CHEMICAL REVIEWS

REVIEW

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Nitric Oxide: A Guardian for Vascular Grafts?

Achala de Mel,[†] Ferid Murad,[‡] and Alexander M. Seifalian*[,†,§,∥]

[†]Centre for Nanotechnology & Regenerative Medicine, Division of Surgery & Interventional Science, and [∥]London Centre for Nanotechnology (LCN), University College London, London, United Kingdom.

[‡]Institute of Molecular and Cellular Signaling, Department of Biochemistry and Molecular biology, George Washington University, Washington D.C.

[§]Royal Free Hampstead NHS Trust Hospital, London, United Kingdom

CONTENTS

1. INTRODUCTION

Cardiovascular disease is a major cause of mortality and morbidity. Current research investigating the roles of nitric oxide (NO) with relevance to the cardiovascular system is an area of immense interest. NO is recognized for its protective role within the vasculature, as well as its influential role in many related physiological systems. In particular, NO is a desirable molecule in research focused on cardiovascular interventions, and thus, more recently, a flurry of research in the field of cardiovascular surgery and biomaterials has placed NO-eluting cardiovascular stents in the spotlight. NO is a diatomic, nonpolar stable free radical with a N−O bond length of 1.15 Å and was first identified by Joseph Priestly in the late 18th century. The unpaired electron, residing in a π^* molecular orbital, confers a great affinity for other biological molecular species with lone pairs of electrons, as well as d_π orbitals of transition metals, notably iron (Fe^{2+}). It was first considered to be a simple gaseous molecule until the 1980s, when it was found to be present in vascular endothelial cells (ECs) and to perform a significant role in facilitating acetylcholine's vasodilatory effect, called endothelial-derived relaxing factor (EDRF).[1,2] Later, it was recognized that this vasodilatory factor was indeed NO.[3] NO is produced and released endogenously by endothelial cells (ECs) at a rate of approximately $(0.5-4.0) \times 10^{-10}$ mol cm^{-2} min^{-1}, and its rapid diffusion across the cell membrane is faciliated by aquaporin-1 channels.[4] It was found that NO is a potent stimulant of soluble guanylyl/guanylate cyclase (sGC), which catalyzes the conversion of guanosine 5′-triphosphate (GTP) to cyclic guanylate monophosphate (cGMP), which, in turn, regulates many significant biochemical pathways in the vascular system.[5−8] NO has been found to interact with a range of metalloprotein-containing structural complexes including hemoglobin[9,10] and has been associated with many biochemical and physiological processes.[11,12] Most interestingly, the cardiovascular protective role of the endothelium has been attributed to NO. The endothelium is in intimate contact with the blood flow and consists of a single layer of ECs that functions as a dynamic organ and covers the entire surface of the circulating system from the heart to the smallest capillary.

Cardiovascular disease is associated with a relatively high degree of mortality and morbidity. Coronary artery bypass graft (CABG) surgery, percutaneous transluminal coronary angioplasty, and stenting are current options for treating occluded or stenosed coronary vessels.[13] For patients requiring bypass surgery, the saphenous vein, internal mammary artery, internal thoracic, or radial artery are the grafts of choice; however, 5−30% of patients have no suitable veins or arteries available because of previous use or diseased vessel walls. Currently, commercially available synthetic bypass grafts are made from either expanded polytetrafluoroethylene (ePTFE) or poly(ethylene terephthalate) (Dacron). Although these are adequate for larger vessels (with internal diameters of >6 mm), they are not suitable for smaller-diameter vascular bypass surgery, resulting in a high rate of graft failure, thus leading to life-threatening conditions or requiring "revision" surgery. For example, bypass graft surgery performed with ePTFE for lower limb occlusion has a patency of 25% after 5 years, and 75% will be blocked and require revision surgery, with a large number leading on to amputation.[14−16] Vascular stents that are used to repair occlusions up to 70% of the native vessels also require a greater modulation of their antithrombogenic effects: despite the success of current modifications to bare-metal stents with cell-proliferation-inhibiting drugs such as sirolimus and paclitaxel, stents have also proved to induce late thrombosis and restenosis.[17−21] Furthermore, there is evidence to suggest that drug-eluting stents might inhibit EC adhesion and proliferation.[22] The overall success of cardiovascular interventions is limited

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because of thrombosis and the formation of intimal hyperplasias (IHs) and ischemia reperfusion injuries (IRIs), which occur during surgical interventions.[23−27]

Thrombotic complications are commonly addressed with antiplatelet therapy,[28] but they are not without their complications. Long-term use of systemic anticoagulant drugs such as heparin and warfarin has been shown to be associated with several side effects, and there are concerns of reduced hemostasis associated with increased risk of bleeding.[29] In addition, a significant number of grafts encounter early graft occlusion despite anticoagulants such as aspirin because of a condition that could be described as aspirin resistance.[30] Thus, there is great interest and potential in the quest to develop cardiovascular devices that resist thrombosis. Vascular interventional procedures are associated with injury to the luminal endothelium by surgical trauma including stretching of the vessels, altered flow dynamics at the site of anastomosis, and distal outflow segments. For example, angioplasty is a controlled traumatic event aimed at causing plaque rupture. However, the absence of a fully functional endothelium (as with synthetic polymeric grafts) or damage to the native endothelium impairs the synthesis of protective NO. The in situ endothelialization of cardiovascular implants including bypass grafts following implantation has been recognized as a favored solution for enhancing graft patency rates.[16,31−33] However, there is a time interval of approximately 6 weeks between bypass graft implantation and complete endothelialization during which the adverse effects associated with platelets such as adhesion, aggregation, and activation can be pronounced. Therefore, the enhancement of the protective mechanisms associated with NO elution in situ postimplantation over time is of keen interest.

Complications associated with ischemia reperfusion injuries are currently addressed with the therapeutic process of preconditioning. The efficacy of preconditioning, which is the process of periodic vascular occlusions followed by reperfusion events, is yet to be confirmed, as the procedure is time-consuming; therefore, an alternative expeditious procedure is desirable. IRIs, which demonstrate a great and complex association with NO but without a clear indication of NO's defined role, are an interesting topic of discussion, as NO might hold the key to the modulation of IRIs. The significant vascular protective features of NO apart from antithrombogenic properties and IRI include antiproliferative and migration inhibitory effects on smooth muscle cells (SMCs), which is often associated with IH[34,35] and antibacterial surface properties.[36,37]

Considering the vast amount of information regarding NO in physiological systems and endeavoring to maintain a focus on cardiovascular interventions, this article is organized under two main themes. First, the article aims to address and highlight the significance of NO in thrombosis and IRIs associated with vascular interventions, as such processes limit the rate of graft patencies. Thus, the article presents both in vitro, in vivo, and clinical studies. Second, the most recent research into the biochemistry of NO formation, regulation, desensitization, and modulation in the circulation is reviewed. This theme also includes a discussion of varied possibilities of functionalizing grafts to activate the NO−sGC−cGMP pathway to obtain potent effects and to enhance the protective functions and elevate the success rate of cardiovascular graft surgery. However, the two themes are not mutually exclusive. References are presented for further consideration by the interested reader for more detailed discussions related to the core chemistry of NO.

2. NITRIC OXIDE IN MODULATING THROMBUS FORMATION

Graft thrombogenicity is a major complication that limits cardiovascular graft patency rates and surgical success. At sites of vessel injury in the absence of NO, cascades of events lead to thrombus formation. Basic mechanisms of platelet adhesion and aggregation that would occur in a natural vessel in the event of vascular injury indicate similar features on a thrombogenic polymer surface (Figure 1). After implantation and exposure to blood flow, serum proteins are instantly adsorbed onto luminal surfaces of the grafts by a dynamic process known as the "Vroman effect", and these proteins act as ligands for platelet receptors. Enhanced platelet interactions with the material surface also can be facilitated by topographical events and irregular flow patterns due to mismatch of mechanical properties between native arteries and bypass grafts. Hence, current research is aimed at studying surface modifications and enhancements in the design of existing cardiovascular implants to achieve optimal and favorable interactions with blood and host tissues.

NO is recognized to be a highly reactive multifunctional molecule with ideal functional properties that is desirable for maintaining graft integrity, particularly in the absence of a fully functioning endothelium (Figure 2). It has been shown that NO can directly influence platelets to induce antithrombotic effects.[38] Figure 3 illustrates the roles of NO following its diffusion from the ECs across the platelet membrane and within the platelets after binding to the heme moiety of sGC. Binding interactions mediated by vWF $\alpha 2\beta 3$ integrin and glycoprotein iβ are required for platelet adhesion and aggregation and were found to be regulated by NO donors.[39] S-nitrosoglutathione (GSNO), a NO donor, was shown to inhibit platelet aggregation on immobilized vWF, but no effect was apparent in platelet adhesion. The protein thrombospondin-1 has been found to act as a promoter of platelet aggregation and inhibitor of antithrombotic activity of NO by exerting an inhibitory effect on the NO−cGMP pathway.[40] NO has also been observed in the phosphorylation of $\alpha 2\beta 3$ integrin interactions and found to mediate platelet adhesion to immobilized fibrinogen and $\alpha 2\beta 1$ interactions with collagen. NO has been shown to directly regulate integrin $\beta 3$ phophorylation and S-nitrosylation of cysteine residues, thus interfering with the platelet−extracellular matrix interactions by reversing the activated integrins on platelets to a more stable inactive state.[41−44] Factor X111 (fibrin-stabilizing factor) is the last factor in the coagulation cascade that stabilizes the platelet plug leading to the formation of a stable clot. NO has been shown to inhibit factor X111 both in vitro and in vivo by S-nitrosylation of one of its highly reactive cysteine residues.[45] Despite the major similarities, NO synthase (NOS) in platelets is distinct from NOS produced in ECs.[46] Arachidonic acid was found to be one such inhibitor that reduces NO and cGMP levels in platelets, thus enhancing their aggregatory effects.[47] Aspirin is known for its antithrombogenic effects and has been found to increase the production of NO by enhancing NOS activity in platelets and decreasing NOS phosphorylation. When NO levels were determined from cGMP measurements, it was found that aspirin had a stimulatory effect on NO synthesis. The increase in synthesis was found to be through the acetylation of NOS, which was determined by liquid chromatography tandem mass spectrometry (LC/MS/MS).[48]

Both endogenous and exogenously introduced NO can efficiently modulate platelet activation under in vivo conditions to

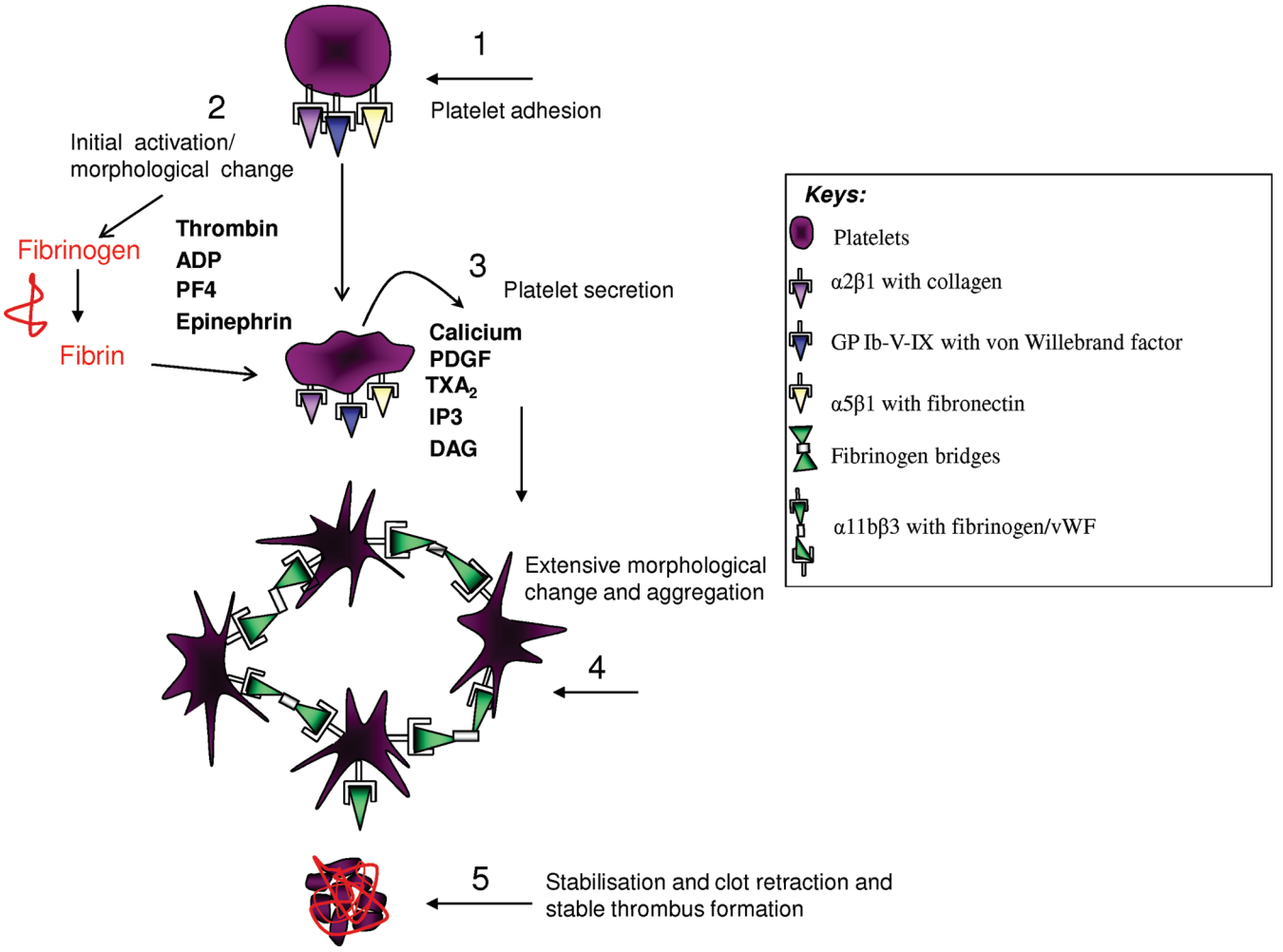

Figure 1. Platelet activities in thrombus formation in the absence of nitric oxide. The role of platelets in thrombus formation includes the following steps: (1) Platelet adhesion, which involves interactions of $\alpha 2\beta 1$ with collagen, GP Ib-IX with the von Willebrand factor (vWF), $\alpha 5\beta 1$ with fibronectin, and $\alpha 11b\beta 3$ (GP11b/111a) with fibrinogen/vWF. (2) Initial activation and shape change, in which adhered platelets initially become activated with the interactions with thrombin, ADP, epinephrine, and platelet factor-4 and thrombin converts fibrinogen to fibrin, which also contributes to platelet activation. NO mediated through protein kinase A has been shown to have a potent effect on inhibiting the shape change of platelets, which is the initial step of platelet activation. (3) Secretion of platelet substance, whereby the primary activation of the preceding factors induces the release of signaling molecules including calcium, PDGF (platelet-derived growth factor), IP3 (inositol 1,4,5-trisphosphate), and DAG (diacylglyerol); the liberation of arachidonic acid; and the conversion to prostaglandins and lipoxygenase products. (4) Extensive shape change and aggregation. Upon activation, platelets undergo a shape change and becomes spherical, elongated with pointed pseudopodia. Thus, platelets respond with distinguishable morphological changes, followed by aggregation with the formation of fibrinogen bridges through interactions particularly with $\alpha 11b\beta 3$ of activated platelets. (5) Thrombus formation. Modified with permission from ref 222. Copyright 2006 Nature Publishing Group.

provide antithrombogenic surface properties.[38,49,50] Patients with advanced coronary risk factors, with conditions related to unstable angina and myocardial infarction (MI), show less NO produced in situ than healthy patients (both endothelium-derived and platelet-derived NO) and impaired responses to NO production and increased platelet activation.[51,52] A study in which healthy volunteers were administered with intravenous infusion of NO synthase inhibitor L-NAME showed increased blood pressure and reduced phosphorylation of platelet vasodilator-stimulated phosphoprotein, a known indicator of NO signaling, whereas the administration of the NO donor glyceryl trinitrate (GTN) restored the levels of markers for platelet activation to normal physiological levels.[53] L-Arginine, which is a precursor of NO, when applied in a porcine model of deep venous thrombosis (DVT), has been demonstrated to preserve endothelial vasoreactivity and reduce platelet adhesion.[54] A great number of articles have presented comprehensive reviews on the potential antithrombogenic properties of NO donors incorporated into biomedical devices.[55,56] Figure 4 is an illustration of the main chemical functionalizing methods that are currently in research aimed at NO elution from vascular grafts.

We have developed[57] and patented[58] a family of nanocomposite polymers that is based on poly(carbonate urea)urethane (PCU)[59] and functionalized with S-nitroso-N-acetylpenicillamine (SNAP) covalently attached to polyhedral oligomeric silsesquioxane (POSS) nanoparticles, which self-assemble on the surface, thus rendering greater hydrophobic properties and relatively prolonged retention in the graft. POSS−PCU was extruded by combining porogen leaching and phase-inversion coagulation

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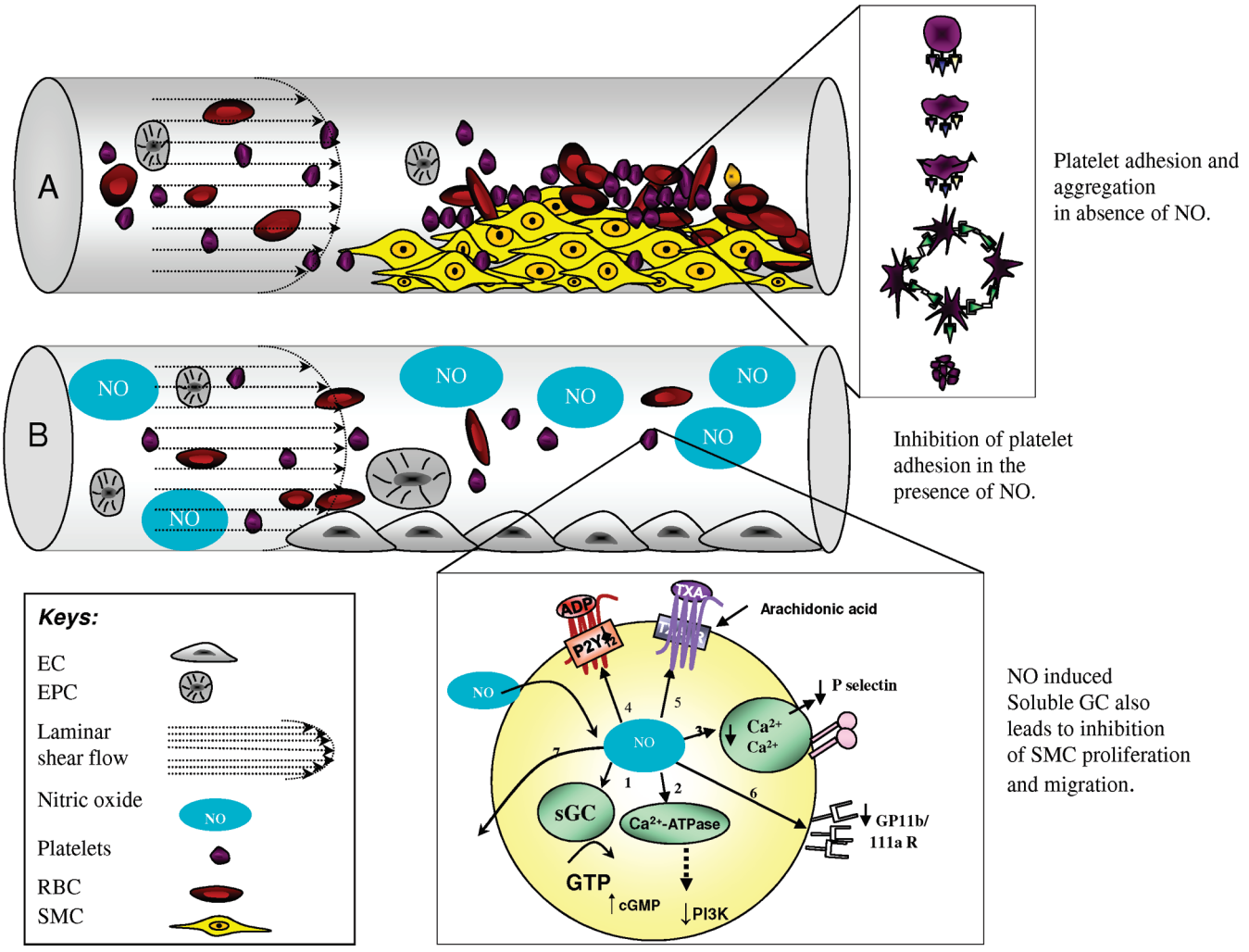

Figure 2. Biological effects on grafts that release NO and pathological events on graft surface in the absence of NO. (A) Small-diameter vascular grafts following implantation tend to fail mainly because of thrombosis and intimal hyperplasia. Biologically and mechanically unoptimized vascular grafts do not promote endothelialization, but they do facilitate the adhesion and proliferation of SMC from the site of anastomosis as a response to injury and lead to overall thrombus formation with the adhesion of RBC and platelets on graft lumen, leading to graft occlusion. Highlighted are the molecular events leading to platelet adhesion, aggregation, and thrombus formation. (B) The presence of NO prevents platelet adhesion and aggregation, SMC proliferation, migration as well as promotes endothelialization thus maintaining a smooth blood flow through vascular grafts. Highlighted are the NO-mediated mechanisms that modulate platelet activities and are discussed further in this section. Modified with permission from ref 223. Copyright 2003 Nature Publishing Group.

techniques into small-diameter conduits[60] to have a porous luminal interior that behaves mechanically similarly to a natural artery with similar viscoelastic properties. The NO-donor-incorporated POSS−PCU grafts have demonstrated excellent antithrombogenic surface properties compared to commercially available ePTFE grafts, when they were tested in vitro as well as in preclinical trials in a large animal (sheep) model under good laboratory practice (GLP)/good manufacturing practice (GMP) conditions (Figure 5) and are currently undergoing clinical trials.

2.1. Stimulation of Endothelial Cell Proliferation and Inhibition of Apoptosis

NO is recognized as a major regulator of endothelial progenitor cell (EPC) mobilization, differentiation, and function. Most interestingly, NO mobilization of progenitor cells has been shown to be specific for EPCs with the ability to distinguish from hematopoietic stem cells.[61] This is certainly of interest with the prospects of employing NO to mobilize high-quality EPCs from the bone marrow, without the potential deleterious side effects of currently used EPC-mobilizing agents where a heterogeneous cell population becomes mobilized. Many other factors invovled in EC migration, such as adhesion and proliferation, are found to be linked to NO. For example, angiotensin II has been demonstrated to promote significant levels of NO and inhibit bone-marrow-derived EPC apoptosis, and these effects have been shown to be reversed when NO inhibitors were applied, thus demonstrating the NO-mediated effect on EPC adhesion and inhibition of apoptosis.[62] Erythropoietin is recognized for its significant roles in the vascular system,[63] and its role in EPC mobilization, re-endothelialization, and prevention of IH has been shown to be through the activation of eNOS.[64,65]

It has been shown that IGFBP-3 can increase eNOS expression in human EPCs, thus leading to NO generation and cell

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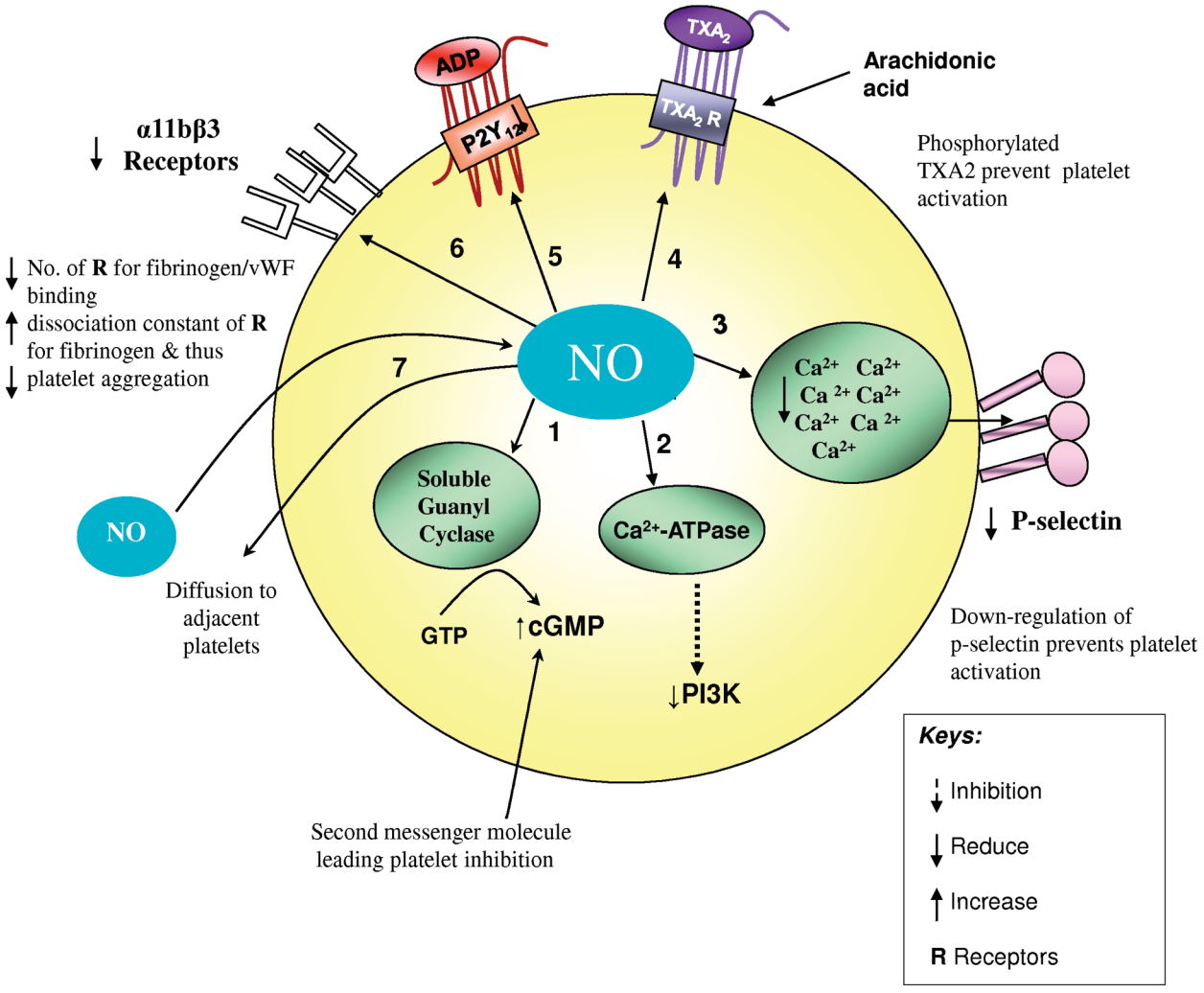

Figure 3. Mechanisms of platelet inhibition mediated by NO. NO-mediated mechanisms that modulate platelet activities are illustrated. NO suppression of platelet activity is mainly mediated through sGC. NO diffuses across the platelet membrane/synthesized from platelet NOS, and within the platelet, it binds to the heme moiety of sGC. NO acts on several targets that are recognized by antiplatelet drugs, as shown in the figure: (1) generation of cGMP by NO binding to the heme moiety of sGC and facilitation of catalysis of GTP to cGMP, which acts as a second messenger; (2) enhancement of calcium ATPase-dependent refilling of intracellular calcium stores and inhibition of PI3K activation; (3) as a result of 1 and 2, suppression of intracellular Ca^{2+} and downregulation of P-selectin expression; (4) induction of phosphorylation of thromboxane A2 (TXA2) receptors and prevention of TXA2-mediated activation, as well as reduction of TXA2 synthesis through inhibition of the conversion of arachidonic acid to prostaglandin G2 and H2; (5) action of cGMP-dependent protein kinases to inhibit platelet P2Y12 and thus suppress platelet activation; (6) modulation of fibrinogen cross-linking through $\alpha 11b\beta 3$ by downregulation of $\alpha 11b\beta 3$ available for fibrinogen and inhibition of $\alpha 11b\beta 3$−vWF binding, as well as increased dissociation of $\alpha 11b\beta 3$ fibrinogen; and (7) diffusion to adjacent platelets and prevention of thrombosis.

migration.[66] A study performed in eNOS-deficient mice has demonstrated that eNOS expression in bone marrow cells is a major regulator of EPC (CD34+/Flk-1 + progenitor cell) mobilization, where the NOS-null mice were not able to recover from defective neovascularization following a bone marrow transplant but positively responded to an intravenous (IV) infusion of wild-type EPCs, thus demonstrating the impaired mobilization of bone marrow in NOS knockouts.[67] It has also been shown that CO-dependent accelerated EC proliferation and bone-marrow-derived EPC mobilization is mediated by NO.[68] The direct introduction of NO has an effect in enhancing EC adhesion and proliferation, and hence, NO-exuding materials have the potential to promote graft endothelialization.[69] The nitrates derived from pentaerythritol trinitrate (PETriN) and isosorbide dinitrate (ISDN), which induce lower oxidative stress on EPCs, have led to greater increased levels in the circulation.[70]

Hyperglycemia associated with diabetes mellitus is known to be linked to endothelial dysfunction and production of reactive oxygen species (ROS) and reduced EC superoxide dismutase (SOD) activity, and this is in parallel with EPC dysfunction. This influences NO bioavailability, and thus vascular conditions with a NO deficit become aggravated through interactions of available NO with the rapid production of free radicals, leading to higher oxidative stress.[71] Homocystein, which is a thiol-containing amino acid, is known to cause endothelial dysfunction by causing EC apoptosis and vascular lesion formation. NO in the form of exogenous donor SNAP and adenoviral transfer of iNOS gene transfer has been shown to suppress endothelial damage induced by homocystein, and NO donors

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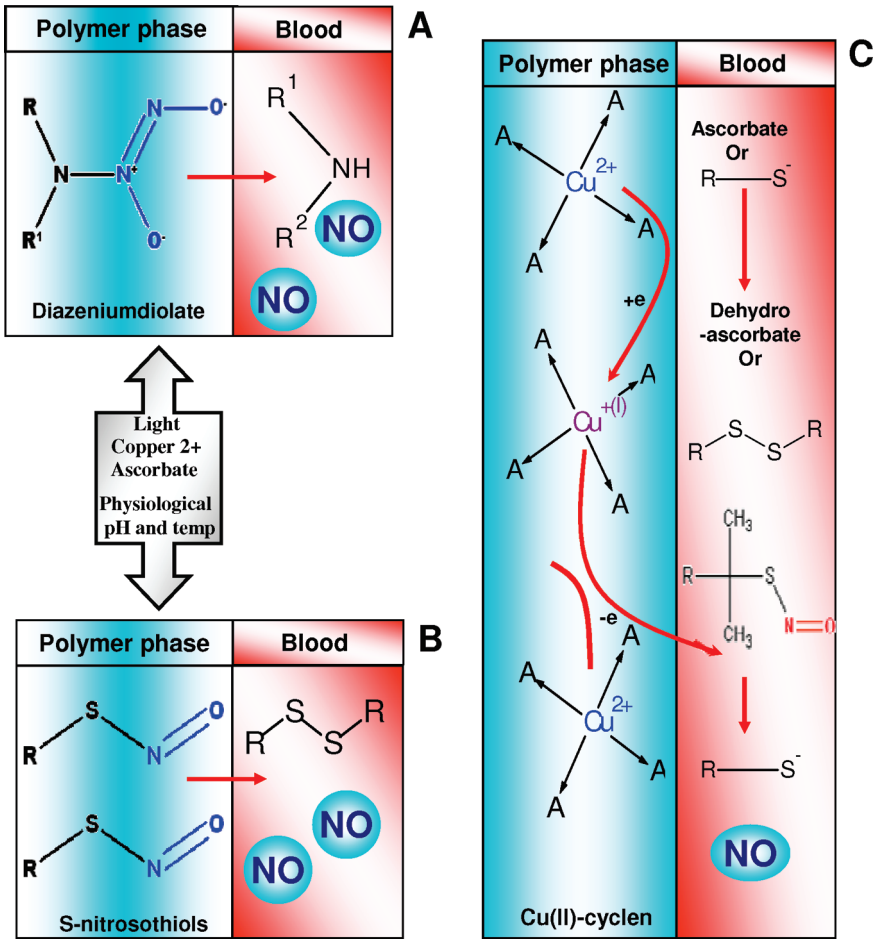

Figure 4. Current methodologies for NO donor incorporation into polymers. Polymer phase (in blue) indicates the compounds that can be integrated into polymeric implants, to interact with the compounds naturally present in blood (in red) to produce NO when the implants are introduced into the vascular system. Immobilization of (A) diazeniumdiolate $\{[N(O)NO]_2\}$ and (B) S-nitrosothiols (RSNO) in polymers, which act as direct NO donors and can promote NO release under physiological conditions. These reactions can be catalyzed by factors such as light, copper, and reducing agents such as ascorbate. (C) Endogenous catalysis of NO formation by immobilized metal ions serves as an alternative to direct NO donors. Immobilization of lipophilic copper complexes[224] such as Cu(II)-cyclen is illustrated. These metal-immobilized polymers can produce NO when in contact with plasma nitrosothiols . NO release is facilitated in the presence of a reducing agent such as ascorbate and glutathione. Organoselinium has also been immobilized in polymers to facilitate NO release by a similar mechanism.[225] This illustration presents an overview of the possible methods of direct synthesis of NO that can be adapted for use in a range of cardiovascular implants. However, these basic methods need to be adapted and optimized to suit particular polymers and the cardiovascular implants of interest.

also suppressed the expression of stromal-cell-derived factor, which is induced by homocystein.[72]

3. ROLE OF NO IN ISCHEMIA REPERFUSION INJURY (IRI)

Patients suffering from acute myocardial infarction (MI) can be effectively treated with prompt restoration of the blood flow with thrombolysis, percutaneuos coronary interventions, or bypass graft surgery. However, in the 1960s, a study in a canine model identified a paradox, where the expeditious myocardial reperfusion following coronary ligation caused additional injuries to the cardiomyocytes; this is referred to as reperfusion injury.[73] However, delayed reperfusion has not proven to be beneficial and has been shown to aggravate organ injury.[74] A recent study performed to determine the optimal time for CABG following MI was found to be 3 days and had a better outcome compared to that performed after 2 days.[75] There is a high mortality rate for patients with acute MI associated with reperfusion injury, which leads to poor clinical outcomes including mortality. Histological evidence of IR has been reported in approximately 25−45% of patients who died after CABG surgery.[27]

IR injury due to cardiovascular surgery is different from naturally occurring MI and is associated with characteristic changes in functional and molecular features, including reperfusion arrhythmias; microvascular dysfunction; low cardiac output; myocardial stunning; altered cytosolic and mitochondrial Ca^{2+} levels; mitochondrial metabolism; permeability; cytoskeletal fragility; and alterations in noncardiomyocytes, which include platelets, ECs, and inflammatory reactions.[76,77]

Hypergeneration of free radicals including superoxide (O_2^-), peroxynitrite ($ONOO^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH) is a major mechanism of concern, as these can trigger cellular damage by interacting with proteins and lipids and opening mitochondrial permeability pores, which leads to a cascade and cyclic increase in free-radical generation.[78] Free-radical oxidative stress notably leads to depletion of tetrahydrobiopterin

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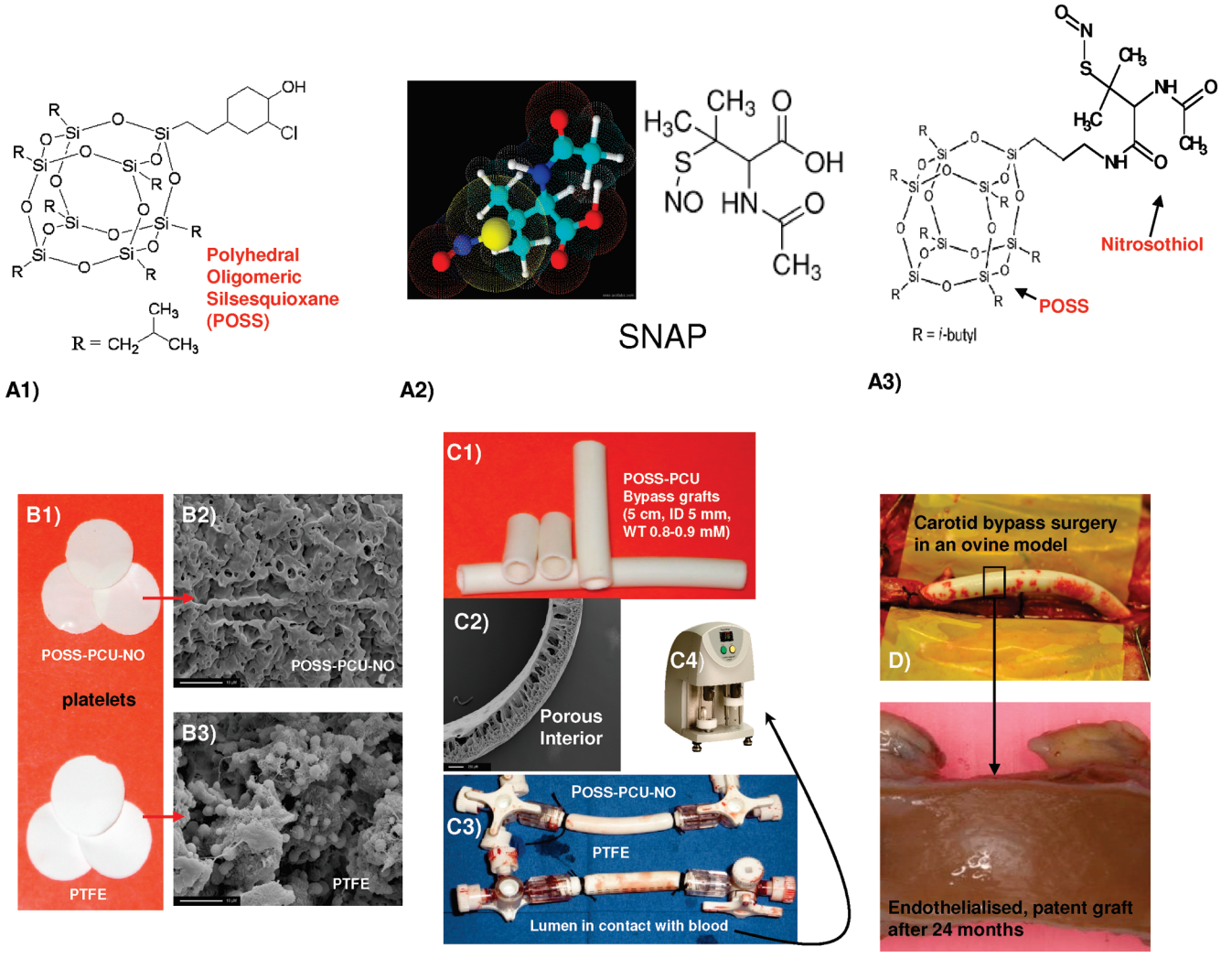

Figure 5. NO-eluting, compliant, small-diameter vascular bypass graft. Small-diameter vascular bypass grafts synthesized using POSS—PCU with a novel form of SNAP that is conjugated to POSS nanoparticles, which renders greater retention in the polymer and controlled release over a relatively longer period of time in the presence of pulsatile blood flow.[226] (A1) POSS-nanoparticle-incorporated polyurethane urea polymer used to fabricate cardiovascular implants with greater biocompatibility, biostability, and ideal mechanical properties (A2) SNAP, a nitrosothiol that is a potent NO donor. (A3) Our group has designed a novel SNAP that is conjugated to POSS nanoparticles and then incorporated into POSS—PCU polymer. Anchoring SNAP to hydrophobic POSS renders the molecule more stable in water, which is highly favorable during the process of graft fabrication, which involves extensive periods of contact in water. In addition, POSS has been shown to facilitate surface assembly, and thus, SNAP adhesion to POSS enables surface assembly of SNAP, thus facilitating release of NO in the presence of catalysis. (B1) Porous nanocomposite polymer and ePTFEs are introduced with platelets on the surface and incubated at 37 °C on a shaker. SEM images of the washed polymer samples following the platelet incubation are shown. (B2) Porous interior of POSS—PCU—NO with no platelets attached. (B3) Highly aggregated platelets that form pseudopodia on ePTFE, thus demonstrating a thrombogenic tendency. (C1) Small-diameter vascular bypass grafts synthesized using POSS—PCU—NO. (C2) SEM image of the porous interior of a cross section of a graft. (C3) Vascular graft lumens introduced with whole blood and incubated at 37 °C on a gentle rotator. (C4) Kinetics and thrombogenic characteristics of blood that was in contact with graft surfaces were tested using TEG. (D) A 75% patency was observed at the end of the preliminary studies in a large animal model. Currently, the grafts are undergoing preclinical trials in sheep under GLP to proceed to clinical applications.

(H_4B)[79] and dysfunctioning of NOS, thus reducing the synthesis of NO by uncoupling the enzyme and leading to O_2^- and free-radical synthesis instead of NO.[80] L-Arginine and H_4B have been shown to ameliorate IR-induced endothelial dysfunction in patients with coronary artery disease, as evidenced by restored endothelium-dependent vasodilation.[81] NO acts in hypoxic conditions to lead to greater myocardical oxygen delivery and consumption,[82] and cytochrome c oxidase has been found to play a major role in regulating NO. At higher oxygen concentrations, cytochrome c oxidase is predominantly in an oxidized state and binds reversibly to NO when compared with low oxygen states.[83,84] NO is primarily recognized to be a protective from IRI, and removal of NO has been shown to elevate IR injury in experimental models,[85,86] but its role in protection from IR injury is not without controversy. However, the protective effects of NO in IRI have been recognized to be possibly associated with a range of reactions including reaction with superoxides, vascular smooth muscle relaxation, antiplatelet activating factor, antiadhesion molecules, cytokine modulation, vascular permeability modulation, protection of the microvasculature integrity, antimicrobial effects, antiapoptosis effects, and antiendothelin effects.[87] Table 1 presents a summary of recent

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Table 1. NO-Inducing Molecules in Models of Ischemia Reperfusion Injury (IRI)[a]

bioavailability/activation of NO	mechanism of IR induction	model	outcome, influence of NO
BAY-58 (1−50 nM) infused for 60 min before R[211]	30 min I + 120 or 180 min R	rabbit	significant ↓ of infarct size in isolation and in situ
arginase inhibitor *N*-omega-hydroxy-nor-L-arg (nor-NOHA)[231]	30 min of coronary artery ligation + 120 min of R	rat	↓ infarct size by 79−39% ↑ plasma nitrite; 10× ↑ in the citrulline/ornithine ratio = arginine utilization toward NOS
NAC administered intravenously during operation[232]	standard CABG operation procedure	20 human CABG operation	lower levels of luminol, lucigenin CL, and TNF-α CK-MB enzyme ↓ inflammatory response, ↓ myocyte death and myocardial dysfunction
HMR1766 (1 μmol/L) or DEAnonoate (0.5 μmol/L) used to activate sGC[77]	in vitro induction of I	cultured coronary EC monolayers and isolated saline-perfused rat hearts	↑ Ca^{2+} sequestration in the ER and attenuation of the R-induced ↑ in cytosolic [Ca^{2+}]. inactivation of the EC contractile machinery ↓ edema development
NO, NOS3 gene transfer[233]	occlusion of the LAD artery for 45 min + 4 or 72 h of R	IR injury in a porcine model	attenuated IR injury, Significantly smaller MI size in AdNOS3 than in control ↓ inflammatory cell infiltration.
S-nitrosocysteine (CysNO) Perfused with CysNO (10 μM) for 30 min before I[234]	30 min of I + 120 min of R	9−12-week-old rat hearts	↑ tissue *S*-nitrosothiol (RSNO) improved postischemic contractile dysfunction, and attenuated necrosis
intra-arterial infusion of equimolar concentrations 6*R*-5,6,7,8-tetrahydro-L-biopterin (6*R*-H_4B) and its stereoisomer 6*S*-5,6,7,8-tetrahydro-L-biopterin (6*S*-H_4B)[235]	20 min of I + 15 min of R	48 nonsmoking healthy volunteers	continuous infusion of 6*R*-H_4B, 6*S*-H_4B during IR preserved the response to ACh dilation and protection from RI
intravenous NAC[236]	NAC orally before CABG, 150 mg/kg bolus of intravenous NAC before skin incision + perfusion at 12.5 mg/(kg h) over 24 h	100 patients	no improvement in clinical results or biochemical markers
L-arg or L-NAME[237]	45 min of I followed by 2 h of R	rat (1) sham laparotomy, (2) IR, (3) IPC, (4) L-arg + IR, (5) L-NAME + IPC + IR	IPC, ↑eNOS expression both IPC and L-arg (compared with IR) ↑ NO_x, improved serum liver enzymes. effects prevented by L-NAME
introducing NAC, plasma RSNO was observed[238]	60 min of liver I + 7 h of R	rabbit	NAC prevented increase in RSNOs

[a] Key: ↑, increased; ↓, decreased; I, ischemia; R, reperfusion.

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studies performed to investigate the role of NO in experimental models of IR.

Ischemia-induced acidosis is known to reduce guanylyl cyclase (cGC) activity, thus creating a NO-unresponsive state despite the high levels of NO; therefore, cGMP activation (although possibly not solely because of an increase in NO concentration) is considered as a promising strategy for lowering MI.[88] Xanthine oxidase (XO), a metalloflavoprotein, is involved in superoxide synthesis and H_2O_2 free-radical generation during reperfusion using xanthine and hypoxanthine, which is produced in abundance during ischemia and thus plays a central role in inducing oxidative injury. NO has been shown to inhibit XO. High concentrations of NO are not considered favorable and are involved in mediating peroxynitrite formation. However, free radicals in IR injury might not all be detrimental and might actually play a cardioprotective role,[89] for instance, peroxynitite and NO in the presence of superoxide can inhibit xanthine oxidase in a dose-dependent manner through the disruption of the molybdenum active site of the enzyme.[90] Ischemic preconditioning is a proposed mechanism to modulate the detrimental effects of IR, which is found to occur through upregulation of NOS[91] and the absence of eNOS, resulting in the removal of the cardioprotective effect of ischemic preconditioning.[92] The upregulation of NOS has been shown to be possibly linked to recruited EPC,[93] although it has been shown that exogenous NO can enhance EPC upregulation. iNOS expressed in cardiomyocytes in the late phase of ischemic preconditioning has shown to impart cardioprotective effects through decreased expression in free radicals and inhibition of mitochondrial permeability.[94] Overexpression of eNOS has shown to maximally protect against IRI in a similar fashion to preconditioning in a study in which the infarct sizes were compared in a mouse heart model.[95] Overexpression of nNOS in cardiacmyocytes has also shown to be cardio protective through nitrite mediated inhibition of mitochondrial function in addition to reduction in reactive oxygen species resulting a significantly reduced infarct size.[96]

During impaired ability of NOS, NO can become available through nitrates and nitrites, thus conserving the cellular energy used in oxidation.[97,98] Nitrates and nitrites, which are available from dietary sources and also from endogenous stores, are formed as oxidation products of NO (Figure 6). The main reduction mechanisms of nitrates and nitrites that lead to NO synthesis include reduction by deoxyhemoglobin, cytochrome c oxidase, protons, and XO, which have been reviewed in detail elsewhere.[99−101] The mechanisms of NOS phosphorylation can also be considered as protective against IRI associated with myocardial injury as human erythropoietin, which plays a role in such NOS modulation has been shown to be protective while being involved in enhancing coronary endothelial NO synthesis.[102]

Elevation of NO and cGMP has been observed during sustained ischemia, which might be a feedback mechanism. The summary of recent studies (Table 1) underscores a clear role for NO in reducing IRI, as well as a possible role in ischemic preconditioning. However, the detailed mechanisms of these procedures are yet to emerge and thus warrant further studies to clarify the role of NO.

4. BIOCHEMISTRY OF NO: VASCULAR NO SYNTHESIS AND BIOAVAILABILITY

NO synthesis in the cells is catalyzed by three isoforms of NO synthase (NOS), namely neuronal NOS (nNOS or NOS1),

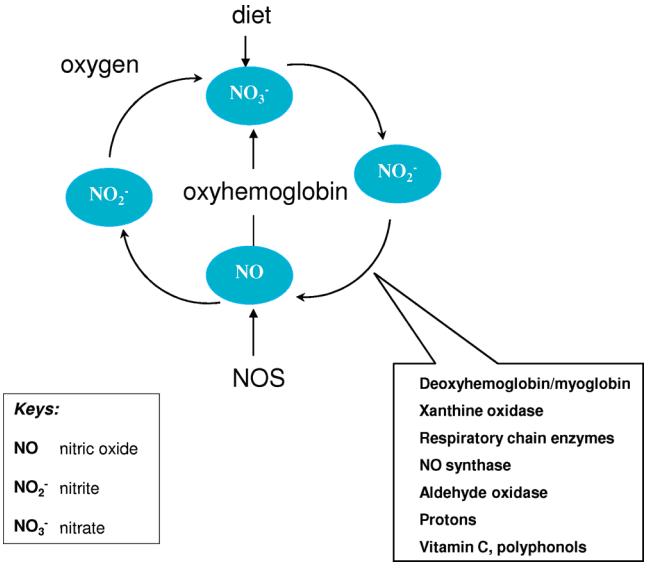

Figure 6. Main mechanisms of NO synthesis in addition to NOS. The bioactivity of NO is acutely terminated by its rapid oxidation to nitrite and nitrate. In blood and tissues, nitrite can be further metabolized to NO and other biologically active nitrogen oxides by enzymatic and nonenzymatic pathways, most of which are greatly enhanced under hypoxic conditions. Adapted with permission from ref 100. Copyright 2009 Nature Publishing Group.

inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3).[103,104] Thus, a great deal of research focuses on the mechanisms of regulating the synthesis of NO as diagnostic and prognostic approaches to vascular disease. Table 2 presents the distinct features between the NOS isoforms, and Figure 7A illustrates NOS, which is a heme−thiolate enzyme that shares features with cytochrome P450, except that it has a unique association with H_4B and calmodulin. NO is generated by oxidation of the terminal guanidino nitrogen of L-arg in the presence of dimerized NOS (Figure 7B).[105,105] L-Arginine, which has a major role as a NOS precursor, has significant implications in coronary atherosclerosis. Availability of L-arg (which is found to be 1−2 mmol/L in freshly isolated ECs) is therefore a critical factor in determining eNOS activity.[106]

Various endogenous NOS inhibitors such as asymmetrical dimethylarginine (ADMA) and N-guanidino monomethyl arginine (NMMA) have been shown to be involved in vascular disease states including atherosclerosis and to impart proatherogenic conditions, which are associated with reduced levels of NO.[107,108] ADMA, as an endogenous competitive inhibitor of NOS, a robust prognostic value for mortality after MI in a study performed with blood serum of 249 patients with acute MI whose circulating levels of ADMA were observed.[109] NO can potentially subject NOS to a feedback inhibition as NO is a heme diatomic ligand. However, transport of NO away from the site of synthesis and competitive binding of O_2 might be involved in controlling the rate of inhibition. Dimethylarginine dimethylaminohydrolase (DDAH) is known to catalyze ADMA to citrulline, and thus, endothelial dysfunction has been shown to suppress DDAH activity, thereby allowing an abundant presence of ADMA and leading to reduced NOS activity.[110]

Table 2. Forms of NOS, Their Cellular Locations, and Ca^{2+}/CaM Dependence and Inhibitors

enzyme	cellular distribution	Ca^{2+}/CaM dependence	NOS inhibitors
eNOS, NOS3[239]	caveolin of ECs, platelets, RBC, cardiac myocytes, and others	yes (membrane-bound eNOS) less dependent (Golgi-associated eNOS)[240]	NG-nitro-L-arg methyl ester (L-NAME asymmetrical dimethylarginine (ADMA)
nNOS	neuronal tissue, cardiac myocytes (sarcoplasmic reticulum), endothelium of blood vessels[242]	yes	N-guanidino monomethyl arginine (NMMA)
iNOS	macrophages, liver, SMC (in soluble form)	no	NG-nitro-L-arginine (LNNA)[241] S-methyl-L-thiocitrulline (nNOS-selective inhibitor)

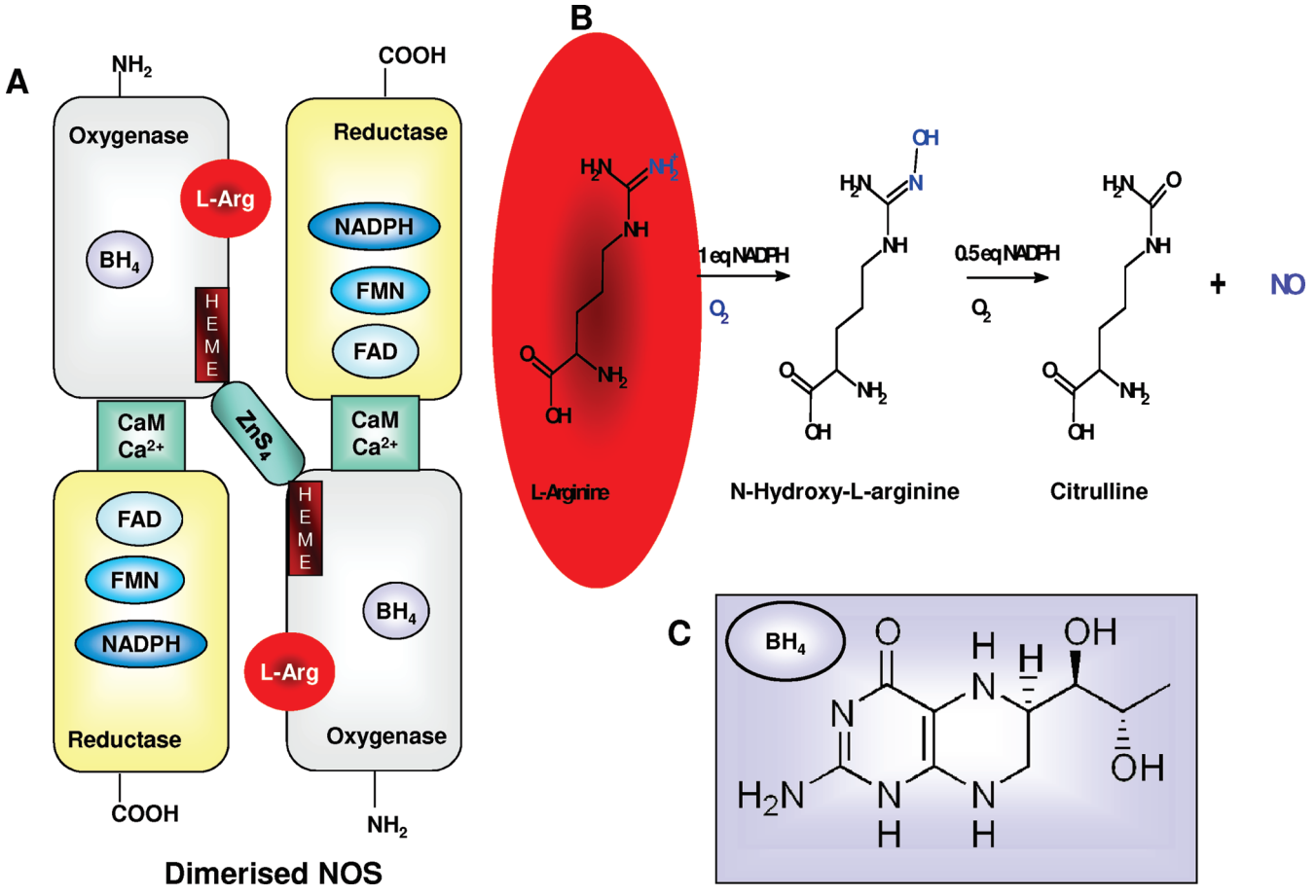

Figure 7. Nitric oxide synthase. (A) eNOS ranges from 135 to 160 kDa and has a dimeric structure. The dimerization is catalyzed by the binding of heme. Zn binding in the dimer interface help stabilize the dimer.[227] This dimerization process was found to be crucial to the catalytic activity and H_4B.[228] H_4B poses a permanently bound position to NOS as it cycles between a fully reduced and an electron-oxidized form. eNOS is the major form of NOS that is involved in the vascular system and is highly dependent on calcium, thus facilitating calcium-concentration-specific modest synthesis of NO. (B) NO is generated through oxidation of the terminal guanidino nitrogen of L-arginine (L-arg) in the presence of dimerized NOS with the association of cofactors, including nicotinamide adenine dinucleotide phosphate hydrogen, flavin adenine dinucleotide, and flavin mononucleotide. In addition to the essential cofactors, chemical stimuli such as acetylcholine, bradykinin, estrogensphingosine 1-phosphate, H_2O_2, and angiotensin II and mechanical stimuli such as laminar shear stress and cyclic strain can also influence eNOS activation. (C) NO synthesis catalyzing cofactor, tetrahydrobiopterin H_4B.

Administration of L-arg is generally accompanied with a favorable effect of vascular protection, and polymerized L-arg has demonstrated a significant enhancement in coronary blood flow as observed in a rat model of cardiac transplantation,[111,112] as well as therapeutic effects in IRI. However, a paradox arises as the levels of L-arg in ECs are generally present in adequate amounts to saturate NOS. In addition, L-arg can be resynthesized from L-cit, which is a byproduct of L-arg, thus presenting a surplus of L-arg for endothelial NOS. Nonetheless, introduction of exogenous L-arg inevitably enhances the NO-mediated vascular protective features, suggesting that L-arg might act on more NOSs other than the ECs such as red blood cells (RBC) and platelet NOS and also might present a strong positive competition against NOS inhibitors. The phosphorylation of specific residues such as Ser633/635 and Ser1177/1179 on eNOS has been shown to enhance its activity with increased NO

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production. Recent studies have shown that AMP-activated protein kinase (which is primarily recognized for its role in regulation of cellular energy balance) is the primary kinase that phosphorylates eNOS Ser633/635.[113,114] AMPK can directly phosphorylate Ser1177 as well as Ser633, and both are associated with eNOS activation.

Extraction of the saphenous vein from patients undergoing bypass surgery with a minimal handling technique has been found to present advantages over the conventional methods by facilitating the preservation of peri-adventitial tissue and endogenous NOS to a greater extent. Thus, such grafts are capable of releasing greater levels of NO while offering a greater potential for enhanced patency rates.[115] A recent study that compared the use of endoscopic radial artery harvesting using vascular strippers with the "no touch, minimal handling technique" on 200 participants for CABG surgery demonstrated that the minimally invasive surgical procedure retained greater integrity of the endothelium, thus preserving NO activity.[116] However, the presence of peri-adventitial adipose tissue in vessels, in addition to promoting ROS synthesis, has been shown to inhibit the synthesis of coronary endothelium-derived NO through protein kinase C (PKC)-β-dependent, site-specific phosphorylation of Thr459, an inhibitory site of eNOS.[117] Thus, these findings might suggest a need to modify the protocols for handling periadventitial tissue of vessels extracted for bypass surgery for greater retention of NO activity.

Biofunctionalization and surface modification of the polymers with L-arg for vascular grafts or stent coating is a relatively novel concept, and our group is currently investigating this concept using POSS−PCU nanocomposite polymer.[118] The preliminary results are promising, with potent antithrombogenic surface properties (Figure 8). The hypothesis has led to a study in the absence of a functional endothelium, where NOS in platelets and RBC-produced NO was tested in poly(ethylene terephthalate) polymers,[119] although the use of glutaraldehyde as in this study might be suboptimal for cross-linking molecules with nitrogen-containing functional moieties. Arginine was also hypothesized to inhibit the inhibitory role of ADMA, which is a competitor for NOS, thus facilitating the synthesis of NO localized to blood-contacting surface of the vascular grafts. The study, which is at its initial state, requires further investigation to clarify the exact source of NO synthesis (i.e., platelets or RBC), as well as the effect of RBC scavenging that is recognized as one of the main NO-limiting mechanisms. This would require further investigation with isolated platelets to decipher the role of arginine incorporated into the polymer on distinct blood components. Although L-arg-associated reactions are more upstream of the NO−sGC−cGMP pathway, it could be hypothesized that this would allow more "natural" NO synthesis, thus limiting any disadvantages associated with rapid overstimulation of sGC. However, the current study has not provided clear evidence as to how L-arg reaches NOS, which is primarily located in the cytosol, or whether L-arg primarily interacts with the membrane-bound NOS as found to be present in RBC.

4.1. NOS Catalyzing Cofactor, Tetrahydrobiopterin (H_4B)

Tetrahydrobiopterin (Figure 7C) has been recognized as one of the indispensable redox-active cofactors[120] that is required for optimal functioning of the EC[121,122] because of its role in enhancing NO production from NOS.[123] Direct redox-active supplementation of H_4B has restored NO-mediated vasodilatation that was impaired in chronic smokers.[124] High glucose levels in diabetes are often associated with high levels of superoxide, decreased levels of NO, and increased total eNOS protein levels, but in an inactive monomeric form. Adenovirus-mediated gene transfer of the rate-limiting enzyme for de novo H_4B synthesis has been found to restore NOS activity and increase NO synthesis.[125] A similar effect was observed when subjects who were induced with an oral glucose challenge were directly supplemented with H_4B.[126] Folic acid, which induces H_4B, has been shown to reverse the dysfunctional state of NOS caused by continuous treatment with nitroglycerine. This was observed in a randomized, placebo control trial with 16 male volunteers who received oral folic acid after continuous administration of transdermal GTN[127] and also in another study with 24 volunteers[128] in in vivo studies with endothelial H_4B, which regulates eNOS and has been shown to inhibit vascular-injury-induced IH and accelerated atherosclerosis by reducing vascular inflammation.[129]

Uncoupling of NOS affects the bioavailability of NO as a result of altered production of NOS and rapid degradation due to the reactions with free radicals.[80] Uncoupled NOS produces $O_2^{\cdot -}$ as electrons move from the reductase domain to the heme, which should reach L-arg and get diverted to molecular oxygen. The switch from NO to $O_2^{\cdot -}$ production, the result of uncoupled eNOS, is triggered by oxidative depletion of H_4B.

Reduced levels of L-arg, which can occur due to increased activity of arginase (which converts arginine into ornathine instead of citrulline), can lead to NOS uncoupling. This condition has been observed in aged rats with vascular stiffness and was found to be reversed with the introduction of arginase inhibitors.[130] Increased C-reactive protein, which is a marker of endothelial dysfunction, is known to decrease NO synthesis in ECs. This mechanism is linked to reduced H_4B levels and uncoupling of eNOS.[131] Ascorbate has been shown to preserve H_4B,[132] and dihydrofolate reductase (DHFR), which can regenerate H_4B from H_2B, has shown to have a significant role in coupling eNOS.[133] The ratio of H_4B to H_2B in plasma is considered as a possible marker of endothelial dysfunction.[134]

4.2. Hemodynamics, RBCs, and Point of NO Return

Hemodynamics have a significant influence on EC, and cardiovascular molecular events affect the patency rates of cardiovascular grafts. Cells within cardiovascular tissues are continuously exposed to physical forces, including (1) shear stress, tangential frictional forces acting on ECs and SMCs due to blood flow and transmural interstitial flow; (2) luminal pressure, cyclic normal force exerted by blood pressure; (3) mechanical stretch, cyclic circumferential stress also attributable to blood pressure; and (4) tension in the longitudinal direction on vascular system[135] acting on the ECs that line the vessels. Shear stress has been shown to be an eNOS expression regulating factor,[136,137] and shear-stress-induced upregulation of Kruppel-like factor 2/4 (KLF2/4) has been found to be a key mediator of eNOS expression.[138,139] Shear stress in the case of laminar flow where the profile of blood velocity is parabolic is expressed as

$$\tau = 4\mu Q/\pi r^3 \quad (1)$$

where μ is the viscosity, Q is the flow rate, and r is the vessel radius.[140]

Vascular shear stress of large conduit arteries typically varies between 5 and 20 dyn/cm^2. However, significant instantaneous values range from negative measures to nearly 40 dyn/cm^2

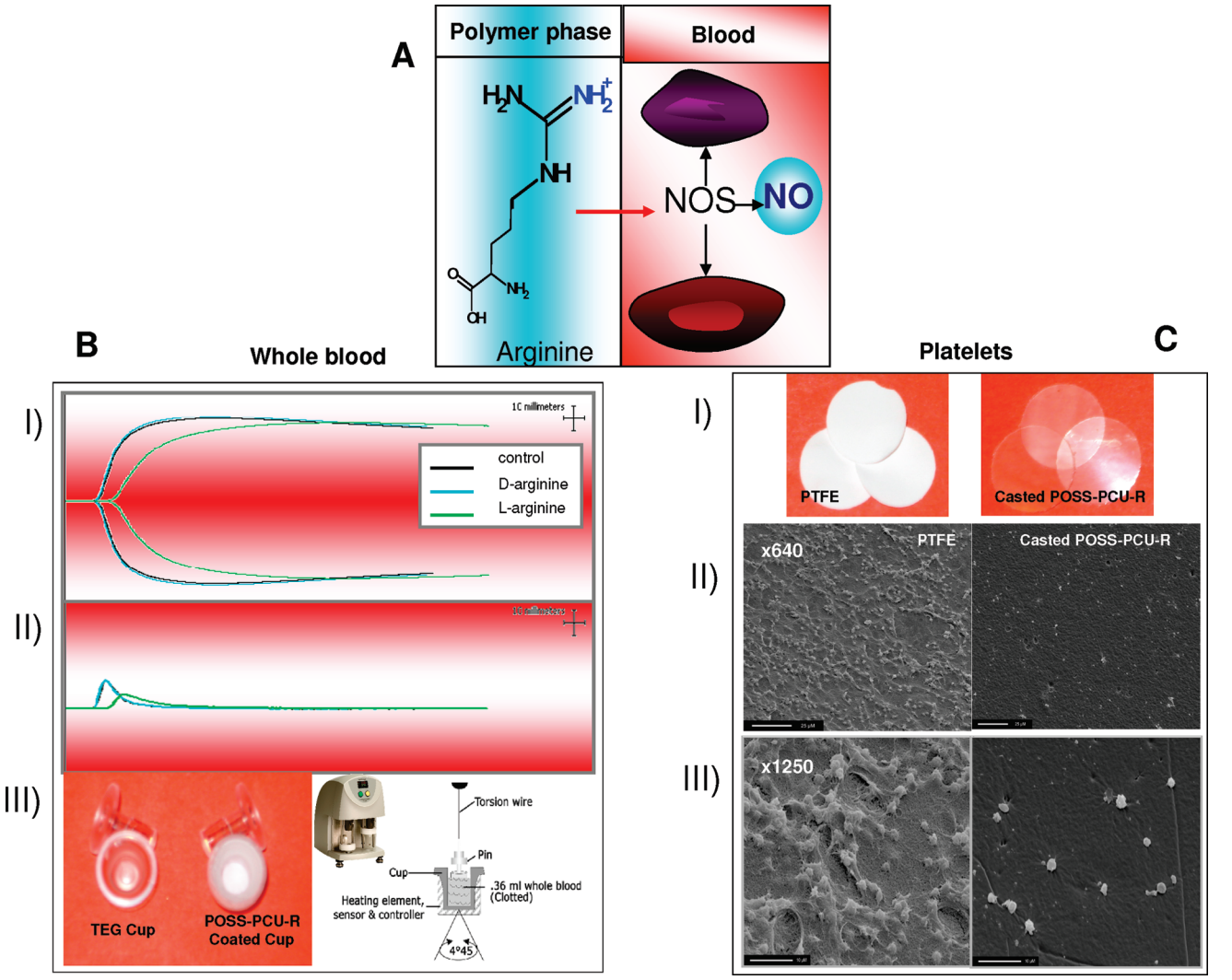

Figure 8. Arginine-incorporated polymer for greater antithrombogenic properties. (A) Hypothesized mechanism of action of arginine incorporated into cardiovascular polymer with POSS−PCU as a model. Localized arginine is hypothesized to interact with cytosolic or membrane-bound NOS in RBC and platelets. (B) Arg−POSS−PCU was tested with whole blood using thromboelastography,[229,230] which can determine whole blood kinetics. This demonstrated characteristics of reduced thrombogenicity in the presence of L-arg, and the specificity was confirmed with no such improvement with D-arginine, which remained as POSS−PCU. (Bi) Features observed with the TEG traces with Arg−POSS−PCU include decreased angle (rate of clot formation) and increased *R*, reaction time related to the time for initial clot formation. (BII) Reduced time for maximum rate for thrombus formation, as well as reduced total thrombus formation. (Biii) TEG tests were performed on TEG cups coated with arginine-incorporated polymer. The machine induces rotation of the cup against an attached pin in the presence of 340 μL of blood to obtain a kinetic trace of coagulation.[229] (Ci) Platelet adhesion tested on Arg−POSS−PCU casted in the form of nonporous sheets as used for various cardiovascular grafts such as heart valve and stent coatings and ePTFE. (Cii) SEM image of polymers after incubation with platelets. Reduced adhesion of platelets was observed on Arg−POSS−PCU compared to (Ciii) ePTFE, which is one of the materials that is currently commercially available for cardiovascular applications. Remarkably, on POSS−PCU, there are very few platelets that appear round and without any pseudopodia, such as are present in abundance on ePTFE.

during states of increased systolic cardiac output with a characteristic pulsatile flow.[141] The S-nitrosylation in ECs has been shown to be shear-force-dependent where ECs subjected to lower (12 dyn/cm^2) shear stress induced less S-nitrosylation as compared to those with higher shear stress (25 dyn/cm^2) during the systolic phase of a cardiac cycle.[136] Shear-flow-induced S-nitrosylation was suppressed in ECs pretreated with an eNOS inhibitor, confirming that S-nitrosylation induced by shear flow is eNOS-dependent.[142] Arginase, which takes L-arg as a substrate, competes with eNOS, thus determining vascular NO synthesis. Interestingly, it has been shown that altered shear-stress-mediated vascular disease states can be mediated by upregulation of arginase and reduced NO production.[143]

In addition to acting as a kinetic force to stimulate NOS, blood flow can regulate the interaction with RBCs and influence the bioavailability of NO. NO interactions with RBCs that enclose hemoglobin can take the form of "sink, store, and synthesis"

sink $\quad Hb-Fe(II)O_2 + NO \rightarrow Hb-Fe(III) + NO_3^-$

store $\quad Hb-Fe(II) + NO \leftrightarrow Hb-Fe(II)NO$

$\quad Hb(\beta 93-Cys) + NO \leftrightarrow Hb(\beta 93-Cys-NO)$

synthesis $\quad Hb-Fe(II) + NO_2^- + H^+ \rightarrow Hb-Fe(III) + NO + OH^-$

$\quad$ L-arginine $\rightarrow$ *NOS* $\rightarrow$ *NO*

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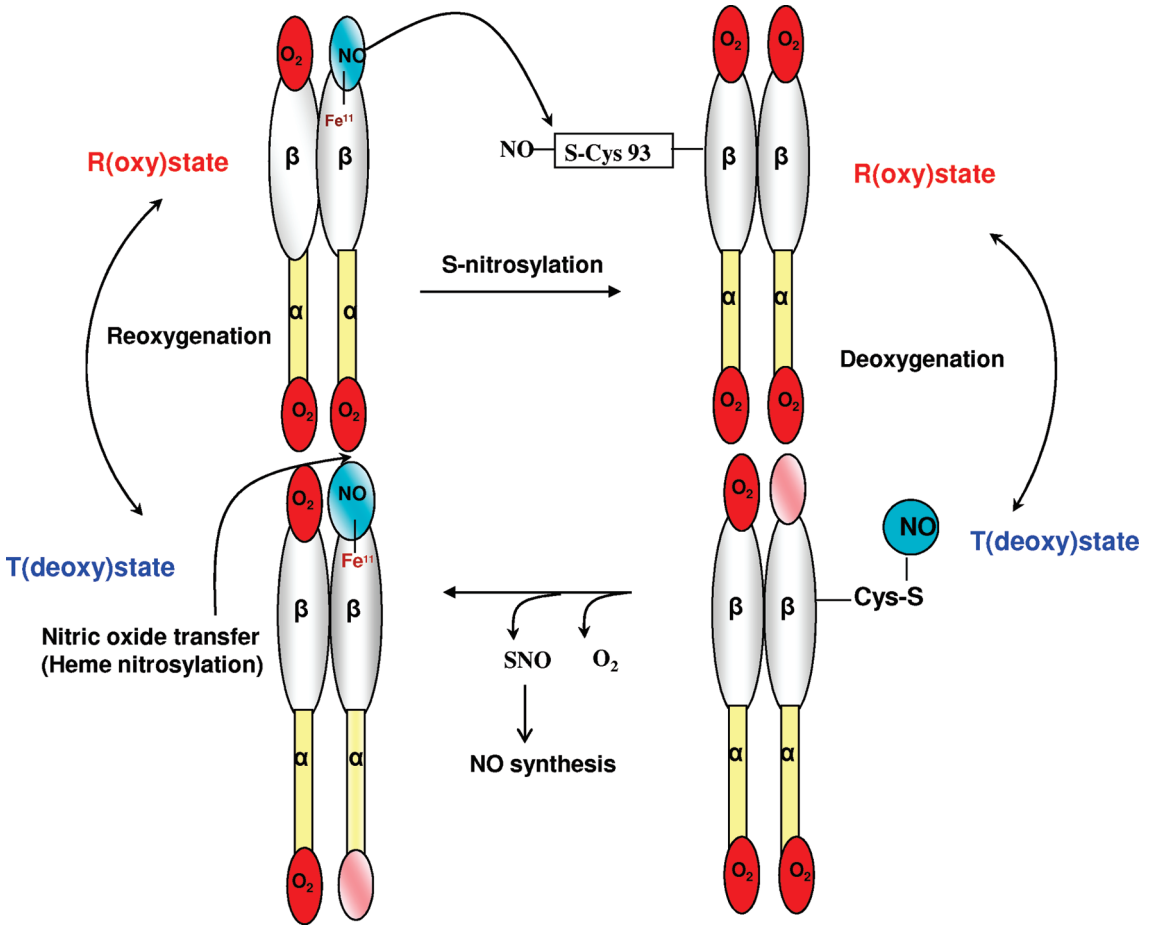

Figure 9. Red blood cell interactions with NO include (1) NO binding to the deoxygenated heme in oxygenated red blood cells, forming iron-nitrosyl hemoglobin (Fe^{II}—NO); (2) oxy-hemoglobin scavenging NO and transferring it to the β-globin Cys-93 residue to form SNOHb (NO transport); (3) hemoglobin deoxygenation and structural transitions from R (oxy) to T (deoxy) facilitating the release of NO; and (4) T-state Hb reacting with NO species and undergoing Hb nitrosylation. Note: In the R state, Cys β93 is enclosed in a hydrophobic pocket, and the heme pocket is more accessible. In the T state, Cys β93 is exposed to reactions, and the heme pocket is less accessible.

where Hb represents deoxyhemoglobin; Hb—Fe(II)NO, nitrosyl hemoglobin; Hb—Fe(III), methemoglobin; and NO_2^-, nitrite.

Figure 9 illustrates the interactions of NO with Hb, thus highlighting the reactions involved in scavenging and storing, and these reactions are recognized to be pH- and O_2-saturation-dependent.[144] NO has a great affinity for free oxy-globins, and RBCs that enclose Hb are known as scavengers of NO, but cell-free Hb is approximately 1000 times more active in acting as a potent NO sink. RBCs are also involved in nitrite uptake and have been shown to involve both HNO_2 diffusion and AE1-mediated transport.[145] Free hemoglobin administration is recognized as a blood substitute; however, this treatment strategy is hampered by the onset of associated hypertension due to uptake of NO by oxy-hemoglobin.[146] As a solution, mutations to the oxygen binding sites have been introduced and hinder NO depletion, leading to the modulation of vasoconstriction effects.[147]

Nitrites are reduced to NO, and myoglobin has been shown to function as an endogenous nitrite reductase at low oxygen levels. A study demonstrated that myoglobin-knockout mice cannot generate nitrite-dependent NO and thus performed poorly in cardiovascular functions including nonrecovery from experimental ischemia.[148] RBCs also serve to directly synthesize NO catalyzed by NOS that has been recognized to have similarities to endothelial NOS.[149] RBCs in flow can detect shear forces, which, in turn, modulate their NOS,[150] and an increase in NOS activity was speculated under hypoxic conditions. Endothelial NOS-mediated NO synthesis becomes limited in small-diameter vessels, and also in the absence of an endothelium, RBCs can be recognized as considerable contributors of NO, in addition to possible NO synthesis by the breakdown of nitrates. Erythrocytic hemoglobin has also shown to have a major role in nitrite conversion to NO.[151,152] NO scavenged by RBCs was found to be recycled as nitrites and nitrates.[98] S-Nitrosylation, particularly of heme proteins, can be a form of NO-limiting mechanism and also extends as a form of NO storage that facilitates long-lasting paracrine effects in the vascular system.[153]

The dynamics of blood flow, including RBCs in relation to NO diffusion and the endothelium or a NO-functionalized polymeric surface as the origin of NO production, is illustrated in Figure 10. If the two main facts that (1) the endothelium-derived NO is the main source of NO and (2) the endothelium is in close proximity to RBCs in circulation where the interactions are of relatively fast kinetics are considered with Fick's second law of diffusion, while recognizing RBCs as a major NO scavengers, the bioactive NO concentration appears to be insufficient to produce a significant

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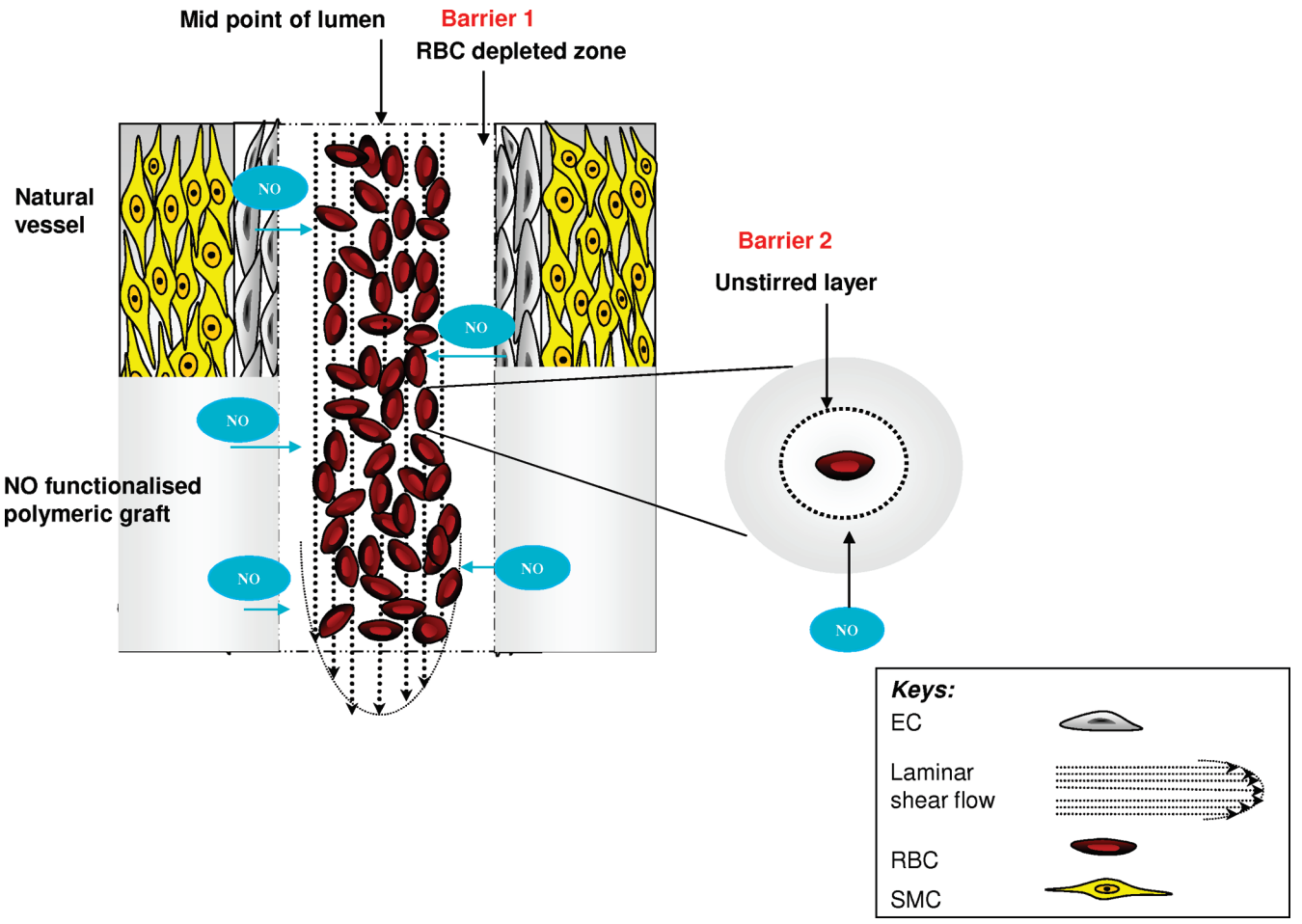

Figure 10. Dynamics of blood flow in relation to NO diffusion. Postulated diffusional barriers for NO experienced in hemodynamic flow as it diffuses from the endothelium or NO-functionalized polymer across a cell-depleted zone. In addition to the marked barriers, hemoglobin compartmentalization also serves as a barrier for NO to reduce the rate of reaction with intraerythrocytic hemoglobin.

response. Nevertheless, a substantial concentration of NO still evidently acts as a substrate for sGC. Few possible reasons for this vascular availability of NO that can migrate outward despite the strong scavenging effect of RBC have been presented.[154] The flow rate is found to be greater toward the inner center of a vessel than closer to the endothelium, thus directing a greater concentration of RBCs toward the center of the lumen. The RBCs have intrinsic diffusional barriers and also serve to compartmentalize hemoglobin, thus reducing the reactions with NO.[155,156] Recent studies designed to measure the NO flux across vessel walls in varying oxygen concentrations demonstrated that the diffusion rate of NO can be determined by vascular oxygen levels.[157] Erythrocyte's ability to transfer oxygen is facilitated by NO, and transfused blood has been shown to be prone to NO depletion, leading to complications associated with ischemia, despite restoration of blood levels.[158] However, it has been shown that the presence of SNO−hemoglobin is not essential for NO-dependent vasodilation in hypoxic conditions.[159]

Limited patencies have been observed in vascular grafts even in the presence of a functional endothelium but disturbed flow conditions. Therefore, the loss of the vascular protective role in altered flow suggests a dominant role for vascular flow in modulating functional NO concentrations. A graft optimized for NO elution might not present its best biological influence if the graft experiences disturbed flow due to nonoptimal mechanical properties, which, in turn, could possibly lead to reduced bioavailability of NO with nonoptimal interactions with RBCs leading to greater scavenging, thus emphasizing the need to match the viscoelastic properties of vascular graft to native artery.[24,160] Therefore, it is of great significance to evaluate the bioavailability and dynamics of NO release[161] from a functionalized bypass graft. NO elution determined in relation to graft performance in the presence of a pulsatile flow system will enable at relatively more realistic values for NO release to be derived compared to static in vitro determination of NO release from a representative sample of functionalized polymer.

In collaboration with Maastricht University, we have developed a system to assess the elasticity or, in index terms, compliance of the viscous component of the vessel wall, as well as the shear stress of pulsatile blood flow in artery and bypass graft.[162] The changes in diameter can be determined continuously using a duplex ultrasound scanner with a vessel wall tracking system and pressure with a Millar or pen micropressure catheter (Figure 11). From these data, hysteresis loops (pressure−diameter) can be generated for single cardiac cycles by plotting diameter against blood pressure, and from the figure elasticity, the viscous component of the vessel can be computed (Figure 11).

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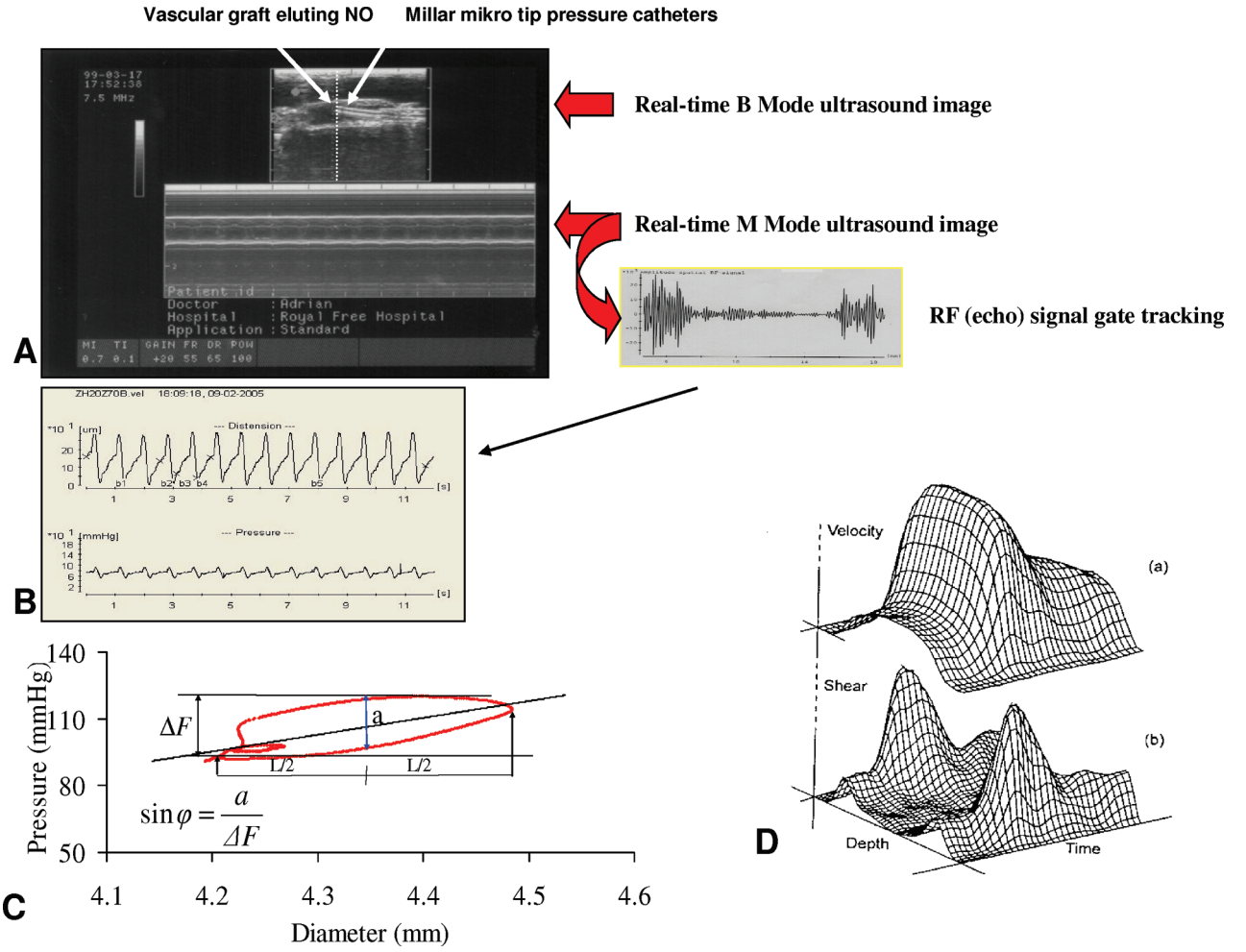

Figure 11. NO bioavailability in the presence of hemodynamic flow. (A) B- and M-mode images of bypass graft mounted within the flow circuit. Note the intraluminal pressure tip catheter. Resultant radiofrequency (RF) signal generated by anterior and posterior walls of imaged bypass graft. (B) Distension−time and pressure−time curves generated by wall tracking system. (C) Diameter−pressure hysteresis within a cardiac cycle can reflect the viscosity characteristics of the bypass graft wall [$\sin(\theta)$], and compliance could be calculated according to formula. (D) Shear rate distribution within the bypass graft (depth) is computed from the dependent velocity distribution by means of $|dv/dr|$ per blood flow velocity profile. Eluting NO is monitored within the flow circuit continuously with an intraluminal NO probe placed inside the graft.

The common method of assessing the elasticity of the vascular system is computation of the compliance index (C), derived as

$$C = \frac{10^4(D_s - D_d)}{D_d(P_s - P_d)} \quad (2)$$

where D is the cross-sectional diameter; P is the pressure inside the vessel; and subscripts s and d represent systolic and diastolic, respectively.[60]

A duplex ultrasound Doppler vascular wall tracking system can be used to monitor wall shear rate caused only by the axial fluid velocity; the shear rates (σ) over time (t) can be estimated as

$$\sigma(t) = \frac{1}{2}\left\{\left|\frac{dv(r,t)}{dr_0}\right|_{max} = \left|\frac{dv(r,t)}{dr_l}\right|_{max}\right\} \quad (3)$$

where $v(r,t)$ is the estimated blood flow velocity profile (Figure 11) and r_0 and r_l are the radial positions measured from the vessel axis to the near and far walls, respectively.[163]

NO concentrations of a functionalized graft can vary when exposed to physiological flow conditions according to the mechanical properties of the vascular graft of concern. Therefore, availability of the above significant parameters with the measurements of blood viscosity and hematocrit will enable the calculation of a true estimate of bioavailable NO concentrations of a graft functionalized with NO donors when exposed to physiological flow conditions.

5. NO TOXICITY, INACTIVATION, AND DESENSITIZATION

Glycerine trinitrate (GTN) is well recognized for its vasoprotective effect, particularly in relieving angina, through its activation of sGC- and cGMP-dependent protein kinase activity. Despite the favorable effects of GTN in relieving conditions of cardiovascular discomfort, GTN therapy used during vascular interventions has been found to have adverse outcomes by inducing systemic hypotension, headache, tachyphylaxis, and endothelial dysfunction in the arteries and thus negatively affecting the graft patency rates.[164,165] However, despite the

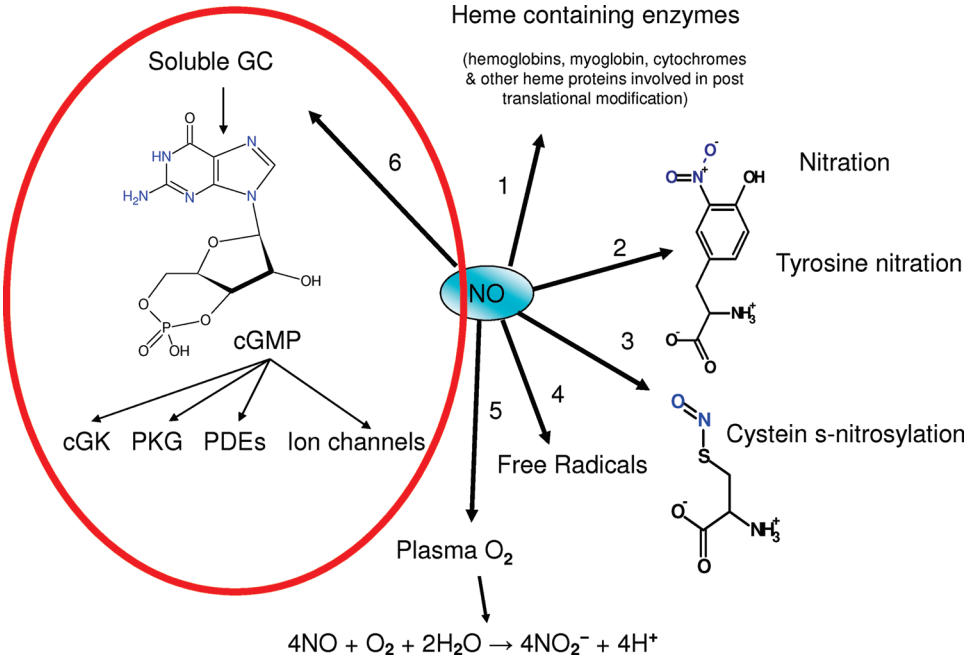

Figure 12. Summarized illustration of the main, significant categories of NO reactions in the vascular system. The reactions include (1) reactions with metal centers (mainly heme); (2) S-nitrosylation, or the interaction of NO with cysteine sulfahydryls/thiol, where a nitrosyl group is added post-translationally; (3) nitration (protein tyrosine); (4) free-radical interactions; (5) reactions with plasma O_2; and (6) synthesis of cGMP through the catalysis of sGC by NO, then leading to the activation of protein kinases and phosphodiesterases.

recognized high risks associated with GTN treatment and CABG patients with unstable angina, it was found that pre-operative treatment demonstrated no alteration in hospital outcomes.[166] The mechanism of activation of sGC by GTN is not clearly defined, but it is suggested that this activation can be through the formation of intracellular nitrite by the enzyme mitochondrial aldehyde dehydrogenase.[167] It has been found that GTN-induced ROS can inhibit bioactivation of GTN by thiol oxidation of aldehyde dehydrogenase and contribute to nitrate tolerance.[168] In addition, it has been shown that GTN treatment leads to increased S-nitrosylation of sGC, leading to desensitization and the development of nitrate tolerance.[169] A reduction in a key protein, leucine zipper-positive (LZ+) isoform of myosin phosphatase target subunit 1 (MYPT1), was observed in nitrate-tolerant arteries, which was also onset by NO donors and cGMP analogues, leading to overall suppressed cGMP-dependent protein kinases (PKGs).[170]

A multitude of reasons, including increased scavenging of NO by superoxide radicals and inactivation of sGC, can render NO resistant to platelet aggregation. It is rather commonly observed in patients with cardiovascular diseases including aortic stenosis, coronary heart disease, stable angina pectoris, diabetes, and hypertension. The decreased sensitivity to NO has been found to be due to both impaired production of NO and impaired responsiveness. Platelet studies of these patients showed a reduced response to NO antiaggregatory effects.[171] A study performed to evaluate the responses of platelets from 51 patients with acute coronary conditions to NO donors led to "platelet NO resistance" or the degree of platelet responsiveness to be considered as a diagnostic marker of acute coronary syndromes, therefore enabling better patient treatments.[51] Circulating monocyte−platelet aggregates, which are an indication of platelet activation, showed a strong positive correlation with BP, and NO donors decreased monocyte−platelet aggregates in normotensive subjects but were unable to respond in hypertensives, indicating a reduced sensitivity.[172]

The use of nitrates as NO is hampered by the development of tolerance. Both nitrate−NO conversion and increased consumption of NO by free radicals can be perceived as nitrate tolerance.[173] In addition to contributing to oxidative stress, these drugs can act only through the reduced heme state of sGC, and the stress-induced oxidized state of sGC cannot respond to binding of NO, thus rendering a nonresponsive state. Overexposure to exogenous NO can reduce NO responsiveness.[174] Repeated induction of NO has been implicated in the desensitization of NO downstream responses by sGC desensitization, reduced synthesis or increased uptake of cGMP, and increased activity of phosphodiesterase-5.[175]

Increased synthesis of NO has been implicated in cytotoxicity and adverse outcomes in the vascular system. A pronounced effect of this situation can be observed in IRI where iNOS becomes activated. Although there is no direct evidence to suggest cytotoxicity of pure NO, there is evidnece for second-order toxicity, which depends on the concentrations of ROS. As a response to injury, vascular cells and activated platelets produce free radicals. Superoxide (O_2^{-}), in particular, scavenges NO to form unstable metabolite peroxinitrate (NO_3^{-}). Peroxinitrate can generate nitrogen dioxide ($\bullet NO_2$) and carbonate radical ($CO_3^{-\bullet}$) which can cause tissue injury. Peroxinitrate is a strong and selective oxidant and is involved in a range of reactions that are generally considered damaging, including lipid oxidation,[176] inhibition of mitochondrial respiration, and protein tyrosine modifications to create nitrotyrosines, thus alterations of enzyme activities leading to apoptosis, necrosis, and cytotoxicity.[177−179] Quercetin, which is an antioxidant and a scavenger of peroxinitrite, has been shown to attenuate systemic inflammation after cardiopulmonary bypass procedures.[180] Both peroxynitrite and peroxynitrates produce $\bullet NO_2$, but peroxynitrate has a

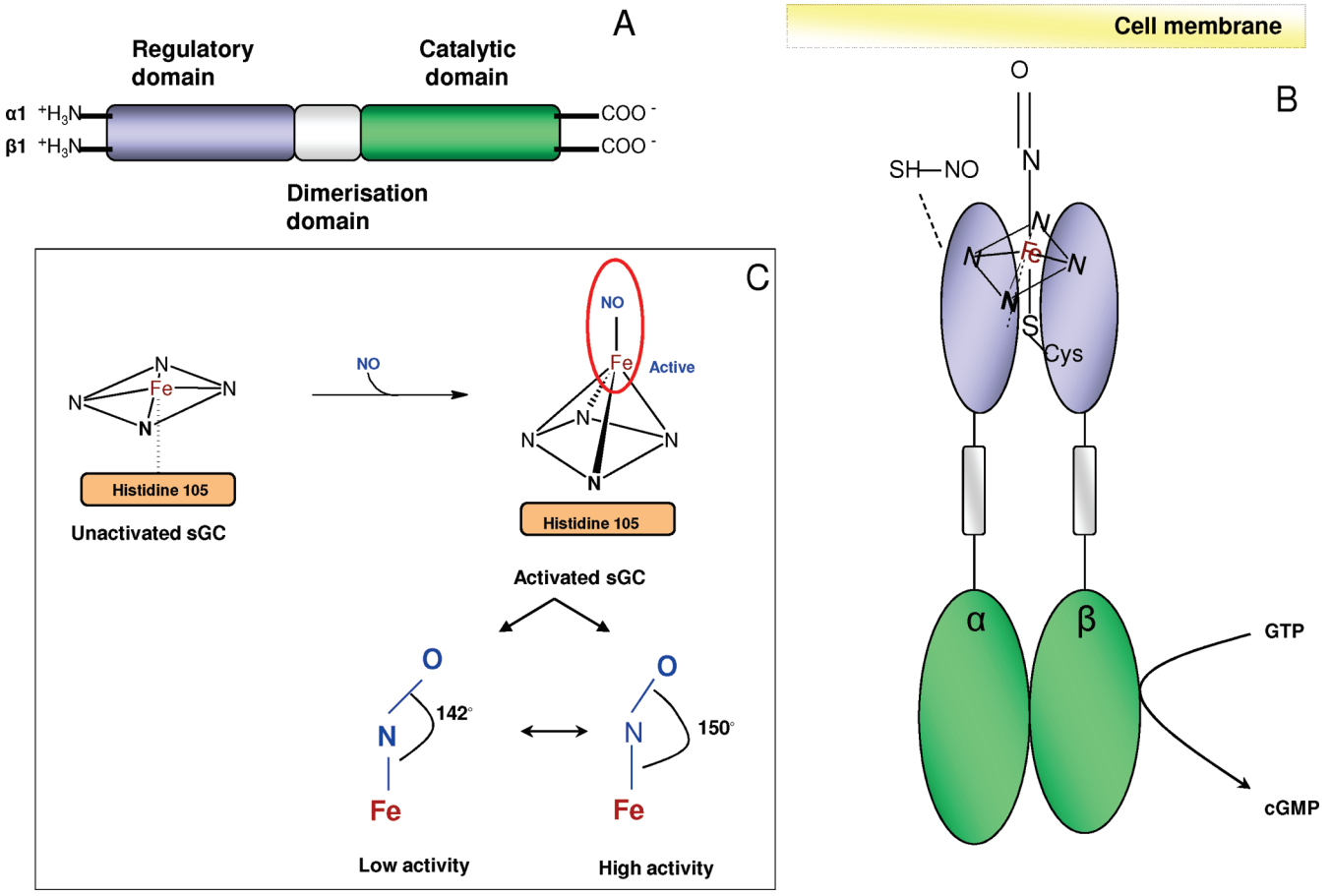

Figure 13. NO interactions with sGC and synthesis of cGMP. (A) Domain structure: sGC is a heterodimeric protein with the C-terminus serving as the catalytic domain and the N-terminus as the regulatory domain, which is sensitive to NO. (B) Structural organization: sGC is a protein present in the cytosol that is a hemeoprotein (a metalloprotein containing a heme prosthetic group) with subunits of α and β with a ferrous prosthetic group. NO interactions with sGC at a site that is alternative to heme (i.e., on a cysteine) are also recognized. The synthesis of cGMP through the catalysis of sGC with NO then leads to the activation of protein kinases and phosphodiesterases to modulate varied biochemical pathways that regulate vascular functions.[199] (C) Reactive site of sGC: Iron is ligated to histadine 105 of the β subunit; NO binds to the heme of sGC and forms a transient six-coordinated NO-bound state that progresses, upon heme—His bond breakage, to a five-coordinated NO-bound activated state. The degree of activation of sGC as high (where the FeNO bond angle is greater) and low (where the FeNO bond angle is smaller) could vary according to the amount of NO present, as well as other sGC stimulators.

stronger O—O bond and is considered less toxic. The chemistry of peroxynitrites and peroxynitrates has been reviewed in detail.[181]

$$NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^{-} \quad (4)$$

Superoxide dismutase (SOD) is a strong antioxidant, and its role includes uptake of superoxide to protect from harmful effects. Despite the well-known protective effects of NO, it has been recognized that NO's unfortunate affinity for superoxide leads to a competition with SOD and to the formation of peroxinitrate, which then operates cytotoxic interactions. One might be inclined to assume that a great degree of synthesis of NO from exogenous sources might be harmful, as this might imply a greater degree of interactions with superoxide to form peroxynitrites. However, it has been shown that the reaction between superoxide and NO is optimal with equimolar concentrations, and it could be suggested that an excess of NO would be favorable.[182] Interestingly, the involvement of heme oxygenase-1 (HO-1) leads to increased NO resistance. Recent research performed with human lung epithelial cells suggests a protective role in NO resistance where high and toxic concentrations of NO are modulated by HO-1.[177]

Providing some reassurance and presenting a potential solution, it has been shown that the apoptotic, cytotoxic effects that are apparently caused by NO are, in fact, distinctly caused by peroxinitrite formation, whereas exogenous NO produced by SNAP induces favorable cytostasis in SMCs.[183] Although the paradox of NO's toxicity is not completely resolved, the above evidence somewhat blunts the initial impression of NO as a double-edged sword. Thus, mechanisms to reduce peroxinitrite formation would be of interest to obtain a greater benefit from NO production and synthesis.

6. NO—SGC—CGMP: THE CARDIOVASCULAR TRINITY?

The specificities of NO interactions with biomolecules largely depend on the concentrations and anatomic distributions. Figure 12 is a summary of the main categories of reactions of NO in the vascular system. NO in modest concentrations interacts with molecules with heme irons such as hemoglobins, myoglobins,[184] and cytochromes, as well as other heme

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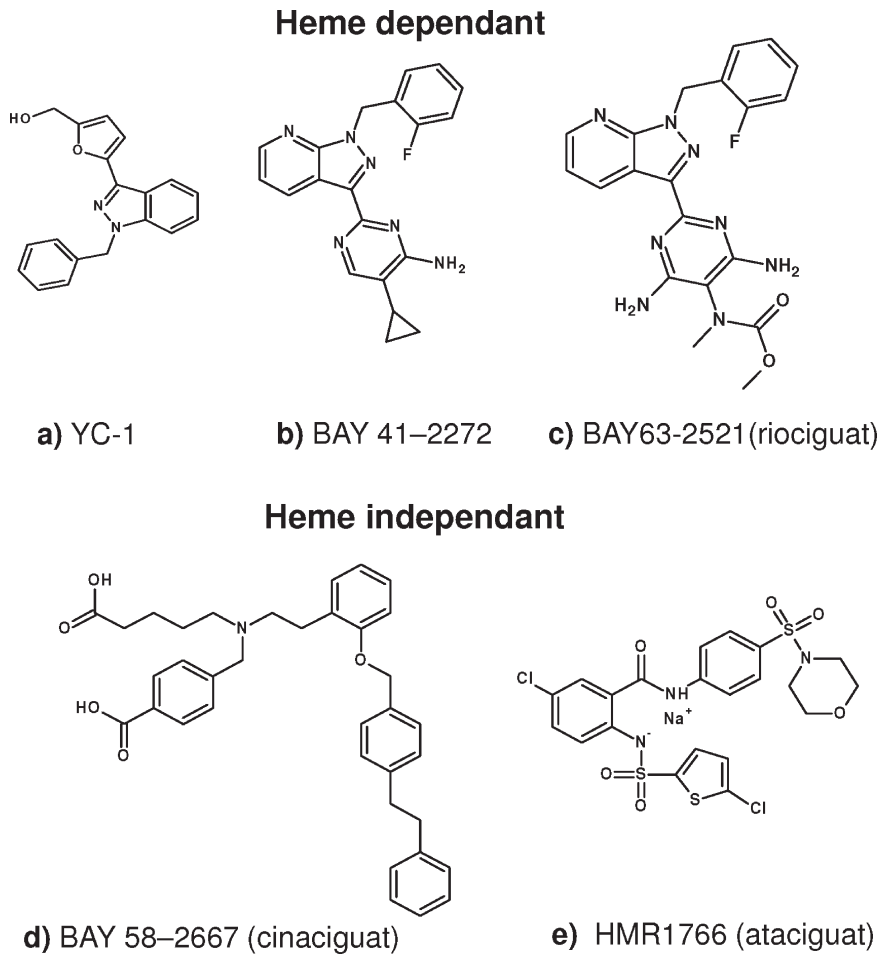

Figure 14. NO-independent stimulators of soluble guanylate cyclase. Alternative sGC stimulators are currently under investigation in clinical trials. These novel compounds, which are still undergoing clinical trials, are promising, with the ability to resist oxidative stress-induced intolerance and desensitization of sGC. Some compounds are heme-dependent, namely, (a) YC-1, (b) BAY 41-2272, and (c) BAY63-2521 (riociguat), whereas others are heme-independent, namely, (d) BAY 58-2667 (cinaciguat) and (e) HMR1766 (ataciguat) and can act synergistically with NO to stimulate, activate, and prevent ubiqutin-mediated degradation.[214].

proteins involved in post-translational modification. The primary mode of action of NO with the greatest impact in the cardiovascular system is through sGC, leading to NO−cGMP signaling and modulating significant processes such as platelet functions, host-defense activities, stress responses, inhibition of SMC proliferation, and cardiovascular homeostasis.[185−187] A recent review presents a thorough discussion of the influence of cGMP in the cardiovascular system and its activation of downstream factors, including cGMP-dependent PKGs, cGMP-gated cation channels, and PDE,[188] thus demonstrating that the endothelial dysfunction is essentially the disruption of the NO−sGC−cGMP pathway.

sGC is an intracellular receptor that is a heterodimeric enzyme consisting of α and β subunits where the C-terminus serves as the catalytic domain and the N-terminus as the regulatory domain that is sensitive to NO. The β subunit contains the catalytic domains and a heme−nitric oxide/oxygen (H−NOX) unit to which the heme prosthetic group is of particular interest, as NO binding to this domain mediates the conversion of GTP to cGMP.[189] NO binds to the heme of sGC and forms a transient six-coordinated NO-bound state that progresses, upon heme−His bond breakage, to a thermodynamically stable five-coordinated NO-bound activated state. Recent studies have demonstrated that this initial activation is transformed to a high-activity state with excess NO that interacts at nonheme sites.[190] Figure 13 is a simplified illustration of the activation of sGC by NO.

Theoretical and computational studies have shown an essential role for the αFβ1 loop and αA−αC in the distal subdomain in the mechanism of sGC.[191] The complexity of sGC activation by NO is also known to be modulated by GTP and ATP and allosteric activators such as YC-1. GTP and ATP bind to both the catalytic site and the allosteric site in vitro and thus function as substrates as well as allosteric modulators.[192] The heme prosthetic group is known to be highly susceptible to oxygen free radicals such as superoxides and peroxinitrites, thus inducing oxidation of the active heme state to an oxidized form (Fe^{3+}), leading to reduced affinity for interaction with NO, as well as acute inactivation and ubiquitin-mediated degradation. This oxidation is greatly increased with organic nitrates, thus emphasizing the limitations of using organic nitrates and other NO donors. The synthesis of cGMP through the catalysis of sGC with NO then leads to the activation of protein kinases and phosphodiesterases to modulate varied biochemical pathways that regulate vascular function. Interestingly, it has also been found that PKG is involved in inhibiting sGC by phosphorylating sGC on Ser64 of its α1 subunit.[193] A recent article presented a

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detailed discussion of NO−cGMP signaling and cellular responses to NO.[187]

However, NO can also mediate cGMP-independent reactions that can have a significant influence in the cardiovascular system. NO-mediated physiologically relevant reactions by cGMP-independent pathways are determined by the lack of inhibition by inhibitors of soluble guanylate cyclase (SGC) such as ODQ (1*H*-[1,2,4]oxadiazolo[4,3-a] quinoxalin-1-one), which inhibits heme-protein-dependent processes. Thrombospontin has also been shown to inhibit the sGC.[194] However, butyl isocyanide, which blocks the heme site of sGC, demonstrated the ability of NO to activate the enzyme, thus suggesting a heme-independent active site. An oxidation-sensitive nonheme binding site has recently been found to be of significance. The thiol modifying reagent methyl methanethiosulfonate was found to specifically inhibit NO activation of sGC by blocking a nonheme site, thus assigning a significant role to cysteine of sGC in controlling NO binding. Thus, NO interacts with both a reduced thiol and ferrous iron on sGC.[195] Despite the ability for both NO and CO to interact with sGC, its sophisticated structure enables specificity in affinity and activity in the regulation of the two, and sGC has been shown to favor NO over CO. Interestingly, Raman studies have shown that the presence of YC-1 can facilitate the stimulation of sGC by CO[191,196] A recent crystal structure study of the molecule demonstrated that the specificity is determined by the mechanism of heme binding, which involves a pivoting and bending thus leading to differing rates of activation.[197] The evidence of ligand discrimination and thus differential binding of NO, CO, and O_2 to sGC through HNOX has been obtained through theoretical and computational studies based on molecular dynamics,[191,198] as well as Raman spectroscopic studies.[199] In vivo distinction between O_2 and NO is significant, as O_2 is generally present at relatively higher concentrations. The absence of tyrosine and glutamine, as well as hydrogen-bond donors, in the distal heme binding pocket that leads to relatively fast dissociation of O_2 has been shown to be a critical feature in this process of favoring NO over O_2.[200,201]

Compounds that mimic NO to activate the downstream factors present a favorable option to the issues surrounding NO-associated cytotoxic product synthesis, as well as a solution to desensitization. HNO, the reduced form of NO, presents a greater degree of tolerance to desensitization and has less affinity for free radicals and thus is free from cytotoxic interactions with superoxide. HNO also activates the sGC pathway through interaction at the heme site and is independent of NO formation, as NO scavengers demonstrated no significant influence on sGC stimulation with HNO. However, at higher concentrations, HNO has been shown to be highly "thiophilic", thus rapidly reacting with cysteine thiols. Therefore, HNO presents minimal potential for excessive stimulation and tolerance, and therefore, recent studies support HNO as a promising alternative to direct NO donors for cardiovascular therapy.[202−204] Irvin et al. published a discussion of HNO and its donors for therapeutic options for cardiovascular disease conditions.[205]

Investigation of NO-independent sGC stimulators has become a relatively recent trend, and many novel compounds have been synthesized.[206,207] Figure 14 presents some pyrazolopyridinylpyrimidine-derived molecules, which are NO-independent cGMP synthesizers that are dependent[208] or independent[209] of a reduced heme group at His-105. BAY 58-2667 (cinaciguat), which can directly activate sGC, has been shown to have an influence on hemodynamics similar to that of nitroglycerin. Studies performed with Ns H-NOX proteins, which represent the ligand binding of the H-NOX domain of sGC, have demonstrated that binding of BAY 58-2667 causes a stabilizing effect on the heme cavity and protects sGC from degradation during activation, thus overcoming the dysfunctional state of sGC with an oxidized heme.[210] BAY 58-2667 was recently assessed in a phase 1 clinical trial to determine its safety and pharmacokinetic properties. Oral induction of cinaciguat was found to improve histopathological lesions, cardiac performance, and impaired cardiac relaxation; reduce oxidative stress; ameliorate intracellular enzyme release; and decrease cycloxygenase 2, transforming growth factor-β and β-actin mRNA expression in rats who were subcutaneously treated with isoproterenol (85 mg/kg) to induce MI.[88] BAY 58-2667 (cinaciguat) has also been shown to reduce MI in a rabbit model in situ, as well as in isolated rat heart.[211] These NO-independent sGC stimulators have shown that they inhibit the expression of tissue factor, thus modulating the procoagulant activity.[212] The combine use of NO donor, DETA-NONOate, NOC-18, and BAY 41-2272 was found to have a more profound effect in stimulating cGMP than the separate agents,[213] where this stimulation of the NO−cGMP pathway signaling has been shown to modulate the differentiation of ECs into myocardial cells. These NO-dependent sGC activators have different mechanisms of activation[199] and act on distinct sites of sGC in the modulation of activation and stabilization.[214] For instance, studies with BAY 41-2272, a pyrazolopyridine derivative molecule with structural similarities to YC-1, have shown the cysteine 238 and cysteine 243 region in the α_1-subunit of sGC to be responsible for catalysis.[206]

However, nitration and S-nitrosylation are two other mechanisms that are independent of the NO−sGC−cGMP pathway, and it is still necessary to clarify whether these processes can be synergized with the NO−cGMP pathway. Tyrosine nitration, particularly α-actinin, has been recognized as a cGMP-independent mechanism of modulating platelet adhesion.[215] Nitrosylation is known to be regulated by the enzyme GSNO reductase, which

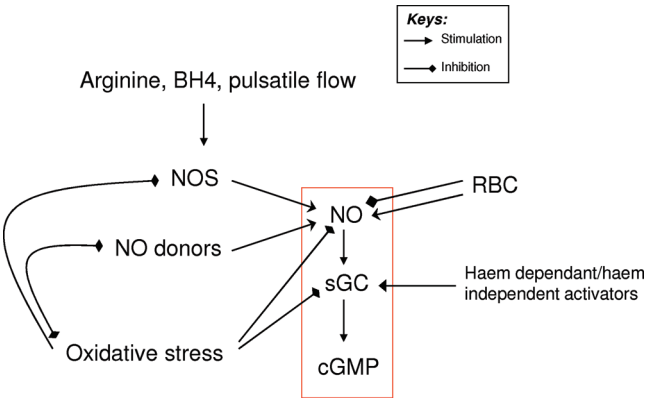

Figure 15. Summary of currently recognized factors for inducing and inhibiting NO, as well as factors directly influenced by NO. Pulsatile flow is a mechanical factor and arginine and BH4 are chemical factors that influence NOS and catalyze NO synthesis. NO donors and RBC can also induce NO synthesis, although RBC can also act as a scavenger of NO and inhibit NO-dependent activities. Oxidative stress acts to inhibit NO, NOS, and sGC, which is the main enzyme that NO activates to synthesize cGMP. sGC can be directly activated by heme-dependent/heme-independent activators, independent of NO, and is not influenced by the inhibitory effects of oxidative stress.

lowers levels of SNO proteins that are generally in balance with S-nitrosoglutathione. A study performed with the deletion of S-nitrosoglutathione reductase gene ($GSNOR^{-/-}$) in mice has demonstrated reduced myocardial infarct size, preserved ventricular function, and overall cardiac protection with tissue oxygenation, thus demonstrating that GSNO is cardioprotective.[216] S-Nitrosylation is the interaction of NO with cysteine sulfahydryls/thiols, where a nitrosyl group is added post-translationally, and has been recognized to modulate intracellular signaling and enzymatic activity, thus influencing cardiovascular effects[217] including conformational changes to platelet integrins.[218,43,219,220] NO, in addition to proteins, also modifies lipid mediators, particularly prostaglandins and lipooxygenases, thus modulating lipid-derived signaling pathways, which have significant impact on cardiovascular regulation.[221]

7. PERSPECTIVE

NO−sGC−cGMP-pathway-associated cardiovascular protective features can be achieved by upregulating three major possibilities: (1) modulation of NOS-mediated NO production (L-arg, cofactors, and downregulation of NOS inhibitors), (2) direct NO donors (also by in situ catalysis of NO from nitrosothiols), and (3) NO-independent sGC activators. Figure 15 summarizes the significant factors that influence NO−sGC−cGMP cardiovascular unity, as discussed in section 6.

Cardiovascular disease conditions are associated with a high level of oxidative stress and reduced overall levels of NO due to decreased or no synthesis and relatively increased scavenging. Therefore, optimal methods of inducing NO elution from vascular grafts required for surgical treatment of cardiovascular conditions have become of great interest, and numerous methods of NO elution are being investigated. L-Arginine has proven to be a potent NO synthesizer, but it is still in its infancy in terms of being accepted for its role in NO synthesis associated with vascular grafts. Further studies are also required for establishing methods of greater retention of water-soluble L-arg within grafts while ensuring that Arg interaction with cytosolic NOS will not be hindered. However, optimization of functional concentrations of NOS cofactors such as H_4B and greater understanding of the NOS enzyme kinetics are warranted.

Despite the heightened interest and many studies that were performed to observe NO release from polymers related to vascular grafts, proportionately fewer or no studies have been carried on to clinical trials, thus highlighting the need for greater investigations of the biochemical and biological fate of NO in vivo. Some of the main concerns associated with direct NO donors apart from the major issues associated with oxidative stress include the following: (1) A high concentration of NO in blood prompts more rapid uptake by hemoglobin and might counteract the localized high concentrations of NO eluted by direct donors. (2) Donors cannot be regenerated, and thus, the antithrombogenic role of the NO modification is limited to the half-life reactivity of the donors concerned (i.e., there is a greater need for modification of release rates of NO). Furthermore, current studies investigating NO-inducing mechanisms seem to have common uncertainties regarding the duration of NO retention and the appropriate NO donor concentrations required to retain their activity in NO-eluting vascular grafts when exposed to pulsatile blood flow. These concerns should be addressed with the scavenging effect of RBCs and also with barriers to scavenging so that the grafts with too high a concentration of NO, which could induce toxic effects or desensitization of downstream pathways, or grafts with too low a concentration to have a significant effect can be efficiently regulated. Hemeodynamics, with its influence on the relative distance of erythrocytes (as vehicles for hemoglobin) in vascular flow, has a major role in determining the bioavailability of NO from implanted vascular grafts. order In to obtain the best of RBC interactions with NO, it would be of interest to determine methods by which NO reaches to "a point of no return" with the interactions with RBCs and also to delineate and possibly facilitate the mechanisms to trigger "points of NO return" from RBCs. Hence, appropriate measures can be taken to modulate the scavenging effects of RBCs and also to promote NO from RBC NOS will be of interest.

Oxidative stress particularly affects the heme moiety of sGC and oxidizes the iron and renders it nonresponsive to NO, thus predisposing the enzyme to ubiquitin degradation. Therefore, despite many great beneficial roles, issues such as vascular tolerance and toxicity could prompt NO to be perceived as demanding and less reliable in diseased conditions. Molecules that can resist the oxidative insults to sGC and activate cGMP downstream pathways, such as nitroxyl and a group of pyrazolopyridines that can resist scavenging by superoxide and that can interact with sGC independent of the state of heme and also prevent ubiquitinated degradation, can be of interest as candidates that compete with NO for its claim as the guardian of the vascular grafts. However, these novel molecules are yet to receive approval (FDA) for clinical use.

8. CONCLUDING REMARKS

This article underlines the impact of NO on the attenuation of thrombotic events and a possible role in IRI in studies associated with cardiovascular grafts. NO-activated pathways have a clear role in preventing thrombosis, but the mode of action of NO in IRI is less clear. NO acts primarily through its interactions with sGC, a heterodimeric heme protein leading to cyclic guanosine-3′,5′-monophosphate (cGMP), which acts as a second messenger and modulates the above cardiovascular protective roles. A range of possibilities have been investigated as potential NO-modulating factors associated with cardiovascular grafts, including direct NO donors, L-arg, the functionality of NOS, cofactors such as H_4B, blood components, and the influence of hemodynamics. Considering that NO should not be dissipated during the process of fabrication and before being introduced into the vascular system, compatible methods of graft/polymer fabrication need to be sorted. Hemodynamics has a major role in determining the bioavailability of NO from implanted vascular grafts. Therefore, determination of graft compliance in parallel with NO−sGC−cGMP path activator concentration in physiological pulsatile flow while considering the modulation of roles of RBCs will be of interest. Molecules that can activate cGMP while being unaffected by possible desensitization or toxic effects of NO recently have attracted attention. However, decades of established reputation in protecting the cardiovascular system leave NO to reign as the guardian of vascular grafts for the foreseeable future.

AUTHOR INFORMATION

Corresponding Author

*Tel.: 0044 20 7830 2901. E-mail: a.seifalian@ucl.ac.uk.

BIOGRAPHIES

Achala de Mel obtained her B.Sc. (Honors) in Biochemistry and Biological Chemistry from the University of Nottingham in 2004, followed by a Master's degree in "Mechanisms of Vascular Disease" at the William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London (http://www.smd.qmul.ac.uk). Subsequently, she spent a brief period at the University of Oxford as a member of the Phosphorylation Dependent Signaling group of the Structural Genomic Consortium. Her Ph.D. thesis was titled "The development of a small diameter vascular bypass graft using stem cells and nanotechnology", she obtained her Ph.D. at the Centre for Nanotechnology & Regenerative Medicine, UCL Division of Surgery & Interventional Science, University College London, under the supervision of Professor Seifalian. Currently, she is a Research Fellow in the same department working on the development of nitric oxide eluting nanomaterials for cardiovascular and tissue engineering applications.

Professor Ferid Murad obtained his B.A. from DePauw University (1958) and his M.D. and Ph.D. from Case Western Reserve University (1965). He completed his residency at Massachusetts General Hospital and a fellowship at the National Institutes of Health. He was made a professor in 1970 at the University of Virginia, and then he moved to Stanford University in 1981. Later, he was appointed the vice president at Abbott Laboratories before he started his own biotechnology company, Molecular Geriatrics Corporation, in 1993. In 1997, Professor Murad joined the University of Texas to create a new department of integrative biology, pharmacology, and physiology, was the Professor and Director Emeritus of The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Disease, and held the John S. Dunn Distinguished Chair in Physiology and Medicine.

In 1998, Professor Murad received the Nobel Prize in medicine for the discovery of the role of nitric oxide in the cardiovascular system. Among his many awards and honors, Professor Murad has received the prestigious Albert and Mary Lasker Basic Medical Research Award, the American Heart Association Ciba Award, and the Baxter Award for Distinguished Research in the Biomedical Sciences from the Association of American Medical Colleges. He has also been awarded the American Society of Clinical Pharmacology Distinguished Research Prize and the President's Scholar Award from the University of Texas-Houston Health Science Center. Currently (since Jan 2011), Professor Murad is a Professor in the Department of Biochemistry and Molecular Biology and the director of the Institute of Molecular and Cellular Signaling at George Washington University.

Alexander Marcus Seifalian is a Professor of Nanotechnology and Regenerative Medicine and the Director of Centre for Nanotechnology & Regenerative Medicine at University College London. He is based within the Division of Surgery & Interventional Science. He completed his education at University of London and University College London Medical School. He is a Fellow of the Institute of Nanotechnology (FIoN) and has published over 325 peer-reviewed research papers, 31 book chapters and 4 families of patents.

During his career, Professor Seifalian has led and managed many large projects with multidisciplinary teams with very successful outcomes in terms of commercialization and translation to patients, including the development and commercialization of a bypass graft for vascular access for hemodialysis; laser-activated vascular sealants that have been commercialized for vascular, liver, and brain surgery; and regeneration of lacrimal ducts using nanomaterials and stem cells.

His current projects involve the development of biocompatible nanomaterials with bioactive molecules, including nitric oxide for cardiovascular implants; the development of nanoparticles for stem cell tracking; and the development of skin, nerve regeneration, and a range of tissue-engineered organs with a current grant sum of £4.2 million. He was awarded the top prize in the field of development of nanomaterials and technologies for the development of cardiovascular implants in 2007 by Medical Future Innovation, and in 2009, he received a Business Innovation Award from the U.K. Trade & Investment (UKTI) in the Life Sciences and Healthcare category.

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ABBREVIATIONS

OH	hydroxyl radical
ADMA	asymmetrical dimethylarginine, $C_8H_{18}N_4O_2$
ADP	adenosine diphosphate, $C_{10}H_{15}N_5O_{10}P_2$
AMPK$_\alpha$-AMP	activated protein kinase
Arg	arginine, $C_6H_{14}N_4O_2$
ATP	adenosine-5′-triphosphate, $C_{10}H_{16}N_5O_{13}P_3$
BAY 41-2272	5-cyclopropyl-2-[1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-pyrimidin-4-ylamine, $C_{20}H_{17}FN_6$
BAY 41-8543	2-[1-[(2-fluorophenyl)methyl]-1*H*-pyrazolo-[3,4-*b*]pyridin-3-yl]-5(4-morpholinyl)-4,6-pyrimidinediamine, $C_{21}H_{21}FN_8O$
BAY 58-2667	(4-[((4-carboxybutyl){2-[(4-phenethylbenzyl)-oxy]phenethyl}-amino)methyl-[benzoic]acid) (cinaciguat), $C_{36}H_{39}NO_5$
CABG	coronary artery bypass grafting
cGMP	cyclic guanylate monophosphate, $C_{10}H_{14}N_5O_8P$
Cit	citrulline, $C_6H_{13}N_3O_3$
CO	carbon monoxide
CysNO	S-nitrosocysteine, $C_3H_6N_2O_3S$
Dacron	poly(ethylene terephthalate)
DDAH	dimethylarginine dimethylaminohydrolase
DETA-NONOate	(Z)-1-[2-(2-Aminoethyl)-N-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate
DHFR	dihydrofolate reductase
DVT	deep venous thrombosis
ECs	endothelial cells
eNOS	endothelial nitric oxide synthase
EPC	endothelial progenitor cells
ePTFE	expanded polytetrafluoroethylene
ER	endoplasmic reticulum
GLP	good laboratory practice
GMP	good manufacturing practice
GPIIb/IIIa	glycoprotein IIb/IIIa inhibitors
GSNO	S-nitrosoglutathione, $C_{10}H_{16}N_4O_7S$
GSNOR−/−	S-nitrosoglutathione reductase null gene
GTN	glyceryl trinitrate/nitroglycerine, $C_3H_5N_3O_9$
GTP	guanosine-5′-triphosphate, $C_{10}H_{16}N_5O_{14}P_3$
H_2O_2	hydrogen peroxide
H_4B	tetrahydrobiopterin, $C_9H_{15}N_5O_3$
Hb	deoxyhemoglobin
Hb-Fe(II)NO	nitrosyl hemoglobin
Hb-Fe(III)	methemoglobin
HbO_2	oxyhemoglobin
His	histidine, $C_6H_9N_3O_2$
HMR1766	5-chloro-2-(5-chloro-thiophene-2-sulfonylamino-N-(4-(morpholine-4-sulfonyl)-phenyl)-benzamide sodium salt (ataciguat), $C_{21}H_{19}Cl_2N_3O_6S_3$
HNO	nitroxyl
H-NOX	heme nitric oxide/oxygen
HO-1	heme oxygenase-1
IGFBP-3	insulin-like growth factor binding protein 3
IH	intimal hyperplasia
iNOS	inducible nitric oxide synthase
IRI	ischemia reperfusion injury
ISDN	isosorbide dinitrate, $C_6H_8N_2O_8$
KLF2/4	Kruppel-like factor 2/4
L-arg	L-argine, $C_6H_{14}N_4O_2$
L-cit	L-citrulline, $C_6H_{13}N_3O_3$
L-NAME	N(G)-nitro-L-arginine methyl ester, $C_7H_{15}N_5O_4 \cdot HCl$
MI	myocardial infarction
NAC	N-acetylcysteine, $C_5H_9NO_3S$
NMMA	N-guanidino monomethyl arginine
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NO_2	nitrite
NOC-18	diazeniumdiolate, NCCN(CCN)N(O)N═O
NOS	nitric oxide synthase
O^-_2	superoxide
ODQ	1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one, $C_9H_5N_3O_2$
$ONOO^-$	peroxynitrite
PCU	poly(carbonate urea)urethane
PDE	phosphodiesterase
PETriN	pentaerythritol trinitrate, $C_5H_9N_3O_{10}$
PKGs	protein kinases
POSS	polyhedral oligomeric silsequioxane
RBC	red blood cells
ROS	reactive oxygen species
Ser	serine, $C_3H_7NO_3$
sGC	soluble guanylyl/guanylate cyclase
SMC	smooth muscle cells
SNAP	S-nitroso-*n*-acetylpenicillamine, $C_7H_{12}N_2O_4S$
SNO	*S*-nitrosohemoglobin
SOD	superoxide dismutase
TXA2	thromboxane A2
vWF	von Willebrand factor
XO	xanthine oxidase
YC-1	3-(5-hydroxymethyl-2-furyl)-1-benzyl indazole, $C_{19}H_{16}N_2O_2$

REFERENCES

(1) Furchgott, R. F.; Zawadzki, J. V. *Nature* **1980**, *288*, 373.
(2) Gruetter, C. A.; Barry, B. K.; McNamara, D. B.; Gruetter, D. Y.; Kadowitz, P. J.; Ignarro, L. *J. Cyclic Nucleotide Res.* **1979**, *5*, 211.
(3) Palmer, R. M.; Ferrige, A. G.; Moncada, S. *Nature* **1987**, *327*, 524.
(4) Vaughn, M. W.; Kuo, L.; Liao, J. C. *Am. J. Physiol.* **1998**, *274*, H2163.
(5) Arnold, W. P.; Mittal, C. K.; Katsuki, S.; Murad, F. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 3203.
(6) Mittal, C. K.; Braughler, J. M.; Ichihara, K.; Murad, F. *Biochim. Biophys. Acta* **1979**, *585*, 333.
(7) Murad, F. *Biosci. Rep* **2004**, *24*, 452.
(8) Malinski, T. *Am. J. Cardiol.* **2005**, *96*, 13i.
(9) McCleverty, J. A. *Chem. Rev.* **2004**, *104*, 403.
(10) Butler, A. R.; Megson, I. L. *Chem. Rev.* **2002**, *102*, 1155.
(11) Murad, F. *Circ. Res.* **2003**, *92*, 339.

(12) The Nobel Prize in Physiology or Medicine 1998: Robert F. Furchgott, Louis J. Ignarro, Ferid Murad. http://nobelprize.org/nobel_prizes/medicine/laureates/1998/ (accessed Jan 5, 2011).
(13) Hlatky, M. A.; Boothroyd, D. B.; Bravata, D. M.; Boersma, E.; Booth, J.; Brooks, M. M.; Carrie, D.; Clayton, T. C.; Danchin, N.; Flather, M.; Hamm, C. W.; Hueb, W. A.; Kahler, J.; Kelsey, S. F.; King, S. B.; Kosinski, A. S.; Lopes, N.; McDonald, K. M.; Rodriguez, A.; Serruys, P.; Sigwart, U.; Stables, R. H.; Owens, D. K.; Pocock, S. J. *Lancet* **2009**, *373*, 1190.
(14) Cheng, K. S.; Mikhailidis, D. P.; Hamilton, G.; Seifalian, A. M. *Cardiovasc. Res.* **2002**, *54*, 528.
(15) Baguneid, M. S.; Seifalian, A. M.; Salacinski, H. J.; Murray, D.; Hamilton, G.; Walker, M. G. *Br. J. Surg.* **2006**, *93*, 282.
(16) de Mel, A.; Bolvin, C.; Edirisinghe, M.; Hamilton, G.; Seifalian, A. M. *Expert Rev. Cardiovasc. Ther.* **2008**, *6*, 1259.
(17) Schofer, J.; Schluter, M.; Gershlick, A. H.; Wijns, W.; Garcia, E.; Schampaert, E.; Breithardt, G. *Lancet* **2003**, *362*, 1093.
(18) Stone, G. W.; Ellis, S. G.; Cannon, L.; Mann, J. T.; Greenberg, J. D.; Spriggs, D.; O'Shaughnessy, C. D.; DeMaio, S.; Hall, P.; Popma, J. J.; Koglin, J.; Russell, M. E. *J. Am. Med. Assoc.* **2005**, *294*, 1215.
(19) Stone, G. W.; Ellis, S. G.; Cox, D. A.; Hermiller, J.; O'Shaughnessy, C.; Mann, J. T.; Turco, M.; Caputo, R.; Bergin, P.; Greenberg, J.; Popma, J. J.; Russell, M. E. *N. Engl. J. Med.* **2004**, *350*, 221.
(20) Hunter, W. L. *Adv. Drug Delivery Rev.* **2006**, *58*, 347.
(21) Kabir, A. M.; Selvarajah, A.; Seifalian, A. M. *Future Cardiol.* **2011**, *7*, 251.
(22) Urban, P.; De Benedetti, E. *Lancet* **2007**, *369*, 619.
(23) Haruguchi, H.; Teraoka, S. *J. Artif. Organs* **2003**, *6*, 227.
(24) Sarkar, S.; Salacinski, H. J.; Hamilton, G.; Seifalian, A. M. *Eur. J. Vasc. Endovasc. Surg.* **2006**, *31*, 627.
(25) Tiwari, A.; Cheng, K. S.; Salacinski, H.; Hamilton, G.; Seifalian, A. M. *Eur. J. Vasc. Endovasc. Surg.* **2003**, *25*, 287.
(26) Sarkar, S.; Schmitz-Rixen, T.; Hamilton, G.; Seifalian, A. M. *Med. Biol. Eng. Comput.* **2007**, *45*, 327.
(27) Weman, S. M.; Karhunen, P. J.; Penttila, A.; Jarvinen, A. A.; Salminen, U. S. *Ann. Thorac. Surg.* **2000**, *70*, 807.
(28) Belch, J. J.; Dormandy, J. *J. Vasc. Surg.* **2010**, *52*, 825.
(29) Johnson, W. C.; Williford, W. O.; Corson, J. D.; Padberg, F. T., Jr. *Vascular* **2004**, *12*, 362.
(30) Poston, R. S.; Gu, J.; Brown, J. M.; Gammie, J. S.; White, C.; Nie, L.; Pierson, R. N., III; Griffith, B. P. *J. Thorac. Cardiovasc. Surg.* **2006**, *131*, 122.
(31) de Mel, A.; Jell, G.; Stevens, M. M.; Seifalian, A. M. *Biomacromolecules* **2008**, *9*, 2969.
(32) de Mel, A.; Punshon, G.; Ramesh, B.; Sarkar, S.; Darbyshire, A.; Hamilton, G.; Seifalian, A. M. *Biomed. Mater. Eng.* **2009**, *19*, 317.
(33) Motwani, M. S.; Rafiei, Y.; Tzifa, A.; Seifalian, A. M. *Biotechnol. Appl. Biochem.* **2011**, *58*, 2.
(34) Ahanchi, S. S.; Tsihlis, N. D.; Kibbe, M. R. *J. Vasc. Surg.* **2007**, *45* (Suppl. A), A64.
(35) Kapadia, M. R.; Chow, L. W.; Tsihlis, N. D.; Ahanchi, S. S.; Eng, J. W.; Murar, J.; Martinez, J.; Popowich, D. A.; Jiang, Q.; Hrabie, J. A.; Saavedra, J. E.; Keefer, L. K.; Hulvat, J. F.; Stupp, S. I.; Kibbe, M. R. *J. Vasc. Surg.* **2008**, *47*, 173.
(36) Nablo, B. J.; Prichard, H. L.; Butler, R. D.; Klitzman, B.; Schoenfisch, M. H. *Biomaterials* **2005**, *26*, 6984.
(37) Engelsman, A. F.; Krom, B. P.; Busscher, H. J.; van Dam, G. M.; Ploeg, R. J.; van der Mei, H. C. *Acta Biomater.* **2009**, *5*, 1905.
(38) Moore, C.; Tymvios, C.; Emerson, M. *Thromb. Haemost.* **2010**, *104*, 342.
(39) Roberts, W.; Michno, A.; Aburima, A.; Naseem, K. M. *J. Thromb. Haemost.* **2009**, *7*, 2106.
(40) Isenberg, J. S.; Romeo, M. J.; Yu, C.; Yu, C. K.; Nghiem, K.; Monsale, J.; Rick, M. E.; Wink, D. A.; Frazier, W. A.; Roberts, D. D. *Blood* **2008**, *111*, 613.
(41) Oberprieler, N. G.; Roberts, W.; Riba, R.; Graham, A. M.; Homer-Vanniasinkam, S.; Naseem, K. M. *FEBS Lett.* **2007**, *581*, 1529.
(42) Oberprieler, N. G.; Roberts, W.; Graham, A. M.; Homer-Vanniasinkam, S.; Naseem, K. M. *Biochem. Pharmacol.* **2007**, *73*, 1593.
(43) Irwin, C.; Roberts, W.; Naseem, K. M. *Platelets* **2009**, *20*, 478.
(44) Roberts, W.; Riba, R.; Homer-Vanniasinkam, S.; Farndale, R. W.; Naseem, K. M. *J. Thromb. Haemost.* **2008**, *6*, 2175.
(45) Catani, M. V.; Bernassola, F.; Rossi, A.; Melino, G. *Biochem. Biophys. Res. Commun.* **1998**, *249*, 275.
(46) Gkaliagkousi, E.; Ritter, J.; Ferro, A. *Circ. Res.* **2007**, *101*, 654.
(47) Signorello, M. G.; Segantin, A.; Leoncini, G. *Biochim. Biophys. Acta* **2009**, *1791*, 1084.
(48) O'Kane, P.; Xie, L.; Liu, Z.; Queen, L.; Jackson, G.; Ji, Y.; Ferro, A. *Cardiovasc. Res.* **2009**, *83*, 123.
(49) Radomski, M. W.; Palmer, R. M.; Moncada, S. *Lancet* **1987**, *2*, 1057.
(50) Loscalzo, J. *Circ. Res.* **2001**, *88*, 756.
(51) Willoughby, S. R.; Stewart, S.; Holmes, A. S.; Chirkov, Y. Y.; Horowitz, J. D. *Arterioscler. Thromb. Vasc. Biol.* **2005**, *25*, 2661.
(52) Katoh, A.; Ikeda, H.; Takajo, Y.; Haramaki, N.; Murohara, T.; Shintani, S.; Kanaya, S.; Yokoyama, S.; Ueno, T.; Honma, T.; Imaizumi, T. *Circ. J.* **2002**, *66*, 837.
(53) Schafer, A.; Wiesmann, F.; Neubauer, S.; Eigenthaler, M.; Bauersachs, J.; Channon, K. M. *Circulation* **2004**, *109*, 1819.
(54) Lin, P. H.; Johnson, C. K.; Pullium, J. K.; Bush, R. L.; Conklin, B. S.; Chen, C.; Lumsden, A. B. *J. Vasc. Surg.* **2003**, *38*, 1396.
(55) Wang, P. G.; Xian, M.; Tang, X.; Wu, X.; Wen, Z.; Cai, T.; Janczuk, A. J. *Chem. Rev.* **2002**, *102*, 1091.
(56) Napoli, C.; Ignarro, L. J. *Annu. Rev. Pharmacol. Toxicol.* **2003**, *43*, 97.
(57) Kannan, R. Y.; Salacinski, H. J.; Butler, P. E.; Seifalian, A. M. *Acc. Chem. Res.* **2005**, *38*, 879.
(58) Seifalian, A. M.; Handcock, S.; Salacinski, H. J. Polymer for use in conduits, medical devices and biomedical surface modification. World Patent WO 2005/070988, 2005.
(59) Ghanbari, H.; de Mel, A.; Seifalian, A. M. *Int. J. Nanomed.* **2011**, *6*, 775.
(60) Sarkar, S.; Burriesci, G.; Wojcik, A.; Aresti, N.; Hamilton, G.; Seifalian, A. M. *J. Biomech.* **2009**, *42*, 722.
(61) Ozuyaman, B.; Ebner, P.; Niesler, U.; Ziemann, J.; Kleinbongard, P.; Jax, T.; Godecke, A.; Kelm, M.; Kalka, C. *Thromb. Haemost.* **2005**, *94*, 770.
(62) Yin, T.; Ma, X.; Zhao, L.; Cheng, K.; Wang, H. *Cell Res.* **2008**, *18*, 792.
(63) d'Uscio, L. V.; Smith, L. A.; Santhanam, A. V.; Richardson, D.; Nath, K. A.; Katusic, Z. S. *Hypertension* **2007**, *49*, 1142.
(64) Santhanam, A. V.; d'Uscio, L. V.; Peterson, T. E.; Katusic, Z. S. *Peptides* **2008**, *29*, 1451.
(65) Urao, N.; Okigaki, M.; Yamada, H.; Aadachi, Y.; Matsuno, K.; Matsui, A.; Matsunaga, S.; Tateishi, K.; Nomura, T.; Takahashi, T.; Tatsumi, T.; Matsubara, H. *Circ. Res.* **2006**, *98*, 1405.
(66) Kielczewski, J. L.; Jarajapu, Y. P.; McFarland, E. L.; Cai, J.; Afzal, A.; Li, C. S.; Chang, K. H.; Lydic, T.; Shaw, L. C.; Busik, J.; Hughes, J.; Cardounel, A. J.; Wilson, K.; Lyons, T. J.; Boulton, M. E.; Mames, R. N.; Chan-Ling, T.; Grant, M. B. *Circ. Res.* **2009**, *105*, 897.
(67) Aicher, A.; Heeschen, C.; Mildner-Rihm, C.; Urbich, C.; Ihling, C.; Technau-Ihling, K.; Zeiher, A. M.; Dimmeler, S. *Nat. Med.* **2003**, *9*, 1370.
(68) Wegiel, B.; Gallo, D. J.; Raman, K. G.; Karlsson, J. M.; Ozanich, B.; Chin, B. Y.; Tzeng, E.; Ahmad, S.; Ahmed, A.; Baty, C. J.; Otterbein, L. E. *Circulation* **2010**, *121*, 537.
(69) Taite, L. J.; Yang, P.; Jun, H. W.; West, J. L. *J. Biomed. Mater. Res. B: Appl. Biomater.* **2008**, *84*, 108.
(70) Thum, T.; Fraccarollo, D.; Thum, S.; Schultheiss, M.; Daiber, A.; Wenzel, P.; Munzel, T.; Ertl, G.; Bauersachs, J. *Arterioscler. Thromb. Vasc. Biol.* **2007**, *27*, 748.
(71) Hamed, S.; Brenner, B.; Aharon, A.; Daoud, D.; Roguin, A. *Cardiovasc. Diabetol.* **2009**, *8*, 56.
(72) Sung, M. L.; Wu, C. C.; Chang, H. I.; Yen, C. K.; Chen, H. J.; Cheng, J. C.; Chien, S.; Chen, C. N. *Circ. Res.* **2009**, *105*, 755.
(73) Jennings, R. B.; Sommers, H.; Smyth, G.; Flack, H.; Linn, H. *Arch. Pathol.* **1960**, *70*, 68.

(74) Prasad, A.; Stone, G. W.; Holmes, D. R.; Gersh, B. *Circulation* **2009**, *120*, 2105.
(75) Weiss, E. S.; Chang, D. D.; Joyce, D. L.; Nwakanma, L. U.; Yuh, D. D. *J. Thorac. Cardiovasc. Surg.* **2008**, *135*, 503.
(76) Garcia-Dorado, D.; Agullo, L.; Sartorio, C. L.; Ruiz-Meana, M. *Thromb. Haemost.* **2009**, *101*, 635.
(77) Kasseckert, S. A.; Schafer, C.; Kluger, A.; Gligorievski, D.; Tillmann, J.; Schluter, K. D.; Noll, T.; Sauer, H.; Piper, H. M.; Abdallah, Y. *Cardiovasc. Res.* **2009**, *83*, 381.
(78) Zweier, J. L.; Talukder, M. A. *Cardiovasc. Res.* **2006**, *70*, 181.
(79) Dumitrescu, C.; Biondi, R.; Xia, Y.; Cardounel, A. J.; Druhan, L. J.; Ambrosio, G.; Zweier, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 15081.
(80) Rosen, G. M.; Tsai, P.; Pou, S. *Chem. Rev.* **2002**, *102*, 1191.
(81) Settergren, M.; Bohm, F.; Malmstrom, R. E.; Channon, K. M.; Pernow, J. *Atherosclerosis* **2009**, *204*, 73.
(82) Victor, V. M.; Nunez, C.; D'Ocon, P.; Taylor, C. T.; Esplugues, J. V.; Moncada, S. *Circ. Res.* **2009**, *104*, 1178.
(83) Taylor, C. T.; Moncada, S. *Arterioscler. Thromb. Vasc. Biol.* **2010**, *30*, 643.
(84) Gladwin, M. T.; Shiva, S. *Circ. Res.* **2009**, *104*, 1136.
(85) Abu-Amara, M.; Yang, S. Y.; Quaglia, A.; Rowley, P.; Fuller, B.; Seifalian, A.; Davidson, B. *Liver Transpl.* **2011**, *17*, 610.
(86) Abu-Amara, M.; Yang, S. Y.; Quaglia, A.; Rowley, P.; de Mel, A.; Tapuria, N.; Seifalian, A. M.; Davidson, B.; Fuller, B. *Clin. Sci. (London)*, **2011**, *121*, 257.
(87) Chan, K. L. *Curr. Med. Chem.: Anti-Inflammatory Anti-Allergy Agents* **2002**, *1*, 1.
(88) Korkmaz, S.; Radovits, T.; Barnucz, E.; Hirschberg, K.; Neugebauer, P.; Loganathan, S.; Veres, G.; Pali, S.; Seidel, B.; Zollner, S.; Karck, M.; Szabo, G. *Circulation* **2009**, *120*, 677.
(89) Penna, C.; Mancardi, D.; Rastaldo, R.; Pagliaro, P. *Biochim. Biophys. Acta* **2009**, *1787*, 781.
(90) Lee, C. I.; Liu, X.; Zweier, J. L. *J. Biol. Chem.* **2000**, *275*, 9369.
(91) Koti, R. S.; Seifalian, A. M.; McBride, A. G.; Yang, W.; Davidson, B. R. *FASEB J.* **2002**, *16*, 1654.
(92) Talukder, M. A.; Yang, F.; Shimokawa, H.; Zweier, J. L. *Am. J. Physiol.: Heart Circ. Physiol.* **2010**, *299*, H437.
(93) Ii, M.; Nishimura, H.; Iwakura, A.; Wecker, A.; Eaton, E.; Asahara, T.; Losordo, D. W. *Circulation* **2005**, *111*, 1114.
(94) West, M. B.; Rokosh, G.; Obal, D.; Velayutham, M.; Xuan, Y. T.; Hill, B. G.; Keith, R. J.; Schrader, J.; Guo, Y.; Conklin, D. J.; Prabhu, S. D.; Zweier, J. L.; Bolli, R.; Bhatnagar, A. *Circulation* **2008**, *118*, 1970.
(95) du Toit, E. F.; Genade, S.; Carlini, S.; Moolman, J. A.; Brunner, F.; Lochner, A. *Eur. J. Pharmacol.* **2007**, *556*, 115.
(96) Burkard, N.; Williams, T.; Czolbe, M.; Blomer, N.; Panther, F.; Link, M.; Fraccarollo, D.; Widder, J. D.; Hu, K.; Han, H.; Hofmann, U.; Frantz, S.; Nordbeck, P.; Bulla, J.; Schuh, K.; Ritter, O. *Circulation* **2010**, *122*, 1588.
(97) Jansson, E. A.; Huang, L.; Malkey, R.; Govoni, M.; Nihlen, C.; Olsson, A.; Stensdotter, M.; Petersson, J.; Holm, L.; Weitzberg, E.; Lundberg, J. O. *Nat. Chem. Biol.* **2008**, *4*, 411.
(98) Zweier, J. L.; Li, H.; Samouilov, A.; Liu, X. *Nitric Oxide* **2010**, *22*, 83.
(99) Lundberg, J. O.; Weitzberg, E.; Gladwin, M. T. *Nat. Rev. Drug Discov.* **2008**, *7*, 156.
(100) Lundberg, J. O.; Gladwin, M. T.; Ahluwalia, A.; Benjamin, N.; Bryan, N. S.; Butler, A.; Cabrales, P.; Fago, A.; Feelisch, M.; Ford, P. C.; Freeman, B. A.; Frenneaux, M.; Friedman, J.; Kelm, M.; Kevil, C. G.; Kim-Shapiro, D. B.; Kozlov, A. V.; Lancaster, J. R., Jr.; Lefer, D. J.; McColl, K.; McCurry, K.; Patel, R. P.; Petersson, J.; Rassaf, T.; Reutov, V. P.; Richter-Addo, G. B.; Schechter, A.; Shiva, S.; Tsuchiya, K.; van Faassen, E. E.; Webb, A. J.; Zuckerbraun, B. S.; Zweier, J. L.; Weitzberg, E. *Nat. Chem. Biol.* **2009**, *5*, 865.
(101) Alzawahra, W. F.; Talukder, M. A.; Liu, X.; Samouilov, A.; Zweier, J. L. *Am. J. Physiol.: Heart Circ. Physiol.* **2008**, *295*, H499.
(102) Mihov, D.; Bogdanov, N.; Grenacher, B.; Gassmann, M.; Zund, G.; Bogdanova, A.; Tavakoli, R. *Eur. J. Cardiothorac. Surg.* **2009**, *35*, 839.
(103) Daff, S. *Nitric Oxide* **2010**, *23*, 1.
(104) Alnaeb, M. E.; Thompson, C. S.; Seifalian, A. M.; Hamilton, G.; Mikhailidis, D. P. *In Vivo* **2007**, *21*, 1069.
(105) Roman, L. J.; Martasek, P.; Masters, B. S. *Chem. Rev.* **2002**, *102*, 1179.
(106) Tousoulis, D.; Boger, R. H.; Antoniades, C.; Siasos, G.; Stefanadi, E.; Stefanadis, C. *Nat. Clin. Pract. Cardiovasc. Med.* **2007**, *4*, 274.
(107) Maas, R.; Xanthakis, V.; Polak, J. F.; Schwedhelm, E.; Sullivan, L. M.; Benndorf, R.; Schulze, F.; Vasan, R. S.; Wolf, P. A.; Boger, R. H.; Seshadri, S. *Stroke* **2009**, *40*, 2715.
(108) Chirinos, J. A.; David, R.; Bralley, J. A.; Zea-Diaz, H.; Munoz-Atahualpa, E.; Corrales-Medina, F.; Cuba-Bustinza, C.; Chirinos-Pacheco, J.; Medina-Lezama, J. *Hypertension* **2008**, *52*, 1051.
(109) Zeller, M.; Korandji, C.; Guilland, J. C.; Sicard, P.; Vergely, C.; Lorgis, L.; Beer, J. C.; Duvillard, L.; Lagrost, A. C.; Moreau, D.; Gambert, P.; Cottin, Y.; Rochette, L. *Arterioscler. Thromb. Vasc. Biol.* **2008**, *28*, 954.
(110) Pope, A. J.; Karrupiah, K.; Kearns, P. N.; Xia, Y.; Cardounel, A. J. *J. Biol. Chem.* **2009**, *284*, 35338.
(111) Kown, M. H.; van Der, S. T.; Uemura, S.; Jahncke, C. L.; Hoyt, G. E.; Rothbard, J. B.; Robbins, R. C. *Transplantation* **2001**, *71*, 1542.
(112) Kown, M. H.; Lijkwan, M. A.; Jahncke, C. L.; Murata, S.; Rothbard, J. B.; Robbins, R. C. *J. Thorac. Cardiovasc. Surg.* **2003**, *126*, 1065.
(113) Chen, Z.; Peng, I. C.; Sun, W.; Su, M. I.; Hsu, P. H.; Fu, Y.; Zhu, Y.; DeFea, K.; Pan, S.; Tsai, M. D.; Shyy, J. Y. *Circ. Res.* **2009**, *104*, 496.
(114) Schulz, E.; Schuhmacher, S.; Munzel, T. *Circ. Res.* **2009**, *104*, 422.
(115) Dreifaldt, M.; Souza, D. S.; Loesch, A.; Muddle, J. R.; Karlsson, M. G.; Filbey, D.; Bodin, L.; Norgren, L.; Dashwood, M. R. *J. Thorac. Cardiovasc. Surg.* **2011**, *141*, 145.
(116) Nowicki, M.; Misterski, M.; Malinska, A.; Perek, B.; Ostalska-Nowicka, D.; Jemielity, M.; Witkiewicz, W.; Zabel, M. *Eur. J. Cardiothorac. Surg.* **2011**, *39*, 471.
(117) Payne, G. A.; Borbouse, L.; Kumar, S.; Neeb, Z.; Alloosh, M.; Sturek, M.; Tune, J. D. *Arterioscler. Thromb. Vasc. Biol.* **2010**, *30*, 1711.
(118) de Mel, A.; Ramesh, B.; Darbyshire, A.; Hamilton, G.; Seifalian, A. An arginine incorporated nanocomposite polymer for vascular bypass grafts. Presented at the *12th Biennial Meeting of the International Society for Applied Cardiovascular Biology*, Cambridge, MA, Sep 22−25, 2010.
(119) Liu, Y.; Yang, Y.; Wu, F. *Appl. Surf. Sci.* **2010**, *256*, 3977.
(120) Wei, C. C.; Crane, B. R.; Stuehr, D. J. *Chem. Rev.* **2003**, *103*, 2365.
(121) Katusic, Z. S.; d'Uscio, L. V.; Nath, K. A. *Trends Pharmacol. Sci.* **2009**, *30*, 48.
(122) Sugiyama, T.; Levy, B. D.; Michel, T. *J. Biol. Chem.* **2009**, *284*, 12691.
(123) Michel, T. *Circulation* **2010**, *121*, 484.
(124) Heitzer, T.; Brockhoff, C.; Mayer, B.; Warnholtz, A.; Mollnau, H.; Henne, S.; Meinertz, T.; Munzel, T. *Circ. Res.* **2000**, *86*, E36.
(125) Cai, S.; Khoo, J.; Channon, K. M. *Cardiovasc. Res.* **2005**, *65*, 823.
(126) Ihlemann, N.; Rask-Madsen, C.; Perner, A.; Dominguez, H.; Hermann, T.; Kober, L.; Torp-Pedersen, C. *Am. J. Physiol.: Heart Circ. Physiol.* **2003**, *285*, H875.
(127) Gori, T.; Burstein, J. M.; Ahmed, S.; Miner, S. E.; Al-Hesayen, A.; Kelly, S.; Parker, J. D. *Circulation* **2001**, *104*, 1119.
(128) Gori, T.; Saunders, L.; Ahmed, S.; Parker, J. D. *J. Cardiovasc. Pharmacol.* **2003**, *41*, 185.
(129) Ali, Z. A.; Bursill, C. A.; Douglas, G.; McNeill, E.; Papaspyridonos, M.; Tatham, A. L.; Bendall, J. K.; Akhtar, A. M.; Alp, N. J.; Greaves, D. R.; Channon, K. M. *Circulation* **2008**, *118*, S71.
(130) Kim, J. H.; Bugaj, L. J.; Oh, Y. J.; Bivalacqua, T. J.; Ryoo, S.; Soucy, K. G.; Santhanam, L.; Webb, A.; Camara, A.; Sikka, G.; Nyhan, D.; Shoukas, A. A.; Ilies, M.; Christianson, D. W.; Champion, H. C.; Berkowitz, D. E. *J. Appl. Physiol.* **2009**, *107*, 1249.
(131) Singh, U.; Devaraj, S.; Vasquez-Vivar, J.; Jialal, I. *J. Mol. Cell Cardiol.* **2007**, *43*, 780.

(132) Kuzkaya, N.; Weissmann, N.; Harrison, D. G.; Dikalov, S. *J. Biol. Chem.* **2003**, *278*, 22546.
(133) Crabtree, M. J.; Tatham, A. L.; Hale, A. B.; Alp, N. J.; Channon, K. M. *J. Biol. Chem.* **2009**, *284*, 28128.
(134) Takeda, M.; Yamashita, T.; Shinohara, M.; Sasaki, N.; Takaya, T.; Nakajima, K.; Inoue, N.; Masano, T.; Tawa, H.; Satomi-Kobayashi, S.; Toh, R.; Sugiyama, D.; Nishimura, K.; Yokoyama, M.; Hirata, K.; Kawashima, S. *Circ. J.* **2009**, *73*, 955.
(135) Tan, A.; Sumpio, B. E.; Lee, S.; Seifalian, A. M. *Curr. Pharm. Des* **2010**, *16*, 3848.
(136) Vara, D. S.; Punshon, G.; Sales, K. M.; Hamilton, G.; Seifalian, A. M. *Curr. Vasc. Pharmacol.* **2011**, *9*, 167.
(137) Boo, Y. C.; Jo, H. *Am. J. Physiol.: Cell Physiol.* **2003**, *285*, C499.
(138) Shen, B.; Smith, R. S., Jr.; Hsu, Y. T.; Chao, L.; Chao, J. *J. Biol. Chem.* **2009**, *284*, 35471.
(139) Wang, W.; Ha, C. H.; Jhun, B. S.; Wong, C.; Jain, M. K.; Jin, Z. G. *Blood* **2010**, *115*, 2971.
(140) Salacinski, H. J.; Goldner, S.; Giudiceandrea, A.; Hamilton, G.; Seifalian, A. M.; Edwards, A.; Carson, R. J. *J. Biomater. Appl.* **2001**, *15*, 241.
(141) Tai, N. R.; Giudiceandrea, A.; Salacinski, H. J.; Seifalian, A. M.; Hamilton, G. *J. Vasc. Surg.* **1999**, *30*, 936.
(142) Huang, B.; Chen, S. C.; Wang, D. L. *Cardiovasc. Res.* **2009**, *83*, 536.
(143) Thacher, T. N.; Gambillara, V.; Riche, F.; Silacci, P.; Stergiopulos, N.; da Silva, R. F. *Atherosclerosis* **2010**, *210*, 63.
(144) Allen, B. W.; Stamler, J. S.; Piantadosi, C. A. *Trends Mol. Med.* **2009**, *15*, 452.
(145) Jensen, F. B.; Rohde, S. *Am. J. Physiol.: Regul. Integr. Comp. Physiol.* **2010**, *298*, R972.
(146) Yu, B.; Bloch, K. D.; Zapol, W. M. *Trends Cardiovasc. Med.* **2009**, *19*, 103.
(147) Fronticelli, C.; Koehler, R. C. *Crit. Care Clin.* **2009**, *25*, 357.
(148) Hendgen-Cotta, U. B.; Merx, M. W.; Shiva, S.; Schmitz, J.; Becher, S.; Klare, J. P.; Steinhoff, H. J.; Goedecke, A.; Schrader, J.; Gladwin, M. T.; Kelm, M.; Rassaf, T. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 10256.
(149) Kleinbongard, P.; Schulz, R.; Rassaf, T.; Lauer, T.; Dejam, A.; Jax, T.; Kumara, I.; Gharini, P.; Kabanova, S.; Ozuyaman, B.; Schnurch, H. G.; Godecke, A.; Weber, A. A.; Robenek, M.; Robenek, H.; Bloch, W.; Rosen, P.; Kelm, M. *Blood* **2006**, *107*, 2943.
(150) Ulker, P.; Sati, L.; Celik-Ozenci, C.; Meiselman, H. J.; Baskurt, O. K. *Biorheology* **2009**, *46*, 121.
(151) Rifkind, J. M.; Nagababu, E.; Cao, Z.; Barbiro-Michaely, E.; Mayevsky, A. *Adv. Exp. Med. Biol.* **2009**, *645*, 27.
(152) Patel, R. P.; Hogg, N.; Kim-Shapiro, D. B. *Cardiovasc. Res.* **2011**, *89*, 507.
(153) Sayed, N.; Baskaran, P.; Ma, X.; van den Akker, F.; Beuve, A. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 12312.
(154) El-Farra, N. H.; Christofides, P. D.; Liao, J. C. *Ann. Biomed. Eng* **2003**, *31*, 294.
(155) Azarov, I.; Huang, K. T.; Basu, S.; Gladwin, M. T.; Hogg, N.; Kim-Shapiro, D. B. *J. Biol. Chem.* **2005**, *280*, 39024.
(156) Vaughn, M. W.; Huang, K. T.; Kuo, L.; Liao, J. C. *J. Biol. Chem.* **2000**, *275*, 2342.
(157) Liu, X.; Srinivasan, P.; Collard, E.; Grajdeanu, P.; Lok, K.; Boyle, S. E.; Friedman, A.; Zweier, J. L. *Free Radic. Biol. Med.* **2010**, *48*, 554.
(158) Reynolds, J. D.; Ahearn, G. S.; Angelo, M.; Zhang, J.; Cobb, F.; Stamler, J. S. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 17058.
(159) Isbell, T. S.; Sun, C. W.; Wu, L. C.; Teng, X.; Vitturi, D. A.; Branch, B. G.; Kevil, C. G.; Peng, N.; Wyss, J. M.; Ambalavanan, N.; Schwiebert, L.; Ren, J.; Pawlik, K. M.; Renfrow, M. B.; Patel, R. P.; Townes, T. M. *Nat. Med.* **2008**, *14*, 773.
(160) Thakrar, R. R.; Patel, V. P.; Hamilton, G.; Fuller, B. J.; Seifalian, A. M. *FASEB J.* **2006**, *20*, 874.
(161) Balbatun, A.; Louka, F. R.; Malinski, T. *Acta Biochim. Pol.* **2003**, *50*, 61.
(162) Cheng, K. S.; Baker, C. R.; Hamilton, G.; Hoeks, A. P.; Seifalian, A. M. *Eur. J. Vasc. Endovasc. Surg.* **2002**, *24*, 383.
(163) Rashid, S. T.; Fuller, B.; Hamilton, G.; Seifalian, A. M. *FASEB J.* **2008**, *22*, 2084.
(164) Schulz, E.; Tsilimingas, N.; Rinze, R.; Reiter, B.; Wendt, M.; Oelze, M.; Woelken-Weckmuller, S.; Walter, U.; Reichenspurner, H.; Meinertz, T.; Munzel, T. *Circulation* **2002**, *105*, 1170.
(165) Thadani, U.; Ripley, T. L. *Expert Opin. Drug Saf.* **2007**, *6*, 385.
(166) Ali, I. S.; Buth, K. J.; Maitland, A. *Am. Heart J.* **2004**, *148*, 727.
(167) Huellner, M. W.; Schrepfer, S.; Weyand, M.; Weiner, H.; Wimplinger, I.; Eschenhagen, T.; Rau, T. *FASEB J.* **2008**, *22*, 2561.
(168) Munzel, T.; Daiber, A.; Mulsch, A. *Circ. Res.* **2005**, *97*, 618.
(169) Sayed, N.; Kim, D. D.; Fioramonti, X.; Iwahashi, T.; Duran, W. N.; Beuve, A. *Circ. Res.* **2008**, *103*, 606.
(170) Dou, D.; Ma, H.; Zheng, X.; Ying, L.; Guo, Y.; Yu, X.; Gao, Y. *Cardiovasc. Res.* **2010**, *86*, 151.
(171) Rajendran, S.; Chirkov, Y. Y. *Cardiovasc. Drugs Ther.* **2008**, *22*, 193.
(172) Gkaliagkousi, E.; Corrigall, V.; Becker, S.; de, W. P.; Shah, A.; Zamboulis, C.; Ritter, J.; Ferro, A. *Eur. Heart J.* **2009**, *30*, 3048.
(173) Gori, T.; Parker, J. D. *Circulation* **2002**, *106*, 2510.
(174) Ring, A.; Mullershausen, F.; Goertz, O.; Koesling, D.; Muhr, G.; Steinau, H. U.; Steinstraesser, L.; Langer, S. *J. Surg. Res.* **2010**, *164*, 169.
(175) Halvey, E. J.; Vernon, J.; Roy, B.; Garthwaite, J. *J. Biol. Chem.* **2009**, *284*, 25630.
(176) Shi, W.; Wang, X.; Shih, D. M.; Laubach, V. E.; Navab, M.; Lusis, A. J. *Circulation* **2002**, *105*, 2078.
(177) Reiter, T. A.; Pang, B.; Dedon, P.; Demple, B. *J. Biol. Chem.* **2006**, *281*, 36603.
(178) Virag, L.; Szabo, E.; Gergely, P.; Szabo, C. *Toxicol. Lett.* **2003**, *140−141*, 113.
(179) Peluffo, G.; Radi, R. *Cardiovasc. Res.* **2007**, *75*, 291.
(180) Hayashi, Y.; Sawa, Y.; Nishimura, M.; Fukuyama, N.; Ichikawa, H.; Ohtake, S.; Nakazawa, H.; Matsuda, H. *Eur. J. Cardiothorac. Surg.* **2004**, *26*, 276.
(181) Goldstein, S.; Lind, J.; Merenyi, G. *Chem. Rev.* **2005**, *105*, 2457.
(182) Radi, R.; Peluffo, G.; Alvarez, M. N.; Naviliat, M.; Cayota, A. *Free Radic. Biol. Med.* **2001**, *30*, 463.
(183) Huang, J.; Lin, S. C.; Nadershahi, A.; Watts, S. W.; Sarkar, R. *J. Vasc. Surg.* **2008**, *47*, 599.
(184) Moller, J. K.; Skibsted, L. H. *Chem. Rev.* **2002**, *102*, 1167.
(185) Murad, F. *N. Engl. J. Med.* **2006**, *355*, 2003.
(186) Boerrigter, G.; Burnett, J. C., Jr. *Circulation* **2009**, *119*, 2752.
(187) Garthwaite, J. *Mol. Cell. Biochem.* **2010**, *334*, 221.
(188) Francis, S. H.; Busch, J. L.; Corbin, J. D.; Sibley, D. *Pharmacol. Rev.* **2010**, *62*, 525.
(189) Derbyshire, E. R.; Marletta, M. A. *Handb. Exp. Pharmacol.* **2009**, *191*, 17.
(190) Cary, S. P.; Winger, J. A.; Marletta, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 13064.
(191) Capece, L.; Estrin, D. A.; Marti, M. A. *Biochemistry* **2008**, *47*, 9416.
(192) Derbyshire, E. R.; Fernhoff, N. B.; Deng, S.; Marletta, M. A. *Biochemistry* **2009**, *48*, 7519.
(193) Zhou, Z.; Sayed, N.; Pyriochou, A.; Roussos, C.; Fulton, D.; Beuve, A.; Papapetropoulos, A. *Arterioscler. Thromb. Vasc. Biol.* **2008**, *28*, 1803.
(194) Miller, T. W.; Isenberg, J. S.; Roberts, D. D. *Br. J. Pharmacol.* **2010**, *159*, 1542.
(195) Fernhoff, N. B.; Derbyshire, E. R.; Marletta, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 21602.
(196) Ibrahim, M.; Derbyshire, E. R.; Marletta, M. A.; Spiro, T. G. *Biochemistry* **2010**, *49*, 3815.
(197) Ma, X.; Sayed, N.; Beuve, A.; van den Akker, F. *EMBO J.* **2007**, *26*, 578.
(198) Zhang, Y.; Lu, M.; Cheng, Y.; Li, Z. *J. Mol. Graph. Model.* **2010**, *28*, 814.

(199) Ibrahim, M.; Derbyshire, E. R.; Soldatova, A. V.; Marletta, M. A.; Spiro, T. G. *Biochemistry* **2010**, *49*, 4864.
(200) Derbyshire, E. R.; Deng, S.; Marletta, M. A. *J. Biol. Chem.* **2010**, *285*, 17471.
(201) Boon, E. M.; Huang, S. H.; Marletta, M. A. *Nat. Chem. Biol.* **2005**, *1*, 53.
(202) Miller, T. W.; Cherney, M. M.; Lee, A. J.; Francoleon, N. E.; Farmer, P. J.; King, S. B.; Hobbs, A. J.; Miranda, K. M.; Burstyn, J. N.; Fukuto, J. M. *J. Biol. Chem.* **2009**, *284*, 21788.
(203) Flores-Santana, W.; Switzer, C.; Ridnour, L. A.; Basudhar, D.; Mancardi, D.; Donzelli, S.; Thomas, D. D.; Miranda, K. M.; Fukuto, J. M.; Wink, D. A. *Arch. Pharm. Res.* **2009**, *32*, 1139.
(204) Fukuto, J. M.; Bianco, C. L.; Chavez, T. A. *Free Radic. Biol. Med.* **2009**, *47*, 1318.
(205) Irvine, J. C.; Ritchie, R. H.; Favaloro, J. L.; Andrews, K. L.; Widdop, R. E.; Kemp-Harper, B. K. *Trends Pharmacol. Sci.* **2008**, *29*, 601.
(206) Stasch, J. P.; Becker, E. M.; Alonso-Alija, C.; Apeler, H.; Dembowsky, K.; Feurer, A.; Gerzer, R.; Minuth, T.; Perzborn, E.; Pleiss, U.; Schroder, H.; Schroeder, W.; Stahl, E.; Steinke, W.; Straub, A.; Schramm, M. *Nature* **2001**, *410*, 212.
(207) Priviero, F. B.; Webb, R. C. *J. Cardiovasc. Pharmacol.* **2010**, *56*, 229.
(208) Stasch, J. P.; Hobbs, A. J. *Handb. Exp. Pharmacol.* **2009**, *191*, 277.
(209) Schmidt, H. H.; Schmidt, P. M.; Stasch, J. P. *Handb. Exp. Pharmacol.* **2009**, *191*, 309.
(210) Martin, F.; Baskaran, P.; Ma, X.; Dunten, P. W.; Schaefer, M.; Stasch, J. P.; Beuve, A.; van den Akker, F. *J. Biol. Chem.* **2010**, *285*, 22651.
(211) Krieg, T.; Liu, Y.; Rutz, T.; Methner, C.; Yang, X. M.; Dost, T.; Felix, S. B.; Stasch, J. P.; Cohen, M. V.; Downey, J. M. *Eur. Heart J.* **2009**, *30*, 1607.
(212) Sovershaev, M. A.; Egorina, E. M.; Hansen, J. B.; Osterud, B.; Pacher, P.; Stasch, J. P.; Evgenov, O. V. *Arterioscler. Thromb. Vasc. Biol.* **2009**, *29*, 1578.
(213) Mujoo, K.; Sharin, V. G.; Bryan, N. S.; Krumenacker, J. S.; Sloan, C.; Parveen, S.; Nikonoff, L. E.; Kots, A. Y.; Murad, F. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 18924.
(214) Hoffmann, L. S.; Schmidt, P. M.; Keim, Y.; Schaefer, S.; Schmidt, H. H.; Stasch, J. P. *Br. J. Pharmacol.* **2009**, *157*, 781.
(215) Marcondes, S.; Cardoso, M. H.; Morganti, R. P.; Thomazzi, S. M.; Lilla, S.; Murad, F.; De, N. G.; Antunes, E. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 3434.
(216) Lima, B.; Lam, G. K.; Xie, L.; Diesen, D. L.; Villamizar, N.; Nienaber, J.; Messina, E.; Bowles, D.; Kontos, C. D.; Hare, J. M.; Stamler, J. S.; Rockman, H. A. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 6297.
(217) Lima, B.; Forrester, M. T.; Hess, D. T.; Stamler, J. S. *Circ. Res.* **2010**, *106*, 633.
(218) Martinez-Ruiz, A.; Lamas, S. *Cardiovasc. Res.* **2007**, *75*, 220.
(219) Morrell, C. N.; Matsushita, K.; Chiles, K.; Scharpf, R. B.; Yamakuchi, M.; Mason, R. J.; Bergmeier, W.; Mankowski, J. L.; Baldwin, W. M., III; Faraday, N.; Lowenstein, C. J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 3782.
(220) Walsh, G. M.; Leane, D.; Moran, N.; Keyes, T. E.; Forster, R. J.; Kenny, D.; O'Neill, S. *Biochemistry* **2007**, *46*, 6429.
(221) Rudolph, V.; Freeman, B. A. *Circ. Res.* **2009**, *105*, 511.
(222) Bhatt, D. L.; Topol, E. J. *Nat. Rev. Drug Discov.* **2003**, *2*, 15.
(223) Evgenov, O. V.; Pacher, P.; Schmidt, P. M.; Hasko, G.; Schmidt, H. H.; Stasch, J. P. *Nat. Rev. Drug Discov.* **2006**, *5*, 755.
(224) Hwang, S.; Meyerhoff, M. E. *Biomaterials* **2008**, *29*, 2443.
(225) Yang, J.; Welby, J. L.; Meyerhoff, M. E. *Langmuir* **2008**, *24*, 10265.
(226) de Mel, A.; Ramesh, B.; Darbyshire, A.; Hamilton, G.; Seifalian, A. Nitric oxide releasing novel nanocomposite material for cardiovascular applications. Presented at the *11th Biennial Meeting of the International Society for Applied Cardiovascular Biology*, Bordeaux, France, Sep 17−20, 2008.
(227) List, B. M.; Klosch, B.; Volker, C.; Gorren, A. C.; Sessa, W. C.; Werner, E. R.; Kukovetz, W. R.; Schmidt, K.; Mayer, B. *Biochem. J.* **1997**, *323* (Pt 1), 159.
(228) Albrecht, E. W.; Stegeman, C. A.; Heeringa, P.; Henning, R. H.; van Goor, H. *J. Pathol.* **2003**, *199*, 8.
(229) Peng, H. T. *J. Biomed. Mater. Res. B: Appl. Biomater.* **2010**, *94*, 469.
(230) Perry, D. J.; Fitzmaurice, D. A.; Kitchen, S.; Mackie, I. J.; Mallett, S. *Br. J. Hamaetol.* **2010**, *150*, 501.
(231) Jung, C.; Gonon, A. T.; Sjoquist, P. O.; Lundberg, J. O.; Pernow, J. *Cardiovasc. Res.* **2010**, *85*, 147.
(232) Orhan, G.; Yapici, N.; Yuksel, M.; Sargin, M.; Senay, S.; Yalcin, A. S.; Aykac, Z.; Aka, S. A. *Heart Vessels* **2006**, *21*, 42.
(233) Szelid, Z.; Pokreisz, P.; Liu, X.; Vermeersch, P.; Marsboom, G.; Gillijns, H.; Pellens, M.; Verbeken, E.; Van de Werf, F.; Collen, D.; Janssens, S. P. *Basic Res. Cardiol.* **2010**, *105*, 169.
(234) Hogg, N.; Broniowska, K. A.; Novalija, J.; Kettenhofen, N. J.; Novalija, E. *Free Radic. Biol. Med.* **2007**, *43*, 1086.
(235) Mayahi, L.; Heales, S.; Owen, D.; Casas, J. P.; Harris, J.; MacAllister, R. J.; Hingorani, A. D. *Arterioscler. Thromb. Vasc. Biol.* **2007**, *27*, 1334.
(236) El-Hamamsy, I.; Stevens, L. M.; Carrier, M.; Pellerin, M.; Bouchard, D.; Demers, P.; Cartier, R.; Page, P.; Perrault, L. P. *J. Thorac. Cardiovasc. Surg.* **2007**, *133*, 7.
(237) Koti, R. S.; Tsui, J.; Lobos, E.; Yang, W.; Seifalian, A. M.; Davidson, B. R. *FASEB J.* **2005**, *19*, 1155.
(238) Glantzounis, G. K.; Rocks, S. A.; Sheth, H.; Knight, I.; Salacinski, H. J.; Davidson, B. R.; Winyard, P. G.; Seifalian, A. M. *Free Radic. Biol. Med.* **2007**, *42*, 882.
(239) Duran, W. N.; Breslin, J. W.; Sanchez, F. A. *Cardiovasc. Res.* **2010**, *87*, 254.
(240) Fulton, D.; Babbitt, R.; Zoellner, S.; Fontana, J.; Acevedo, L.; McCabe, T. J.; Iwakiri, Y.; Sessa, W. C. *J. Biol. Chem.* **2004**, *279*, 30349.
(241) Willmot, M.; Gibson, C.; Gray, L.; Murphy, S.; Bath, P. *Free Radic. Biol. Med.* **2005**, *39*, 412.
(242) Melikian, N.; Seddon, M. D.; Casadei, B.; Chowienczyk, P. J.; Shah, A. M. *Trends Cardiovasc. Med.* **2009**, *19*, 256.