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Nano-medicine and Vascular Endothelial Dysfunction: Options and Delivery Strategies

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Abstract

The endothelium is a thin innermost layer of flat cells which release various mediators including endothelin-1 (ET-1), prostanoids, von Willebrand factor (vWF) and endothelium-derived relaxing factor (EDRF; nitric oxide) to regulate vascular tone. Endothelial nitric oxide synthase (eNOS) is a key enzyme that generates nitric oxide (NO). NO maintains vascular homeostasis and cardiac functions by influencing major vascular protective properties such as anti-platelet, anti-proliferative, anti-migratory, antioxidant and anti-inflammatory action in vessels. Abnormal endothelial production and release of NO lead to vascular endothelial dysfunction (VED) and further leads to pathogenesis in myocardial and other tissues. Numerous pharmacological agents such as angiotensin-converting enzyme inhibitors, statins, calcium channel blockers, ET-1 receptor antagonists, insulin sensitizers, antioxidants and supplements like tetrahydrobiopterin, arginine and folate have been implicated in the treatment of VED, but their therapeutic potency was restricted due to some unavoidable adverse effects. The new era with advances in nanotechnology and its ability to target a specific disease, nano-medicine explored an innovative gateway for advanced therapy for VED. The present commentary reveals the various available, pipeline nano-medicine, their interaction with endothelium and in other associated pathological conditions and their delivery strategies for target-specific treatment of VED.

Keywords Vascular endothelial dysfunction · Nano-medicine · Nanoparticle · eNOS · Cardiovascular disorders

Abbreviations

ACE-1	Angiotensin-converting enzyme 1
ADMA	Asymmetric dimethylarginine
AGE	Advanced glycation end product
Akt	Protein kinase B
BH4	Tetrahydrobiopterin
CAD	Coronary artery disease
CDK	Cyclin-dependent kinase
CETP	Cholesteryl ester transfer protein
CVD	Cardiovascular disease
CXCL12	C-X-C motif chemokine 12
DAMPs	Endogenous damage-associated molecular patterns
EAhy926 cells	Endothelial-like cells
ECs	Endothelial cells
EDRF	Endothelium-derived relaxing factor
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
FAD	Flavin adenine dinucleotide
FMN	Flavin mononucleotide
GGTase-I	Geranylgeranyltransferase-1
GLP-1	Glucagon-like peptide 1
Hif-1α	Hypoxia-inducible factor-1α
HMG-CoA	3-Hydroxy 3-methylglutaryl coenzyme A
HO-1	Heme oxygenase-1
HUVECs	Human umbilical vein endothelial cells
ICAM-1	Intercellular adhesion molecule 1
IGF-1R	Insulin-like growth factor 1 receptor
IL-6	Interleukin-6
JAK	Janus kinase
LOX-1	Lectin-like oxidized low-density lipoprotein receptor-1
LPS	Lipopolysaccharide

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MCP-1	Monocyte chemoattractant protein
MNBs	Magnetic nano-beads
MRI	Magnetic resonance imaging
mTOR	Mammalian target of rapamycin
NADPH	Nicotinamide adenine dinucleotide phosphate
NFk-β	Nuclear factor kappa-β
NO	Nitric oxide
NPs	Nanoparticles
PAK1	p21 protein (Cdc42/Rac)-activated kinase 1
PAMPs	Pathogen-associated molecular patterns
PECAM	Platelet-endothelial cell adhesion molecule-1
PET–MRI	Positron emission tomography–magnetic resonance imaging
PIK3R2	Phosphatidylinositol 3-kinase regulatory subunit beta receptor
PI3K	Phosphatidylinositol-3-kinases
PKA	Protein kinase A
PLGA-PEG	Poly(lactide-co-glycolide)–poly(ethylene glycol) polymer
PPAR	Peroxisome proliferator-activated receptor
PRRs	Pattern recognition receptors
PTPase	Protein tyrosine phosphatase
ROS	Reactive oxygen species
SPIONs	Superparamagnetic iron oxide NPs
SPRED-I	Sprouty-related protein I
S1P	Sphingosine-1-phosphate
TLRs	Toll-like receptors
TNF-α	Tumor necrosis factor-α
USIOPs	Ultra-small superparamagnetic iron oxide particles
VCAM-1	Vascular cell adhesion molecule 1
VED	Vascular endothelial dysfunction
VEGF-A	Vascular endothelial growth factor-A
VSMCs	Vascular smooth muscle cells
vWF	Von Willebrand factor

Introduction

The endothelium is an innermost covering layer of the vasculature lumen. It releases a wide range of signaling factors that regulate vascular tone, cellular adhesion, smooth muscle cell proliferation, pro-thrombotic activity and vascular inflammation [1]. The vascular homeostasis has been regulated by maintaining a balance between vasorelaxation and vasoconstriction via various released mediators. Endothelium vasodilation or relaxation is mediated through nitric oxide (NO), endothelium-derived hyperpolarizing factor and prostacyclin, whereas endothelium vasoconstriction is derived through endothelin-1 (ET-1), angiotensin II, prostanoids and superoxide anion [2]. Early pioneering experiments of Robert Furchgott, John Zawadski, and Louis Ignarro demonstrated the role of endothelium-derived relaxing factor (EDRF) or NO as a vasodilator [3]. NO also plays an important role as the anti-inflammatory, anti-platelet, anti-proliferative and anti-migratory agent [4] which helps to regulate blood pressure, vascular tone, and leukocyte adhesion inhibition. NO is released through endothelium as side product during conversion of L-arginine to L-citrulline by presence of endothelial NO synthase (eNOS) enzyme which further depends on nicotinamide adenine dinucleotide phosphate (NADPH) and requires other cofactors such as Ca^{2+}/calmodulin, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) and tetrahydrobiopterin (BH_4) [5]. The alteration between vasorelaxation and vasoconstriction or thrombotic and thrombolytic properties of the endothelium represents vascular endothelial dysfunction (VED) [6]. Moreover, VED specifically refers to decreased NO bioavailability and increased asymmetric dimethylarginine (ADMA) in vessels which leads to impairment in endothelium-dependent relaxation [7]. Certainly, VED is the major hallmark for several other pathological conditions, but it has limited option for treatment which raises a need to update the therapeutic possibilities. Nanotechnologies have explored numerous applications in clinical field over conventional drug approach including encapsulation of drug content, increase bioavailability and reduce systemic toxicity, and targeted drug delivery [8, 9].

The contemporary innovative approach of nano-medicine plays an emerging role in the detection, treatment, and management of the various pathological states. Nanoparticles (NPs) have been used to detect the potential biomarkers of vascular diseases [10, 11]. The delivery of NPs to the endothelial cells is associated with specific endocytic pathways [12, 13]. In addition to the large surface area, NPs have the promising ability of targeted drug delivery to vasculature by manipulating various physicochemical characteristics including size, surface charge, solubility, and coating materials [14–16]. Thus, achieving the advanced therapeutic outcomes against vascular abnormalities by targeting various nano-formulations has become a thrust area of research in the current scenario [17–19].

Pathogenesis of Endothelial Dysfunction and Target Opportunity for Nanotechnology

The several potential target sites have been explored in last decades which can be pointed for initiation of VED including increased expression or activation of ET-1, Rho-kinase, poly-ADP ribose polymerase (PARP), protein tyrosine phosphatase (PTPase), caveolin-1, cholesteryl ester transfer protein (CETP), lipoprotein lipase, advanced glycation

end product (AGE) and transketolase, geranylgeranyltransferase-1 (GGTase-1), epoxide hydrolase, and Janus kinase [20–25] (Fig. 1), whereas the downregulation of some other protein kinase including protein kinase A (PKA), protein kinase B (Akt), peroxisome proliferator-activated receptor (PPAR), sphingosine-1-phosphate (S1P), angiotensin-converting enzyme 2 (ACE-2) could be responsible to instigate the endothelial dysfunction [26]. All these factors either alter the function of eNOS, NO production and release or counteract oxidative stress, directly or indirectly [26]. Moreover, the eNOS uncoupling may be initiated by the reduced level of BH_4 or substrate L-arginine deficiency. Besides the molecular alterations, numerous pathological conditions are also responsible for inducing endothelial dysfunction, for instance, diabetes mellitus, hypercholesterolemia, hyperhomocysteinemia, obesity, estrogen deficiency, hyperuricemia, and aging [26], whereas some environmental factors including smoking, alcohol consumption, and arsenic intake may also instigate the VED by increasing oxidative stress of body system (Fig. 1) [27–29]. VED has been defined as the root cause of myocardial damage as various pathological events are induced by endothelial dysfunction such as coronary artery disease, chronic heart failure, atherosclerosis, hypertension, and stroke [30].

Recent studies suggest that both acute and chronic inflammation predominantly instigate endothelial dysfunction [26, 31]. Pro-inflammatory/immune effector cytokines have been explored as pioneering therapeutic intents for inhibiting endothelial dysfunction [26, 31]. Endothelial activation and dysfunction involve both innate and adaptive immunity. Acute inflammation in a vessel is vasodilation and increased permeability of microvasculature including plasma proteins, pro-inflammatory cytokines and adhesion molecules [32]. The increased secretion of these adhesion molecules [vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1)] cause the perturbations in endothelial cells and associated cardiovascular disease (CVD) [32]. However, the trigger of cardiovascular inflammation has been explained by innate immunity-derived surveillance mechanisms [33] which involve specialized pattern recognition receptors (PRRs) and their response to pathogen-associated molecular patterns (PAMPs) and endogenous damage-associated molecular patterns (DAMPs) [34]. Interestingly, PRRs involve four major receptor families including membrane-based toll-like receptors (TLRs) which elicit pro-inflammatory and pro-thrombotic responses within the vasculature [35].

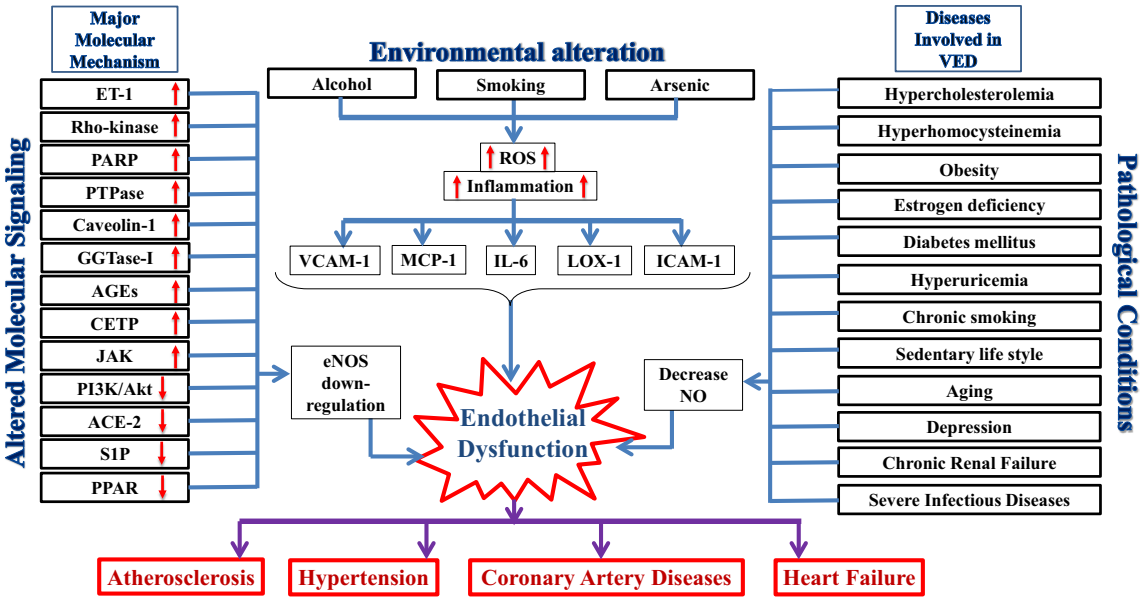

Fig. 1 Various risk factors and signaling pathways involved in pathogenesis of vascular endothelial dysfunction and associated disorders. ET-1 represents endothelin-1, Rho-kinase represents, PARP represents (ADP ribose) polymerase, PTPase represents protein tyrosine phosphatase, GGTase-1 represents geranylgeranyltransferase, AGEs represents advanced glycation end product, CETP represents cholesteryl ester transfer protein, JAK represents Janus kinase, PI3K represents phosphatidylinositol-3-kinases, Akt represents protein kinase B, ACE 2 represents angiotensin-converting enzyme 2, S1P represents sphingosine-1-phosphate, PPAR represents peroxisome proliferator-activated receptor, ROS represents reactive oxygen species, NO represents nitric oxide, eNOS represents endothelial nitric oxide synthase, VED represents vascular endothelial dysfunction, VCAM-1 represents vascular cell adhesion molecule 1, ICAM-1 represents intercellular adhesion molecule 1, MCP-1 represents monocyte chemoattractant protein, IL-6 represents interleukin-6, LOX-1 represents lectin-like oxidized low-density lipoprotein receptor-1

Moreover, the bioavailability of NO could be suppressed by reactive oxygen species (ROS) by altering the expression of several signal transduction molecules including VCAM-1, ICAM-1, IL-6, MCP-1 and lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) [29]. ROS is an inevitable by-product of cellular metabolism. Increase level of ROS alters the various vital molecular signaling pathways including c-Jun N-terminal kinases (JNK), p38 which leads to autophagy. Elevated ROS can also obstruct the major signaling of Akt and down-regulate mammalian target of rapamycin (mTOR) which is directly involved in the synthesis of NO [36]. ROS could also be generated from oxidase and leads to uncoupling of eNOS enzyme.

Most of the current target sites and their ligands would restore the endothelial functions by reducing oxidative stress, improving NO bioavailability through eNOS modulation and altering its mechanistic pathways. However, some of the conventional treatments may have unwanted on-/off-side adverse effects as ET-1 antagonists and insulin sensitizers may cause fluid retention and edema in hypertensive and diabetic patients [37]. Moreover, 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors possess cholesterol lowering, anti-inflammatory effects with a mild tendency to reduce hypertension, but are also linked with new-onset diabetes risk and rhabdomyolysis [38]. Angiotensin-converting enzyme 1 inhibitor (ACE inhibitors) and angiotensin II receptor blockers have been used to treat heart failure with reduced ejection fraction, chronic kidney disease, diabetic nephropathy and hypertension [39]. Instead of these pharmacological use, ACE inhibitors and angiotensin II receptor blockers may cause some side effects including hypotension, angioedema, hyperkalemia, and teratogenicity [40]. The nano-formulations of these conventional drugs may significantly improve the therapeutic ability against various associated complications.

Nano-medicines with a Glimpse on Pros and Cons

Nano-medicine is an advanced version of conventional drug that includes the development and application of nano-materials and nanotechnologies [41]. Indeed, the nano-medicine is an overall consequence of the interaction between interdisciplinary field as nano-science, nano-engineering, and nanotechnology interrelate with life sciences which provide inimitable properties to the product for designing new therapeutic effects and diagnostics use. It is assuming that nano-medicine will develop better options for early detection of diseases, diagnostic kits, specific and potent treatment with a low dose and short duration for improving the quality life [42]. NPs or nano-materials are chief components of nano-medicine which has been found in a different form that reflect their unique role. Nano-sized materials have been used as drug delivery systems including micelle (lipids or synthetic amphiphiles-based monolayered globular structure that can carry hydrophobic agents), liposome (phospholipids bilayers-based spherical vesicles which encapsulate hydrophilic agents), polymer NPs (composites of NPs in polymer matrix which can comprise and control release of both hydrophilic and hydrophobic agents), dendrimer (highly symmetric, spherical molecular structure of monodisperse dendrons having versatile drug delivery approach with high functionality due to their multivalent properties), carbon nanotube (cylindrical nano-structure made up of carbon allotropes and having unique properties of aspect ratio, strength, thermal and electrical conductivity) and metallic NPs (magnetic core coated with hydrophilic polymers that has been used as contrasting agent for biological imaging) [43].

Nano-medicine has been widely used in imaging and diagnosis of molecular and cellular changes in the biological system by computed tomography (CT); magnetic resonance imaging (MRI); imaging of radioactivity, such as positron emission tomography (PET) or single photon emission computed tomography (SPECT). Biological analysis by these smart imaging systems is a common approach today as it has inert nature and may not cause any impairment in living tissues [44]. Encapsulated nano-medicine is highly specific for targeted drug delivery which ultimately improves the onset of drug action and bioavailability and also minimizes the associated side effects and toxicity with reduced cost [45]. The huge application of nano-medicines has been seen in the treatment of cancer where the conventional anti-cancer drugs produce chronic toxic effects due to their non-specific targeting approach to tumor cells. There are several reports available which explored the potential therapy of cancer treatment by using nanotechnology including the use of tumor-specific thermal scalpels and antibody-coated magnetic iron NPs [46]. The application of nanotechnology has extended up to gene therapy to treat the genetic disorders with increased transfection efficiency and minimized cytotoxicity (Chitosan), where NPs-based carrier molecule has been used to exchange the abnormal gene with normal gene [45]. Indeed, several studies have revealed the novel applications in systemic delivery of drugs, tissue regeneration, bone repair, immunity and treatment of life-threatening diseases like cancer, diabetes and vascular diseases [47].

Conversely, some other study reported the health hazards due to unintentional exposure of NPs that may lead to the respiratory problem and pulmonary toxicity. Due to an ultra-small size of NPs, human may get their exposure through the air. Prolong exposure with a high concentration of this polluted air may cause severe breathing problem and have more adverse health effects (chronic obstructive pulmonary disease) in susceptible populations. Moreover, the inhaled NPs can retain into lungs or may translocate to an extrapulmonary site and cause toxicity in other organs (cardiovascular and neuronal cell injury). Some evidence also reported

that NPs have the ability to alter autophagy mechanism that may induce an inflammatory response and oxidative stress [45, 48]. NPs can directly come in contact with human skin and may cause skin irritation. Even the penetration of NPs having size more than 1 μm is limited but it may easily penetrate from wound or rupture skin [49]. Thus, besides the effectiveness and wide application of nano-medicine, it would be mandatory to satisfy the all other concerns of ideal drug including safety, stability, reproducibility, regulatory and ethical requirements.

Instead the presence of pros and cons of NPs use, there is the certain advantage of nano-medicines over the conventional drug therapy which further enhances the clinical application of nano-medicine. Fluorescent imaging is highly sensitive and has an advantage of no interaction with the biological system over the limitation of the conventional approach of using various dyes [44, 45]. Additionally, the smaller size of nano-medicine (NPs having a size < 100 nm) makes them more suitable to be attached with various peptides and can be easily detectable during cellular changes which represent a novel tool to explore molecular signaling with high accuracy. Moreover, the current strategy has been implicated in cell and organ transplantation which reflect the next step of application in the biomedical field [50].

Intervention of Nano-medicine in Major Vascular Diseases

The therapeutic application of nano-medicine embraces the rapid development of nanotechnology. A wide spectrum of NPs ranging from liposomes and niosomes to polymers, hybrid of lipid and polymer as well as organic precursors, carbon nanotubes, quantum dots, metal, metal oxides including superparamagnetic iron oxide NPs (SPIONs) and biological molecules (such as proteins) are being used [51, 52]. The ability of NPs to deliver drugs at the specific targeted site provides an exceptional advantage such as enhanced circulation of NPs due to PEGylation and versatility to encapsulate or entrap drugs to upsurge their solubility as well as preventing exposure to unfavorable conditions [53]. The systemic toxicity of the free drug can also be reduced through targeted drug delivery by a highly localized drug release approach [54]. Additionally, this technique also favors co-delivery of two or more drugs at a target site in a single or multiple matrix system. Therefore, it would also be possible to deliver advanced or less compatible formulations in combination as a single approach which enhances the scope of combinational drug therapies [55]. The major therapeutic application can be explained by its ability to deliver molecules intracellularly for insertion of the desired gene (e.g. siRNA) or foreign DNA in host cells, which provides great flexibility to therapy development [56]. These advantages could provide an advanced therapeutic approach for the treatment of VED and related disorders specifically by targeted drug delivery to endothelial tissue. Endothelial cells cover the huge part of the vascular system which makes the vasculature a wide route for introduction of nano-medicine. Thus, the intense understanding between the interaction of nano-medicine and endothelial cells can evade the unintended side effects or injury. A simple example of an advanced application of nano-medicine may be described by the enhanced therapeutic efficacy of statin against coronary artery diseases (CAD). Additionally, various NPs-based nanotechnologies (fluorescence tomography, PET, MRI, CT scan, optical coherent tomography, photoacoustic tomography and molecular imaging) have been used for imaging of CVD. Moreover, the study also revealed the various therapeutic applications of nano-formulations including neutral liposomes (for macrophage depletion, reduced inflammation), cationic liposomes (for significant improvement in myocardial perfusion), perfluorocarbon NPs (for fibrinolysis), polyelectrolyte NPs (for high transfection efficiency) and polymeric NPs (for inhibition of restenosis) in CVD [57]. The consequences of increased inflammatory changes may further promote the vascular permeability of coronary endothelium. In general, a high dose of a statin has been used for reducing the mortality rate in CAD which itself leads to dose-dependent side effects [58]. However, the advanced approach of nano-medicine revealed that efficacy of statin can be improved by use of statin-loaded vesicles that have functionalized surface by oligonucleotides. These oligonucleotides are highly specific for inflammatory macrophages which significantly reduced the systemic toxicity as well as increase the target specificity of the drug [57]. Secondly, the upregulation of angiogenesis within atherosclerotic plaques can lead to growth and rupturing of plaque which suddenly blocks the coronary artery and results in myocardial infarction [59]. A potent anti-angiogenic agent (Fumagillin) may protect the abnormal neovascularization in atherosclerotic plaques but also endorse the severe neurocognitive impairment. However, the delivery of this anti-angiogenic agent through integrin-targeted NPs has shown significant anti-angiogenic potential with lowered side effect [57, 59, 60]. Furthermore, the involvement of nano-medicine can significantly modulate the intracoronary thrombosis by using encapsulated micellar NPs of natural anti-thrombotic agent [61]. Instead of these, several other applications of nano-medicine are concerned with significant treatment of CAD by minimized the systemic toxicity of potent drugs including biomimetic nano-fibrous scaffolds can accelerate the endothelial regeneration after stent placement [62], and the technique of nano-spinning and nano-patterning has been proposed for tissue-engineered graft material [57, 59–63].

Nano-medicine and Diabetes-Induced VED

The application of nanotechnology in diabetic treatment has been widely used in preclinical and clinical trials [59–63] including an implementation of artificial pancreas beta cells which reduce the need for pancreas transplantation [64]. An artificial beta cell may be a promising therapeutic approach of nanotechnology in clinical studies for diabetic patients as the synthesized smart pancreatic beta cells containing multicompartmental lipid membrane having specially designed insulin-stuffed vesicles with membrane-fusion machinery which are sensitive to glucose [65]. The amount of glucose further triggers the release of insulin from insulin-stuffed vesicles [65]. Oral delivery of insulin with biodegradable polymeric nanospheres is another potential approach which reduces the need of repeated routine subcutaneous injections of insulin and increases patient's compliance [66]. Moreover, various techniques for noninvasive monitoring of blood glucose have been explored from last one decade using various nanotechnologies including optical sensing technique (nanotube near-IR emission, nanotube fluorescence enhancement, graphene catalytic activity, Raman spectroscopy and hydrogel mediated Bragg diffraction), electric sensing technique (nanotube conductance modulation, hydrogen peroxide catalysis via nanotubes, hydrogen peroxide catalysis, enzyme-free glucose catalysis and NPs catalysis of hydrogen peroxide) and magnetic sensing technique (shift in magnetic resonance of a membrane) [67]. The detection of the precise blood glucose level makes glucose monitoring become more convenient. Additionally, a distinct category of NPs including polymeric biodegradable NPs, polymeric micelles, ceramic NPs, liposomes and dendrimer has been used as insulin carriers [68]. For instance, chitosan NPs have been significantly used for non-parenteral drug delivery system due to its biodegradable and biocompatible nature. Thus, encapsulated insulin in carboxylated chitosan grafted poly(methyl methacrylated) NPs shell increases the permeation and absorption of insulin through intestinal epithelium [69, 70]. A recent report has shown the advance application of nanotechnology to deliver the insulin by inhalation where encapsulated dry powder of insulin can be inhaled into the lungs [71].

Contemporary therapeutic options for diabetes mellitus including insulin secretagogues, sensitizers, and sodium-glucose transporter 2 inhibitors (Gliflozins) have been reported for improving not only hyperglycemia but also endothelial function [72, 73]. A target-specific drug delivery with better efficacy and potency may give the maximum therapeutic effects. Biodegradable NPs have been used to deliver proteins (insulin), genes, and other synthesized drug which has systemic toxicity. However, the polymer drug delivery system offers the better option to attain target site with high potency, controlled release, less dosing frequency and minimum side effects of the drug which reflect the features of advanced therapeutic strategy [74, 75]. Metformin is the first-line medication to decrease hepatic glucose levels but also improve the functioning of vascular endothelium. Despite metformin's promising euglycemic potentials, it can be associated with some side effects including gastrointestinal irritation, diarrhea, blurred vision, depression, lactic acidosis and hyperhidrosis that arise a need to regulate the concentration of blood metformin. A recent investigation reports a carbon nanosphere composite which can be used as sensitive metformin sensor to avoid the overdosing [76]. The intestinal hormone glucagon-like peptide (GLP-1) secreted by the intestinal L cells commonly regulates postprandial hyperglycemia and stimulates the insulin secretion from pancreatic beta cells [77]. It has been reported that lipid-based NPs [nano-structured lipid carriers (NLC), lipid nano-capsules (LNC), and liposomes] and poly(lactide-co-glycolide)-poly(ethylene glycol) (PLGA-PEG) NPs can significantly stimulate the secretion of GLP-1 [78]. Moreover, GLP-1 conjugates have also been invented for targeted gene therapy to increase GLP-1 bioavailability [79]. Exendin-4 also exhibits similar potential as GLP-1, as it inhibits pancreatic islet cell death and has a longer half-life in serum, which in turn enhances survival rate and extent of islet cells and aids in insulin production after transplantation. Replacement or regeneration of islet cells could be a potential way of treatment in insulin-dependent diabetes [80]. Ghosh et al. synthesized polymeric NPs embedded with coumarins, the composed PLGA-PEG polymer with Pep I peptide to target the endothelium of microvessels in pancreatic islet cells. These Pep I-NPs have been shown to increase threefold binding in islet capillaries [81]. The correction of islet vascular endothelial cells malfunction with the delivery of plasma sensitive nucleic acid opens a new way in the genetic approach that is ultimately based on NPs targeting techniques. Moreover, various nano-medicine-based gold nano-structures haVE also been used for significant reduction in diabetes and associated endothelial damage that seems to be a promising option [82]. However, more advancement is still needed in these types of techniques as the number of vectors for delivery is limited. Therefore, insulin levels achieved by these techniques need regulation. Moreover, the exendin-4 expression vector (PEI25k/pbeta-SP-Ex-4 complex) incorporated in polyethylenimine dendrimers was developed to ameliorate isolated β cells apoptosis during islet transplantation [83]. All aforementioned methods and techniques of islet cell regeneration and encapsulation are still in the early stages of preclinical development and could be potential alternatives to current therapies. However, elucidation of safety and efficacy of such techniques remains a challenge.

Nano-medicine and Atherosclerosis-Induced VED

Altered endothelium endorses the primary sign of atherosclerosis that may be associated with increased permeability of endothelium, hypertension, shear stress and oxidative stress [84]. From initial lesion formation to the final thrombotic stage, activation and inflammation of the endothelium play an important role in atherosclerosis progression and also in myocardial infarction [85]. The immune system plays a critical role in the development of atherosclerosis. Additionally, triggering of leukocyte recruitment into plaque and subsequent activation of inflammatory cascade has been thought to be initiated by lipids and lipid by-products through activation of endothelium. This fact has been further confirmed by atherosclerotic plaque reduction through the use of anti-inflammatory drugs in animal models [86]. Nano-medicine can provide a vital approach for encapsulation of therapeutic agents which potentially enhanced the bioavailability and delivery to specific immune cells of plaque, ensuring treatment effectiveness.

Atherosclerosis is a major root cause of vascular disease and towering prevalence of mortality associated with ischemic heart diseases worldwide. The deposition of progressive plaque in coronary artery leads to myocardial ischemia or infarction. Nanotechnology has been significantly contributed at the different level (noninvasive management and invasive therapy) of treatment against atherosclerosis and cardiac injury. The noninvasive technique involves to reduction or removal of atherosclerotic plaques by medical treatments, whereas invasive therapy technique involves the mechanical revascularization using drug-eluting stents (DES) and biodegradable stent [57]. In the noninvasive approach, several anti-inflammatory, antioxidant and lipid-lowering drugs have been used to prevent plaque formation and endothelial injury. Surface-activated pravastatin loaded vesicles are the potential treatment for reducing the inflammatory and oxidative injury with minimizing the systemic toxicity of statin as well as 15 times lower toxicity in muscle cells [58]. Furthermore, the use of biomimetic NPs-based synthetic high-density lipoprotein (HDL) [liposomal formulation with dimyristoyl phosphatidylcholine (DPMC)] can significantly increase the HDL level in blood which potentially reduce the plaque formation in the animal study [87]. Integrin-targeted NPs of fumagillin and atorvastatin have also been used for anti-angiogenic effect in atherosclerotic plaques with significant minimization of systemic side effect of the drug [88, 89]. It also has been observed that upregulation of monocytes and macrophages promote the plaque growth and rupture, whereas the NPs-based drug delivery of PPAR-γ agonist (pioglitazone) significantly inhibits the plaque rupture in the animal model [90]. Moreover, NPs-based delivery of short interfering RNA (siRNA) silencing CCR2 also hampered the plaque formation and growth [91]. NPs of iron oxide, glucocorticoids (liposomal glucocorticoid therapy) have also been used to prevent inflammation by removing the macrophages that accumulated in atherosclerotic plaques [92]. Ruptured or degenerated atherosclerotic plaques lead to thrombosis formation and can cause a sudden obstruction in the coronary artery which results in myocardial infarction. Encapsulated micellar NPs of hirudin (natural anti-thrombotic agent) and NPs associated with an irreversible thrombin inhibitor, (D-phenylalanyl-L-prolyl-L-arginyl-chloromethyl ketone) have significantly reduced the intra-arterial thrombosis in an animal study [93].

Instead of noninvasive therapy, several invasive nano-medicine techniques are also used in clinical therapy. After several amendments in stent formation with no failure and side effects, currently biodegradable stent with NPs coating of the antibody has been used to restore the flow of blood [94]. Furthermore, several NPs-based drugs are used to reduce arterial stenosis. For instance, liposomal NPs encapsulated with bisphosphonate have been used to diminish the proliferation by reducing monocytes and macrophages, whereas without NPs encapsulation bisphosphonate could not reach up to the effective dose level in the blood due to its poor cell membrane permeability [95, 96]. In addition, albumin-based NP delivery of paclitaxel (potent mitotic inhibitor and anti-proliferative agent) shows the dose-dependent reduction in stent restenosis [97]. Moreover, nano-medicine for vascular injury at the site of the stent has been delivered in encapsulated liposomal formulations (prednisolone and paclitaxel) shown the reduce restenosis rate in an animal study [98, 99]. Nano-medicine-based nano-fibrous matrix has been developed to accelerate the healing of endothelial at the site of the stent by releasing the NO to stop cell proliferation, platelet adhesion and promote endothelial cell recovery. Besides stent implementation, coronary artery bypass is another invasive technique to restore the obstructed blood flow. Nanotechnology has developed a tissue-engineered vascular graft which is much similar to a normal artery and has been used in coronary artery bypass graft surgery [100]. These studies built hopes to prolong the efficacy of the drug with diminishing the side effect by use of appropriate NPs formulation. The ultimate success of atherosclerotic treatments through targeting vascular endothelium depends not only on the development of novel anti-inflammatory nano-medicine but also on the identification of appropriate target sites, delivery techniques, patient selection, treatment adherence and clinical endpoints.

The diagnostic use of nano-medicine includes the use of the noninvasive assessment of atherosclerotic plaques by MRI using-ultra-small superparamagnetic iron oxide particles (USIOPs) which can quantify the atherosclerotic plaque burden of macrophages and lipid-lowering potential of therapies [101]. Moreover, the cross-linked iron oxide

has been used in monitoring the efficacy of statins through target-specific imaging of adhesion molecules [102]. The release of cytokines for activation of endothelial cells leading to expression of VCAM-1 has been reported [103]. Additionally, detection of inflammation within the plaque has been successfully achieved by NPs targeting VCAM-1 expressing cells [104]. In the progression of atherosclerosis, it has been observed that vasa vasorum (a network of microvessels) undergoes angiogenic expansion with neovascularization reaching into the plaques, which permits the accumulation of NPs. Moreover, the new microvessels showed enhanced permeability due to inflammation [105]. A liposomal NPs preparation containing prednisolone was developed for imaging as well as treatment of atherosclerotic inflammation through a reduction in the macrophage burden [106]. All aforementioned preclinical studies reported the therapeutic potential of nano-medicine in the treatment of atherosclerosis by improving endothelial function from either direct or indirect approaches.

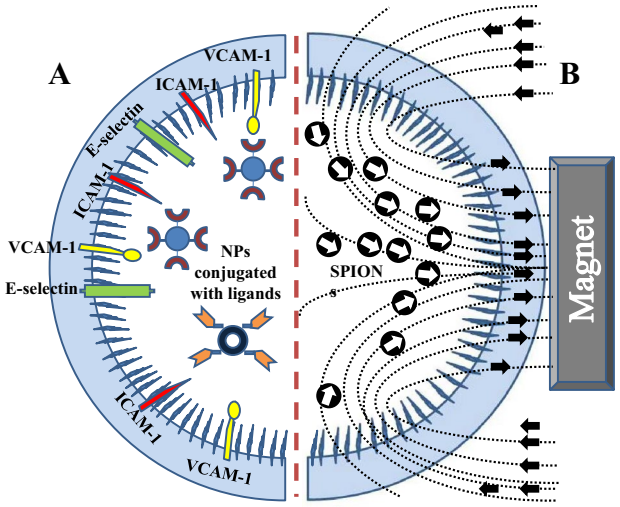

Fig. 2 Various targeting strategies for endothelium **a** molecular targeting, **b** magnetic targeting. *ICAM-1* Intercellular adhesion molecule-1, *NPs* nanoparticles, *VCAM-1* vascular cell adhesion molecule-1, *SPIONs* superparamagnetic iron oxide NPs

Nano-medicine: Innovation in Drug Delivery Strategies for Vascular Endothelium

As mentioned above, only a few nano-medicine techniques are able to make their way to clinical development because merely selecting a drug and its nano-carrier is not enough. The challenge to endothelium drug delivery depends on the varying size of arteries, continuous shear stress by flowing blood and margination from the bloodstream. Various attempts have been made to select appropriate targets and design NPs-formulations but one factor that confirms their effectiveness is the delivery strategy that could enhance NPs binding to the vascular endothelium in micro, small, medium and of course large vessels against continuous flowing blood. Two delivery strategies are most evident in the literature, which are proven to enhance endothelial interaction with NPs; molecular and magnetic targeting (Fig. 2).

Molecular targeting uses the approach of conjugating NPs to specific ligands that activate endothelium to enhance internalization of particles underflow, includes various adhesion molecules such as VCAM-1, ICAM-1, platelet-endothelial cell adhesion molecule-1 (PECAM-1), as well as endothelial selectins. Iron oxide NPs conjugated with peptide sequentially homologous to antigen-4 (a ligand for VCAM-1) have been formulated [107, 108]. This formulation has shown about 12-fold more target to background ratios when compared with VCAM-1 monoclonal antibodies. Further, VCAM-1 expressing endothelial cells were effectively identified in a tumor necrosis factor-alpha-induced inflammatory murine model. The same has been observed in atherosclerotic lesions present in cholesterol-fed apolipoprotein E apoE−/− mice. The recent report has shown the role of anti-ICAM and anti-PECAM conjugated NPs to target endothelium under flow models of endothelial-like cells (EAhy926 cells) and primary HUVECs [109]. However, a relatively slow but effective endocytosis of ICAM-targeted NPs was observed in vivo in mouse pulmonary endothelium. Further, internalization of anti-ICAM-NPs was accelerated by treatment with lipopolysaccharide (LPS) [109]. It is intriguing to note that endothelial cells activation also upregulates selectins, making it a potential molecular target and drug delivery platform in endothelium. In a murine model of inflammation, researches targeted E-selectins by conjugating anti-E-selectin monoclonal antibodies to ultra-small-SPION in vivo for imaging of the endothelium to detect vascular inflammation [110]. Collectively, all these findings suggest that endothelial interacting molecules could be potential targets for drug delivery and internalization of NPs.

In magnetic targeting, an external magnetic field is employed to attract drug-SPIONs conjugate to a specified region of the vascular endothelium. The same has been demonstrated in rabbit and mice tumor models using SPIONs conjugate [111]. Further, research has been extended to a rat myocardial infarction model which showed promising results in term of cardiac repair with intravenous delivery of magnetic nano-beads (MNBs)/adenoviral vectors (Ad)-encoded hVEGF gene and application of an external epicardial magnet to enhance targeting in the ischemia affected area [112]. Studies demonstrated utilization of magnetic targeting in the rat iliac artery [113] and femoral artery [114] with SPIONs conjugate using a strong external magnetic field. Hitherto, experimental studies on the magnetic

targeting of the endothelium are sparse but existing studies showed promising results.

Concluding Remarks

VED is the hallmark of various cardiovascular disorders. Thus, treatment of VED should be beneficial. Various available treatment options bear risks of unwanted adverse effect, which encourages the search for newer treatment strategies, such as nano-medicine, with more target specificity and efficacy. With the availability of numerous endothelium-targeting options, it becomes vital to select a rational nano-medicine design with an appropriate targeted site, ligand, and nano-carrier. Shear stress under continuous flow, varying size of arteries and its margination from bloodstream affects nano-medicine internalization in endothelium. Therefore, various delivery strategies have been developed to provide sufficient transport of medication to specific regions of the vascular endothelium. Despite various unfavorable conditions, molecular and magnetic targeting are two in vivo, potential and evidenced strategies to overcome physiological shear stress. However, further translational studies are scanty as elucidation of safety and efficacy of NPs and their delivery strategies remains a challenge. In order to progress to clinical development, NPs should interact and internalize in endothelium effectively, proven to be non-toxic, more effective than existing options, easy to use and should be cost plus design effective. With considerable bench work but only a handful of clinical studies, nanotechnology still holds hope for the treatment of VED.

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Compliance with Ethical Standards

Conflict of interest No conflict of interest is declared.

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