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Therapeutic Nanoparticle Systems Targeting Smooth Muscle Cells in Cardiovascular Diseases: A Review

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Abstract

Smooth muscle cells (SMCs), as a crucial component of blood vessel walls, play a key role in their structural integrity and functional regulation. They are also involved in maintaining blood pressure, regulating blood flow, and modulating the immune response. However, various factors can trigger a phenotypic shift in SMCs, leading to dysregulation and dysfunction. Such alterations cause the development of numerous cardiovascular disorders. Addressing these types of diseases is of a great importance due to their high prevalence across many societies. Development of nanotechnology has a great contribution in developing advanced and more effective therapeutic strategies for cardiovascular diseases. In this article, we present a comprehensive overview of recent research progress in the field of drug and gene delivery to SMCs. Our aim is to offer valuable insights and guidance for future research endeavors, ultimately contributing to the development of more effective therapeutic approaches for managing cardiovascular disorders.

Keywords: *Smooth muscle cells, Cardiovascular diseases, Drug delivery, Gene Delivery*

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1. Introduction

Cardiovascular diseases (CVDs) are a significant health concern globally due to a combination of genetic, lifestyle, and environmental factors, threatening many lives worldwide [2]. Due to involvement in various conditions' pathogenesis and progression, smooth muscle cells (SMCs) play crucial roles in CVDs. These cells are crucial components of blood vessel walls, including the coronary arteries and aorta, contributing to their structural integrity and functional regulation [3]. However, in some particular situations, such as NOTCH3, signaling deprivation in SMCs may result in severe heart failure, notably when exposed to hypertension, causing changes in cardiac adjustment and the phenotype of SMCs [4]. Notch receptors and their ligands are known to control apoptosis, proliferation, and differentiation and are involved in determining cell fate. Vascular damage affects NOTCH3 expression, which is involved in the proliferation and differentiation of vascular SMCs. Thus, Treatments for cardiovascular disease that specifically target the NOTCH3 gene or downstream molecular pathways may be able to reduce the formation of vascular SMCs and have fewer adverse effects [5].

Additionally, SMCs can be the cause of many CVDs, such as atherosclerosis, via phenotype shifting from contractile to synthetic shape (Figure 1). Hypertension and arterial aneurysms can also occur due to SMCs' potential to regulate blood vessel diameter and generate an immoderate extracellular matrix. Another example of SMC cardiovascular disorders is myocardial infarction, which can emerge, which can emerge by phenotype shifting and immoderate extracellular matrix production ability of these cells [6-8]. Pulmonary arterial hypertension and cardiac and vascular injury are other situations that can originate from SMC's high capacity to control the extracellular matrix and the mechanical properties of the blood vessel wall [9,10]. Thus, designing appropriate therapeutic systems to unravel any dysregulation and dysfunctionality of SMCs seems crucial.

The development of targeted drug delivery systems (TDDS) has revolutionized the treatment of various diseases, particularly those involving muscle tissue. SMCs, in particular, play a crucial role in maintaining blood pressure, regulating blood flow, and modulating the immune response, and their dysregulation can lead to various pathologies ascribed in the upper paragraphs. In this context, the targeted delivery of drug to SMCs has arose as a promising solution for preventing and treating these conditions [11,12].

Targeted delivery allows for the selective inhibition or activation of abnormal SMCs without affecting other cell types, reducing the risk of adverse effects and improving therapeutic efficacy [13]. Moreover, in some cases, drug resistance can emerge in SMCs. Targeted delivery helps to overcome this resistance by directly targeting the cells and minimizing the impact of resistance mechanisms [14]. For instance, according to the study performed by Zha *et al.*, targeted delivery of anti-proliferative agents to SMCs in the vessel wall can help prevent the progression of atherosclerosis [15]. Additionally, targeted delivery can improve the efficacy of therapeutic agents by increasing their concentration at the site of action and reducing systemic toxicity [13,16], as well as allowing for the inquiry for disease progression in a more controlled and specific manner, which can contribute to improved insight into disease etiology and the advancement of more potent therapies [14,17,18]. These various beneficial impacts of TDDS and the necessity of efficient solutions for delivering therapeutics to desired targets resulted in the emergence of specific TDDS called Muscle-targeted drug delivery systems (MTDDS).

These systems can be manipulated to release the therapeutic agent at a specific rate and location, allowing for more precise and effective treatment.

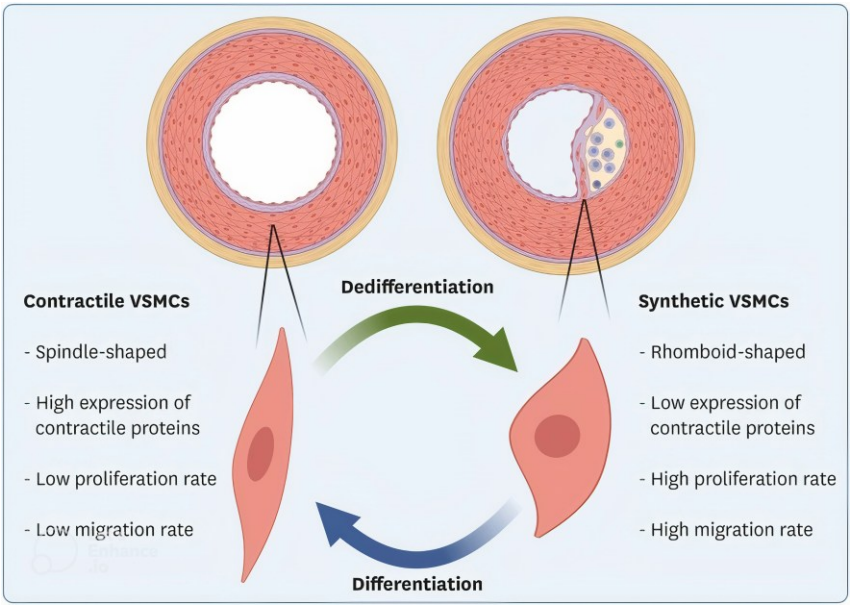

Figure 1. phenotypic switching of VSMCs from contractile in healthy vessels to synthetic in pathological conditions like atherosclerosis. Reproduced from Huynh *et al.* (Ref. [1]). Copyright (2022), licensed under CC BY-NC 4.0 (https://creativecommons.org/licenses/by-nc/4.0/).

MTDDS have been explored for the medication of various conditions, including muscle atrophy, muscle-related pain, muscle dystrophy, and CVDs [19] . In this review, various aspects of muscle-targeted drug and gene delivery systems focused on SMCs are discussed, and different applications of these systems are provided comprehensively to give the experts a wide range of insights in this field.

2. Targeting SMCs

In pharmaceutical research, the precise and accurate delivery of drugs has long posed challenges and complexities. The concept of targeted drug delivery, initially coined as the "magic bullet" by Paul Ehrlich, revolutionized the approach to drug administration. The ultimate goal is to direct drugs specifically to their target cells, tissues, or organs, ensuring the delivery of optimal drug concentrations. Various pharmaceutical formulations have been developed utilizing TDDS to ensure the precise administration of drugs to specific sites, thereby enhancing the desired pharmacological outcomes [20].

There are various parts in SMCs, including ligands and receptors, that MTDDS can target. Androgen receptors are one of the fine examples of SMC receptors that were well investigated in multiple literature. Androgens like testosterone and dihydrotestosterone can target androgen receptors. This can be beneficial in treating CVDs where androgen regulation plays a significant role [21,22]. Thromboxane receptors may also be suitable targets for anti-hypertension drugs. According to the study performed by Sparks *et al.*, the presence of thromboxane receptors in vascular SMCs (VSMCs) was linked to a substantial reduction in angiotensin II-induced hypertension and attenuated vascular remodeling. This phenomenon coincided with diminished urinary thromboxane excretion following chronic angiotensin II exposure. Consequently, these findings indicate that thromboxane receptors in VSMCs play a pivotal role in mediating the effects of thromboxane A_2 on thromboxane receptor agonist-induced shock, aortic vascular remodeling, and hypertension [23].

Atypical Chemokine Receptor 3 (ACKR3) is another possible target for MTDDS. These kinds of receptors,

which are well-reviewed in the literature of Duval *et al.*, play a crucial role in CVDs. ACKER3 participates in β-arrestin and Gαi-protein signalling pathways, interacting with CXCR4 and Connexin 43, and has several natural ligands including CXCL11, CXCL12, MIF, PAMP-12, and opioid peptides as well as synthetic ligands such as VUF11403, VUF11207, TC14012, CCX771, and ACT-1004-1239 [24].

Sortilin, known for its complex metabolic and CVD behaviours, is another important target for MTDDS. Sortilin is a mammalian trafficking receptor that exhibits structural homology to the VPS10 protein. The VPS10 domain of sortilin adopts a 10-bladed β-propeller configuration, enabling it to interact with over 50 distinct protein ligands. This versatility allows sortilin to participate in diverse biological processes, including lipoprotein and lipid metabolism, inflammation, neuronal development and degeneration, and lysosomal degradation pathways. Sortilin follows an intricate intracellular trafficking route, functioning as a receptor in various compartments such as the trans-Golgi network, secretory vesicles, endosomes, the cell surface, and multivesicular bodies. Furthermore, sortilin has been implicated in the pathogenesis of several disorders, including Alzheimer's disease, hypercholesterolemia, Parkinson's disease, inflammatory syndromes, and prion diseases [25]. In addition to the mentioned disorders, sortilin is also known for its related pathways that can result in vascular calcification and other situations such as atherosclerosis, type II diabetes, and hypercholesterolemia, which is also can lead to cardiovascular risk [26,27].

CD44 is another member of MTDDS targets. CD44, a cell adhesion molecule, plays a pivotal role in the pathogenesis of atherosclerosis through multiple mechanisms. It promotes the recruitment of macrophages to atherosclerotic lesions by mediating their migration and adhesion to the endothelium, as evidenced by the significantly reduced lesion size and 90% fewer macrophages in the lesions of CD44-deficient mice than wild-type mice [28,29]. Additionally, CD44 regulates gene expression in focal adhesion formation, angiogenesis, and extracellular matrix deposition, creating a pro-atherogenic environment [30]. The ligation of CD44 by hyaluronan on macrophages provokes the generating of inflammatory mediators such as IL-12, iNOS, and MCP-1, contributing to the progression of atherosclerosis [28,29].

Furthermore, CD44 is upregulated on inflammatory-related cells such as monocytes, lymphocytes, and neutrophils under inflammatory conditions, exacerbating the inflammatory response [31]. HA and CD44 also regulate SMC proliferation and VCAM-1 expression in atherosclerotic lesions, promoting lesion growth and monocyte recruitment [28]. Thus, CD44 is a valuable target, and designing proper MTDDS for it can be precious.

Elastin receptors are another essential target owing to their crucial role in maintaining the elasticity of arteries. Targeting these receptors with synthetic elastic proteins like SEP can help restore the normal function of elastic fibres in the aorta [32]. In addition, Peptide receptors can be targeted by peptides that attach to these receptors. This can be used to deliver cargo to human SMCs, essential in treating CVDs [33]. Platelet-derived growth factor (PDGF) is one the peptides that can be used as a cargo ligand. PDGF has a central role in SMC proliferation and migration, critical processes in developing CVDs such as atherosclerosis and restenosis. In targeted drug delivery, PDGF is used as a ligand to target SMCs, particularly in the treatment of restenosis after angioplasty and stent implantation. This approach is advantageous in treating restenosis after angioplasty or stent implantation. By conjugating drugs or drug-loaded nanoparticles (NPs) to PDGF or PDGF-binding molecules, these pharmaceutical agents can be carefully targeted to SMCs in the impacted artery [33,34]. The REDV peptide is another example of a peptide ligand, considered a specific ligand for vascular endothelial cells and is used to target these cells in DDSs. In the study by Zhou *et al*., the REDV peptide was conjugated with trimethyl chitosan by a double-functional poly (ethylene glycol) conjugator for the targeted delivery of microRNA-126 to VECs. This strategy was developed to enhance the preferential absorption and proliferation of VECs compared to VSMCs [35].

Cell-penetrating peptides (CPPs) can also be promising ligands in this case [33]. These short, cationic peptides have been commonly used as ligands for targeted cargo delivery to several cell types. CPPs

can cross biological barriers, such as cell membranes, and have been engineered to target specific cell types or tissues [36].

3. Drug delivery systems targeting SMCs

DDS have evolved as a promising approach to elevate the therapeutic efficacy of medications while minimizing their adverse effects. These systems selectively transport drugs to the desired site of action within the body, thereby achieving high concentrations at the target location while limiting exposure to non-targeted areas. This precise delivery mechanism optimizes the drug's therapeutic benefits while reducing the risk of off-target interactions and side effects associated with high systemic doses. The fundamental principles of targeted drug delivery involve carefully coordinating the drug's behavior, target site, and the pharmaceutical carrier employed. Ideally, these systems should possess several key characteristics, including non-immunogenicity, non-toxicity, biodegradability, controlled drug release kinetics, biocompatibility, and the ability to be easily eliminated from the body [37]. Various factors, such as drug concentration, particulate location, molecular weight, physiological environment, and carrier properties, must be meticulously considered during developing and optimizing these delivery systems. While targeted DDS offer numerous advantages, they face several key challenges. Achieving successful clinical translation and identifying new therapeutic targets are crucial for their widespread adoption.

Additionally, combining diagnostic and therapeutic capabilities into a single system, known as theranostics, is an active research area. Developing predictive preclinical models and establishing standardization protocols are essential for accurate evaluation and regulatory approval. Furthermore, engineering precision NPs capable of overcoming biological barriers and enabling personalized medicine is a significant focus in the field [37]. Ultimately, selecting the most appropriate targeting strategies, such as active targeting, passive targeting, ligand-mediated targeting, or stimuli-responsive systems, is crucial for maximizing the efficacy and safety of targeted DDS [38].

In targeted therapy, active targeting involves the incorporation of ligands or antibodies onto nanocarriers, enabling them to recognize and bind to specific receptors expressed on target cells or the target microenvironment. This approach enhances the selective accumulation of therapeutic agents at the cell site while minimizing exposure to non-target cells [39]. Passive targeting, on the other hand, exploits the unique physiological characteristics of target cells, such as the enhanced permeability characteristics, allowing NPs to accumulate in target tissues [40] preferentially. Ligand-mediated targeting involves the incorporation of specific ligands onto nanocarriers, enabling them to interact with receptors expressed on target cells or tissues. This strategy can improve bioavailability, enable controlled or triggered release, and enhance targeted delivery to desired sites [41]. Stimuli-responsive targeting utilizes external or internal stimuli, such as pH, temperature, or enzymatic activity, to trigger the payload release and target specific tissues and cells [42]. Smart targeting combines strategies to engineer nanovesicles that recognize and deliver exact bioactive molecules and drugs to specific tissues and cells [43]. Among the mentioned delivery strategies, ligand-mediated targeting is mainly used to deliver therapeutics to SMCs. This method involves ligands (provided in section 2) that specifically bind to receptors on the surface of SMCs, allowing for targeted delivery of therapeutic agents.

Due to the diverse and proper characteristics of NPs that were comprehensively reviewed in previous works, these particles are highly suggested for utilization as a carrier to deliver drugs against different disorders and microenvironments [44-47]. Due to their unique physicochemical properties, NPs are also considered promising DDS for targeted therapy of SMCs[48]. However, it is essential to choose the best NPs to deliver the desired material because the pharmacological and toxicological behavior in vivo of NPs are significantly influenced by their size, shape, surface properties, and elasticity, affecting drug release mechanisms and interactions with cells [49].

One of the proper NPs, in this case, is liposomes. Liposomes, being biocompatible and biodegradable, mimic cell bilayer membranes. They have proven that they can effectively carry therapeutic agents like nitric

oxide to regulate SMC behavior in vascular grafts [50]. Another example in the case of liposomal cardiovascular medicines is liposome encapsulated nitroglycerin (LN). According to the Ardekani *et al.*'s study, LN prevented the excessive formation of mitochondrial superoxide linked to high dosages of nitroglycerin, resulting in a 70-fold improvement in nitroglycerin therapeutic efficacy compared with free nitroglycerin. These results serve as the foundation for thoroughly examining potentially more effective vascular normalization treatments [51].

In addition, Polymeric NPs have emerged as promising carriers for targeted drug delivery to SMCs, a crucial approach for treating vascular diseases like restenosis caused by abnormal proliferation of these cells. Researchers have developed various NP systems leveraging different targeting mechanisms to enhance therapeutic agents' uptake and sustained release in SMCs. Poly(lactic-co-glycolic acid) (PLGA) NPs have been formulated with conjugated targeting ligands such as PDGF-BB peptides to facilitate their uptake by proliferating SMCs. These PDGF-BB-conjugated PLGA NPs demonstrated increased cellular internalization and sustained release of encapsulated drugs like dexamethasone over 14 days in human aortic SMCs [48]. Another example of PLGA loaded therapeutic is Atorvastatin-loaded spray-dried PLGA microparticles (ASPM) developed by Melnik *et al.* recently. The study's generated particles exhibited 76–85% encapsulation efficiency and substantial drug contents. With 10 and 20%, sustained drug release was accomplished for 90 days. The ASPMs' potential for further in vitro and in vivo application was indicated by comparing their before and after γ-ray sterilization, which revealed no discernible effect of the therapy on particle morphology, size, or polymer molecular weight. The ASPM has the ability to counteract the synthetic phenotype of SMCs and reduce their ability to migrate and proliferate [52].

4. Gene delivery systems targeting SMCs

Proliferation and migration of vascular smooth muscle cell (VSMC) has a significant role in intimal hyperplasia, atherosclerosis and restenosis. Through Gene transfer mediated knockdown or over expression of genes this proliferation and migration can be controlled and decrease. Gene delivery is typically accomplished through two main approaches, including virus-mediated gene transfer and nonviral NPs. However, cardiovascular gene delivery has another third approach, named stent-based gene delivery in which endovascular stents serve as a scaffold to efficiently deliver therapeutic genes in to the injured blood vessel wall [53,54].

4.1 Stent-based gene delivery

CVDs, like atherosclerosis, often lead to the narrowing of blood vessels due to the accumulation of fatty plaques. Common treatments for such conditions involve interventional procedures such as percutaneous transvascular balloon angioplasty and stenting. However, a complication of these treatments in long-term is the development of in-stent restenosis, where the dilated arteries become obstructed once again due to deficient recovery of endothelial and uncontrolled proliferation of VSMCs at the site where the stent is implanted. To address this issue, a strategy employed in clinical practice involves the utilization of drug-eluting stents. These stents are coated with drugs such as paclitaxel [55], which are released at the site of implantation to inhibit the excessive growth of VSMCs and reduce inflammation and immune response (Figure 2). However, a drawback of this approach is the lack of specificity in drug delivery, leading to adverse effects on the natural regeneration of endothelial cells away from the immediate implantation site. In addition, there is a risk of blood clot formation inside the stent after implantation, leading to late stent thrombosis and potentially resulting in a heart attack. Studies have demonstrated that scientists can overcome the limitations of current stent technologies and improve their gene loading capacity by incorporating nanomedicine and biomaterials into stent scaffolds. This approach enables localized and sustained delivery of therapeutic genes to the affected vessel wall [56,57].

Coating the surface of stents with natural polymers that are highly biocompatible, non-immunogenic, and easily degradable, to immobilize NPs loaded with therapeutic genes, has shown promising efficiency in the treatment of restenosis. One such example is the use of hyaluronic acid (HA), a natural polymer known for promoting re-endothelialization without significant inflammation. In a study by Lian Che *et al.*, NPs of

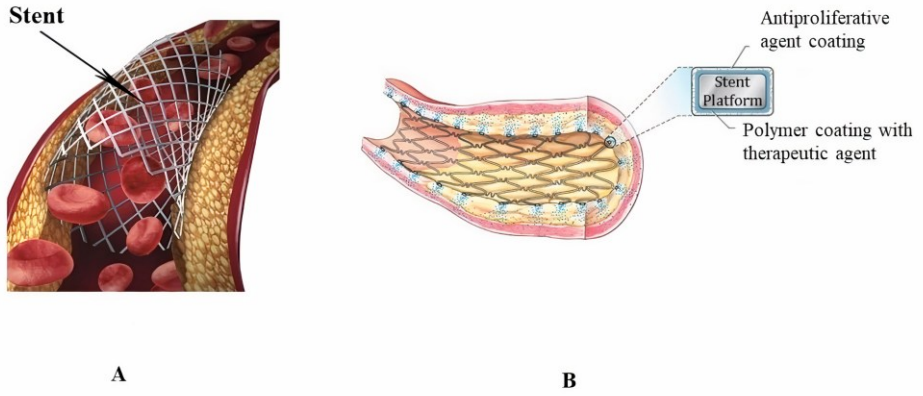

Figure 2. (A) stent implantation in coronary artery with stenosis. (B) a scheme illustrating the methods of gene and drug delivery for preventing in-stent restenosis. It shows how stents can be enhanced by using NPs carrying DNA, siRNA, and miRNA instead of traditional anti-inflammatory or anti-thrombotic medications. Specifically, genes that can disrupt plaque formation around affected areas are attached to the stent's surface to prevent neointimal growth.

Disulfide cross-linked low molecular polyethylenimine (ssPEI) polymers carrying miRNA 145 were immobilized on HA-coated stents. MicroRNA 145 has the ability to inhibit VSMC growth by suppressing proteins involved in its signal transduction cascades, like c-Myc. The results of the study demonstrated that the miRNA 145-eluting stent effectively mitigated in-stent restenosis without any side effects [58]. In a study conducted by Paul *et al.*, a hydrogel-coated stent was developed, incorporating single-walled carbon nanotubes (CNTs) into the fibrin hydrogel. The inclusion of CNTs aimed to enhance the mechanical properties of the hydrogel and promote mitotic division of endothelial cells. The CNT-incorporated hydrogel served as a substrate for delivering NPs functionalized with cell-penetrating Tat peptide, carrying Ang1 and Vegf plasmids. The results demonstrated that the co-delivery of Ang1 and Vegf genes had a significant impact on reducing arterial restenosis. Moreover, using this nano biohybrid stent platform, which consisted of functionalized single-walled carbon nanotube layers and biohydrogel, for local delivery of therapeutic NPs provided a notable enhancement in endothelial regeneration, subsequent inhibition of neointimal proliferation and in-stent restenosis in arteries that underwent balloon injury and stent placement [59]. In another study conducted by Zhu *et al.*, a coated stent was evaluated using dodecylated chitosan NPs loaded with a DNA plasmid for the prevention and treatment of restenosis. The use of chitosan in the stent system offered several advantages, including easy fabrication, good biocompatibility, and low immunogenicity. The results demonstrated that this stent system exhibited remarkable local gene transfection and expression both in vitro and in vivo [60].

Although gene delivery stent systems utilizing synthesized NPs have shown promising results in terms of gene loading capacity and sustained release, effectively preventing in-stent restenosis, studies on gene delivery stent systems employing virus-based vectors have not achieved this goal effectively. Prolonged transduction of vascular tissue using this type of stent system is often affected by several factors, including low initial transduction of the vascular tissue, the presence of anti-AAV2 neutralizing antibodies before and their further induction after the stent placement. These factors collectively decrease the overall therapeutic effects of the gene delivery with these systems. For example, in a study conducted by Hooshdaran *et al.*, a gene delivery stent was formulated using adeno-associated viral (AAV)

vectors loaded with an oxidation-resistant apoA1 mutant gene. Apolipoprotein A1 (apoA1) is a key component of high-density lipoproteins (HDL) known for its anti-atherosclerotic and anti-restenosis properties. However, when apoA1 undergoes oxidative modifications, it loses its beneficial effects. Nevertheless, for the reasons mentioned, their system did not mitigate restenosis effectively in the presence of severe atherosclerotic disease [61]. Indeed, surface modification of adenoviral vectors and the use of biocompatible polymers have shown promise in enhancing the transfection efficiency and therapeutic effects of these vectors in the context of stent implantation. For example, the use of poloxamer 407, a biocompatible polyoxyethyl-polyoxpropyl block copolymer, has been shown to enhance the transfection efficiency of adenoviral vectors in vascular smooth muscle cells (VSMCs) during stent implantation [62].In another study, an adenoviral vector was subjected to modification using cross-linking agent with ester bonds (HL) that reacts with amines and thiols. This modification facilitated the development of a synthetic complex that enabled local and sustained release of adenovirus-gene vectors from metallic surfaces. The complex was composed of metallic surfaces pre-treated with PABT/PEI(PDT), which could then anchor the HL-reacted adenovirus. This controlled release of active adenoviral vectors resulted in enhanced vector stability and efficient transgene expression both in vitro and in vivo. Furthermore, this approach proved effective in inhibiting in-stent restenosis through the local delivery of AdiNOS, an inducible form of the nitric oxide synthase gene. The study's results also demonstrated a significant reduction (56%) in neointimal formation and a remarkable inhibition of restenosis [63]. These findings highlight the potential of surface modification techniques and biocompatible polymers to improve the transfection efficiency and therapeutic outcomes of adenoviral vectors in stent-based gene delivery approaches.

In atherosclerosis, the accumulation of dead cells within the necrotic core of lesions is a significant characteristic. This accumulation is primarily caused by a defect in efferocytosis, which refers to the clearance of apoptotic cells by macrophages. In atherosclerotic plaques under vascular stents, the failure to effectively clear apoptotic cells leads to remain of inflammation. While efferocytosis repair is currently limited to non-stenting therapeutics, Zou *et al.* conducted a study investigating a bioresponsive pro-efferocytotic vascular stent designed for post-stenting healing. They encapsulated exosomes derived from mesenchymal stem cells, which have been found to regulate efferocytosis through pathways such as SLC2a1, STAT3/RAC1, and CD300a, into liposome-based multivesicular chambers. These chambers were then grafted onto vascular stents. The results of the study showed promising outcomes in the prevention of in-stent restenosis. By enhancing efferocytosis and modulating foam cell formation processes, the bioresponsive pro-efferocytotic vascular stent demonstrated potential in addressing this issue [64].

4.2 Virus-mediated gene delivery

In recent years, viral gene delivery vectors such as retroviral, adeno-, and adeno-associated vectors have gained significant attention due to the advantages they add to gene therapy. These vectors, which exist in a wide range of sizes and morphologies, provide high efficiency in transfection of different gene materials to various types of cells, enabling sustained gene expression. However, there are safety concerns in human applications due to their toxic properties, including the risk of insertional mutagenesis, which could lead to the development of malignancies, and the potential for triggering an immune response [65,66]. Many studies have carried out on viral vectors to efficiently deliver genes to target cells while simultaneously addressing these limitations.

We have summarized the research on gene delivery to smooth muscle cells using viral vectors in Table 1. The efficiency of transduction to VSMC by viral vectors can be enhanced by Poloxamer 407 which is a viscous biocompatible polyol. This additive may provide slower kinetics of vector release or bring the viral vector into closer proximity to the cell surface, both of which could contribute to the enhanced transduction efficiency by 10-100 times [62,67,68].

4.3 Non-viral mediated gene delivery

Non-viral vectors are another category of carriers used for gene delivery, offering several advantages over viral systems. These include biocompatibility, reduced

Table 1. Summary of Gene Delivery Methods Using Viral Vectors in Smooth Muscle Cell Research

Trans element	gene	Viral vector	Function	Animal strains	Dose/ Route	Ref.
siRNA	CCN1 (knochdown)	lentivirus	By supporting VSMC adhesion and stimulate chemotaxis through $\alpha_6\beta_1$ integrin, CCN1is involved in neointimal hyperplasia and the development of atherosclerosis.	rat carotid artery balloon injury model	Fifty picograms (p24 antigen) of indicated lentiviral vectors diluted to a total volume of 100 µL/ instilled into the arterial segment	[69]
DNA	SERCA2a (over expression)	chimeric AAV (AAV2.5)	SERCA2a arrest VSMC proliferation in G0/G1 through normalization of calcium cycling in VSMCs and shutting of the calcium-dependent transcription factor nuclear factor of activated T lymphocyte (NFAT)	Carotid artery balloon-injured rat	$B10^{10}$ diluted to a total volume of 100 ml/was instilled between the two clamps, and the external carotid artery	[70]
cDNA	Angiopoietin-1 (Ang1) and Ang2	AAV	Both have protective benefits against inflammation and cell death in cardiac allografts. While Ang1 induces SMC activation, Ang2 lacked the SMC activating effects.	Carotid artery balloon-injured rat	2.0×10^11 genomic particles/ Intramyocardial injection	[71]
cDNA	Cellular repressor of E1A-stimulated gene (CREG) (overexpression)	Recombinant adenovirus	CREG inhibits proliferation which is downregulated in SMC in balloon-injured carotid artery.	Carotid artery balloon-injured rabbit	Ad-CREG, 1×10^{10} pfu/ mL	[72]
cDNA	AT_1 receptor antisense (overexpression)	adenovirus	AT_1 receptor antisense attenuated the cellular actions of ANG II mediated by the AT1 receptor.	*In vitro* study	1×10^9 or 5×10^9 viral particles/ml	[73]
shRNA	Yes-associated protein (YAP)-1 and tafazzin (TAZ) (overexpression)	adenovirus	YAP1/TAZ signaling overexpresses USP15, which promotes the process of pulmonary artery SMC proliferation and migration in pulmonary hypertension.	Rats and mice	1×10^{12} vector genomes/ml, intravenous injection	[74]

toxicity, non-immunogenic, high loading capacity, cost-effectiveness, versatility and the potential for large scale production. Non-viral vectors comprise lipid-based systems, polymer-based vectors inorganic materials, and or a combination of different types. They can be easily prepared, modified, and surface functionalized in various ways [65,75,76]. Most of these systems carry a positive charge to attract and condense the large fragments of nucleic acids, and form complexes by electrostatic force. For example, Cheang *et al.* developed an inorganic nanocomposite particle composed of calcium carbonate/phosphate for delivery of a reporter gene into human VSMC, in which utilized Ca^{2+} ions to bind the phosphate groups of DNA through electrostatic interactions. This was the first time that this nanocomposite particle was used for gene transfection in a stable state [77].

Most gene delivery studies on SMC have utilized polymer-based vehicles of different types [78-80]. PEI cationic polymers are a potential nanocarriers for siRNA delivery to vascular tissue and VSMCs when used for a short period of exposure. Although their cytotoxicity profile is unfavorable, they are widely used as nanocarriers due to their strong gene complexation and high transfection efficiency [80-82]. Another polymer used in gene delivery to VSMCs is chitosan, a natural carbohydrate polymer derived from the outer shells of crustacean such as shrimp and crab. while this polysaccharide has favorable biodegradable and biocompatible properties with no toxicity, its water solubility in physiological conditions is poor. Chitosan derivatives exhibit improved solubility compared to chitosan, making them more effective for gene delivery. WAN *at al.* achieved an enhanced solubility by modifying chitosan with hydroxybutyl groups attached to its hydroxyl and amino reactive sites. Using these NPs, they efficiently transferred siRNAs against tissue factor (TF) into human umbilical VSMCs. TF is an essential cofactor in triggering of coagulation. As TF is involved in promoting thrombosis and inducing the migration and proliferation of VSMCs, it is a potential therapeutic target for CVDs. The knockdown of TF resulted in inhibited proliferation and enhanced apoptosis in VSMCs [83]. In another study, Xia *et al.* utilized chitosan NPs to deliver siRNA targeting platelet-derived growth factor B (PDGF-B), an important factor in the proliferation and migration of SMCs into the neointima. Although the transfected siRNA suppressed proliferation of VSMCs and consequently reduced intimal thickness and area in the rabbit iliac artery injury model, its effectiveness was lower compared to when it was delivered using cationic liposomes [84]. To achieve an efficient gene silencing with chitosan it is important to consider and optimize not only the properties of chitosan derivatives but also factors such as its molecular weight, degree of deacetylation, and N/P ratio [85].

In a different approach, Cheng *et al.* developed a complex nanocarrier composed of gold NPs (Au), polyethylenimine and carboxymethyl hexanoyl chitosan (PEI-Au/CHCA) to deliver a novel and smart plasmid. They select protein kinase C-delta (PKCδ), an apoptosis-inducing protein, as a therapeutic target and place it under the control of an SMC-specific promoter, human early growth response 1 (hEGR1) promoter, to selectively inhibit the proliferation and migration of SMCs. Under induced inflammation conditions, healthy SMCs and epithelial cells were not affected by the inhibitory effect of the loaded nanocarriers [86].

Similarly, Lacin *et al.* fabricated PEG-lated NPs poly (St/PEG-EEM/DMAPM) to deliver plasmid DNA encoding TIMP-2 (Tissue inhibitor of matrix metalloproteinase-2) to SMC. They aimed to inhibit the activity of matrix metalloproteinases (MMPs), which resulted in reduced SMC proliferation and migration [87].

One approach in gene therapy for cardiovascular restenosis is to enhance competitiveness of endothelial cells over SMCs. An essential point in restenosis prevention is to maintain endothelial layer which can be achieved by promoting endothelium regeneration. Hepatocyte growth factor (HGF) is a multifactional cytokine that can accelerate the proliferation of ECs and, through maintaining healthy endothelial layer, prevent SMC proliferation. Using a multilayered gene delivery system, Chang *et al.* delivered plasmid DNA encoding HGF into umbilical vein endothelial cells and human umbilical artery SMCs. They employed layer-by-layer self-assembly technique to immobilize protamine sulfate and HGF-pDNA on the surface of substrates, constructing a multilayered film. Although this functional delivery system successfully enhanced HGF expression in both cell types, it had an inverse effect on endothelial cells compared to SMCs,

resulting in a selective promotion of endothelial cell proliferation [88].

5. Molecular Imaging (Theragnostic NP delivery to SMC)

Molecular imaging in CVDs is of great significance for non-invasive detection, tracing, and quantification of single-cell populations. modalities for molecular imaging including magnetic resonance imaging (MRI), positron emission tomography (PET), and acoustic imaging methods. Among them MRI provides numerous advantages including high imaging depth, spatial resolution, and soft tissue contrast, while being limited by low sensitivity. However, current gold-standard small molecule contrast agents are not cell-specific, relying on non-specific uptake to facilitate imaging of biologic processes. To address this limitation, researchers have utilized targeted NPs with magnetic or paramagnetic feature allowing for the visualization of NP delivery, the quantification of local drug dosage, and determination of the depth and extent of damage after of balloon angioplasty and stenting [89].

Contrast agents, pharmaceuticals that enhance the visibility of tissues or orans in MRI, are either superparamagnetic iron oxide (SPIO) complex or soluble paramagnetic metal chelates. The first group consists of a crystal nuclei of iron oxide coated with polymers like polysaccharides. Paramagnetic agents include metal ions with unpaired electrons such as iron (Fe^{2+}, Fe^{3+}), manganese (Mn^{2+}, Mn^{3+}), and gadolinium (Gd^{3+}) that is the better option compared to others due to its intrinsic features [90,91].

Lanza *et al.* developed a tissue factor-targeted NP system using Gd for the delivery of antiproliferative drugs, including doxorubicin and paclitaxel, to vascular smooth muscle cells. They incorporated Gd onto the surface of perfluorocarbon NPs to provide a prominent T1-weighted MR signal [49].

However, since in SPIO -based contrast agents there are thousands of iron atoms that create an inherent amplification, they provide higher contrast effects compared to Gd [92]. SPIO NPs have several desirable properties, including good stability, low toxicity, and high biocompatibility, which means they do not induce inflammation. However, the presence of these magnetic NPs was found to have a negative impact on the viability of the smooth muscle cells (SMCs) [93,94].

Wang *et al.* coated the SPIO complex with polyethylene glycol (PEG) polymers to carrying Pik3cb short hairpin RNA (shRNA) to investigate their effect in inhibiting restenosis. Phosphoinositide 3-kinase (PI3K) has shown specific functions in cell proliferation and metabolism. Activated PI3Ks then activate AKT1 which initiates a signaling cascade that promotes cell survival, proliferation and metabolism. Studies have indicated that the class IA isoform p110β of PI3K is a therapeutic target for restenosis treatment that may develop after percutaneous transluminal coronary angioplasty. The formulated ultrasound-triggerable NP in their study was able to remarkably downregulate pAkt protein expression when compared to the administration of the naked Pik3cb shRNA in a rat model with balloon-induced vascular injury and as a result, decreased neointimal thickening, which is the hallmark of restenosis [95]. In a study by Yabin Wang *et al.,* SPIO NPs were coated with meso-2,3-Dimercaptosuccinic acid (DMSA) to synthesize a multimodal molecular imaging probe that simultaneously carries Profilin-1 siRNA to VSMCs. Profilin-1 is a family member of small actin-binding proteins that promotes actine polymerization and cytoskeleton reorganization. Profilin-1 has been identified as an overexpressed protein in VSMCs, making it a promising molecular target for the study and treatment of atherosclerosis. The researchers leveraged this overexpression in a two-pronged approach: first, they targeted profilin-1 to visualize and detect atherosclerotic lesions in vivo non-invasively through imaging techniques; and second, they aimed to silence the expression of profilin-1 as a potential therapeutic strategy to attenuate the progression of the atherosclerotic process. By focusing on this overexpressed protein in VSMCs, the researchers sought to enhance both the diagnostic capabilities and the development of new treatment options for this cardiovascular disease. The developed magnetic NPs were biocompatible NPs that could perform dual MR and optical imaging of atherosclerotic plaque in mice [96].

Another potential molecular target found in VSMCs within atherosclerotic plaques is osteopontin, which is

overexpressed in these cells. The upregulation of this protein promotes the VSMCs phenotypic shifting from a contractile phenotype to a synthetic phenotype, which significantly boosts the progression of atherosclerotic plaques. Building on this knowledge, Ma *et al.* fabricated a type of theragnostic NP platform based on human serum albumin to achieve precise identification and noninvasive detection of atherosclerotic plaques, as well as deliver a sirtuin 1 (Sirt1) activator called SRT1720 as a potential therapeutic. Unlike the previous examples that utilized magnetic resonance imaging, this theragnostic NP platform was based on near-infrared (NIR) fluorescent imaging. Despite the different imaging modality, this NP system also demonstrated high potential for the accurate identification and noninvasive detection of atherosclerotic plaques [97].

The researchers found that using materials with magnetic properties could accelerate the process of tissue remodeling and regrowth in tissue-engineered vascular grafts (TEVGs). This is because the magnetic properties can help facilitate the capturing and adhesion of cells at the site of the injured blood vessel. To explore this further, Arias *et al.* developed a magnetic bacterial cellulose material and used it to target SMCs in vitro. They formed iron oxide (Fe_3O_4) NPs along the fibers of the bacterial cellulose and then coated the fibers with dextran to preserve the embedded Fe_3O_4 NPs from oxidation. [98]. However, the dextran coating layer resulted in less cell coverage compared to other reported systems, such as magnetized steel stent wires [99].

6. Conclusion

Vascular smooth muscle cells (VSMCs) are essential for the integrity and functionality of blood vessels. Under pathological conditions and stimuli such as inflammation, differentiated VSMCs can undergo phenotypic changes from a contractile to a synthetic state, acquiring enhanced proliferation and migration capabilities. This transformation is particularly evident following stent implantation, leading to in-stent restenosis.

Numerous studies have focused on controlling and inhibiting the proliferation and migration of smooth muscle cells (SMCs) by developing various nano-based delivery systems for therapeutic agents. These agents include antiproliferative drugs such as paclitaxel, as well as genetic materials (plasmid DNA, siRNA, etc.).

A promising application of nanoparticles is in drug-eluting stents, where the incorporation of NPs allows for localized and sustained release of therapeutic agents directly at the site of implantation. By coating stents with natural polymers that are biocompatible and biodegradable, researchers can effectively immobilize NPs loaded with therapeutic genes, enhancing the treatment of restenosis while minimizing adverse effects on surrounding endothelial cells.

Additionally, nano-based delivery systems have been utilized for molecular imaging, enabling the delivery of small molecule contrast agents. This approach facilitates the visualization of nanoparticle delivery, quantification of local drug dosages, and assessment of the extent of damage following balloon angioplasty and stenting.

There is a diverse array of NP options, including polymeric nanoparticles and viral-based systems, each presenting unique challenges and considerations. The selection of an appropriate NP is contingent upon several factors, including the desired drug release kinetics, target cell type, and the physicochemical properties of the nanoparticle itself. Promising candidates such as liposomes, viral-based nanoparticles, micelles, and polymeric nanoparticles have shown potential for targeted drug delivery to SMCs, which is crucial for effective treatment of vascular diseases.

However, further research is imperative to optimize these delivery systems and address the hurdles associated with their clinical translation. By advancing our understanding of these nanotechnology-based approaches, we can improve therapeutic outcomes for patients suffering from vascular conditions.

7. Reference

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