJ, Biessen EA. Therapeutic potential of a synthetic peptide inhibitor of nuclear factor of activated T cells as antirestenotic agent. Arteriosclerosis, Thrombosis, and Vascular Biology. 2006;26(7):1531-7.

[48] Blindt R, Vogt F, Astafieva I, Fach C, Hristov M, Krott N, et al. A novel drug-eluting stent coated with an integrin-binding cyclic Arg-Gly-Asp peptide inhibits neointimal hyperplasia by recruiting endothelial progenitor cells. Journal of the American College of Cardiology. 2006;47(9):1786-95.

[49] Huang G, Zhou Z, Srinivasan R, Penn MS, Kottke-Marchant K, Marchant RE, et al. Affinity manipulation of surface-conjugated RGD peptide to modulate binding of liposomes to activated platelets. Biomaterials. 2008;29(11):1676-85.

[50] Blindt R, Vogt F, Astafieva I, Fach C, Hristov M, Krott N, et al. A novel drug-eluting stent coated with an integrin-binding cyclic Arg-Gly-Asp peptide inhibits neointimal hyperplasia by recruiting endothelial progenitor cells. Journal of the American College of Cardiology. 2006;47(9):1786-95.

[51] Joner M, Cheng Q, Schonhofer-Merl S, Lopez M, Neubauer S, Mas-Moruno C, et al. Polymer-free immobilization of a cyclic RGD peptide on a nitinol stent promotes in-

tegrin-dependent endothelial coverage of strut surfaces. Journal of Biomedical Materials Research, Part B. Applied Biomaterials. 2012;100(3):637-45.

[52] Kramer J, Ruf RG, Schmidt S, Axthelm C, Strasser R, Janssen G, et al. The CAIRP (CAndesartan for In-stent Restenosis Prevention) trial—a multicenter study of AT1-receptor blocker therapy in coronary stenting. Journal of Invasive Cardiology. 2008;20(5):205-10.

[53] Stoll M, Steckelings UM, Paul M, Bottari SP, Metzger R, Unger T. The angiotensin AT2-receptor mediates inhibition of cell proliferation in coronary endothelial cells. Journal of Clinical Investigation. 1995;95(2):651-7.

[54] Yin M, Yuan Y, Liu CS, Wang J. Development of mussel adhesive polypeptide mimics coating for in-situ inducing re-endothelialization of intravascular stent devices. Biomaterials. 2009;30(14):2764-73.

[55] Ceylan H, Tekinay AB, Guler MO. Selective adhesion and growth of vascular endothelial cells on bioactive peptide nanofiber functionalized stainless steel surface. Biomaterials. 2011;32(34):8797-805.

[56] Miyake T, Nakagami H, Morishita R. Nucleic acid drugs for preventing restenosis after coronary revascularization. Current Topics in Medicinal Chemistry. 2012;12(15): 1613-20.

[57] Cordes KR, Sheehy NT, White MP, Berry EC, Morton SU, Muth AN, et al. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. Nature. 2009;460(7256): 705-10.

[58] Cheng Y, Liu X, Yang J, Lin Y, Xu D-Z, Lu Q, et al. MicroRNA-145, a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation. Circulation Research. 2009;105(2):158-66.

[59] Wang J, Liu K, Shen L, Wu H, Jing H. Small interfering RNA to c-myc inhibits vein graft restenosis in a rat vein graft model. Journal of Surgical Research. 2011;169(1):e85-e91.

[60] Zhang Y, Wang Z, Gemeinhart RA. Progress in microRNA delivery. Journal of Controlled Release. 2013;172(3):962-74.

[61] Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene< i> lin-4</i> encodes small RNAs with antisense complementarity to< i> lin-14</i>. Cell. 1993;75(5): 843-54.

[62] O'Sullivan JF, Martin K, Caplice NM. Microribonucleic acids for prevention of plaque rupture and in-stent restenosis: "a finger in the dam." Journal of the American College of Cardiology. 2011;57(4):383-9.

[63] Menghini R, Stöhr R, Federici M. MicroRNAs in vascular aging and atherosclerosis. Ageing Research Reviews. 2014.

[64] Yang J, Jiang H, Chen S-S, Chen J, Li W-Q, Xu S-K, et al. Lentivirus-mediated RNAi targeting CREB binding protein attenuates neointimal formation and promotes re-endothelialization in balloon injured rat carotid artery. Cellular Physiology and Biochemistry. 2010;26(3):441-8.

[65] Park H-J, Yang F, Cho S-W. Nonviral delivery of genetic medicine for therapeutic angiogenesis. Advanced Drug Delivery Reviews. 2012;64(1):40-52.

[66] Chaabane C, Otsuka F, Virmani R, Bochaton-Piallat M-L. Biological responses in stented arteries. Cardiovascular Research. 2013:cvt115.

[67] Santulli G, Wronska A, Uryu K, Diacovo TG, Gao M, Marx SO, et al. A selective microRNA-based strategy inhibits restenosis while preserving endothelial function. Journal of Clinical Investigation. 2014;124(9):4102.

[68] Kim D, Lee D, Jang YL, Chae SY, Choi D, Jeong JH, et al. Facial amphipathic deoxycholic acid-modified polyethyleneimine for efficient MMP-2 siRNA delivery in vascular smooth muscle cells. European Journal of Pharmaceutics and Biopharmaceutics. 2012;81(1):14-23.

[69] Zhao Y, Srivastava D. A developmental view of microRNA function. Trends in biochemical sciences. 2007;32(4):189-97.

[70] Van Rooij E, Olson EN. MicroRNAs: powerful new regulators of heart disease and provocative therapeutic targets. Journal of Clinical Investigation. 2007;117(117 (9)): 2369-76.

[71] Urbich C, Kuehbacher A, Dimmeler S. Role of microRNAs in vascular diseases, inflammation, and angiogenesis. Cardiovascular Research. 2008;79(4):581-8.

[72] Ji R, Cheng Y, Yue J, Yang J, Liu X, Chen H, et al. MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation. Circulation Research. 2007;100(11):1579-88.

[73] Cheng Y, Zhang C. MicroRNA-21 in cardiovascular disease. Journal of Cardiovascular Translational Research. 2010;3(3):251-5.

[74] Selcuklu SD, Donoghue MA, Spillane C. miR-21 as a key regulator of oncogenic processes. Biochemical Society Transactions. 2009;37(4):918.

[75] Zhang Z, Li Z, Gao C, Chen P, Chen J, Liu W, et al. miR-21 plays a pivotal role in gastric cancer pathogenesis and progression. Laboratory Investigation. 2008;88(12): 1358-66.

[76] Ribas J, Ni X, Haffner M, Wentzel EA, Salmasi AH, Chowdhury WH, et al. miR-21: an androgen receptor-regulated microRNA that promotes hormone-dependent and hormone-independent prostate cancer growth. Cancer Research. 2009;69(18):7165-9.

[77] Fleissner F, Jazbutyte V, Fiedler J, Gupta SK, Yin X, Xu Q, et al. Short communication: asymmetric dimethylarginine impairs angiogenic progenitor cell function in pa-

tients with coronary artery disease through a microRNA-21-dependent mechanism. Circulation Research. 2010;107(1):138-43.

[78] Liu X, Cheng Y, Zhang S, Lin Y, Yang J, Zhang C. A necessary role of miR-221 and miR-222 in vascular smooth muscle cell proliferation and neointimal hyperplasia. Circulation Research. 2009;104(4):476-87.

[79] Reynolds A, Leake D, Boese Q, Scaringe S, Marshall WS, Khvorova A. Rational siRNA design for RNA interference. Nature Biotechnology. 2004;22(3):326-30.

[80] Xia H, Mao Q, Paulson HL, Davidson BL. siRNA-mediated gene silencing in vitro and in vivo. Nature Biotechnology. 2002;20(10):1006-10.

[81] Qu Y, Shi X, Zhang H, Sun W, Han S, Yu C, et al. VCAM-1 siRNA reduces neointimal formation after surgical mechanical injury of the rat carotid artery. Journal of Vascular Surgery. 2009;50(6):1452-8.

[82] Puranik AS, Dawson ER, Peppas NA. Recent advances in drug eluting stents. International Journal of Pharmaceutics. 2013;441(1):665-79.

[83] Santiago FS, Khachigian LM. Nucleic acid based strategies as potential therapeutic tools: mechanistic considerations and implications to restenosis. Journal of Molecular Medicine. 2001;79(12):695-706.

[84] Patil Y, Panyam J. Polymeric nanoparticles for siRNA delivery and gene silencing. International Journal of Pharmaceutics. 2009;367(1):195-203.

[85] Che H-L, Bae I-H, Lim KS, Song IT, Lee H, Muthiah M, et al. Suppression of post-angioplasty restenosis with an Akt1 siRNA-embedded coronary stent in a rabbit model. Biomaterials. 2012;33(33):8548-56.

IntechOpen