Expert Opinion

New biomaterials for the sustained release of nitric oxide: past, present and future

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Nitric oxide (NO), the 1992 'Molecule of the Year', is the focus of immense medical and scientific exploration. Interest in NO has grown exponentially since the initial and relatively recent discovery that NO is the long sought after endothelial relaxing factor. There is intense research that is continuing to expose the extensive physiologic impact of NO in virtually all organ and tissue systems under both normal and pathological conditions. Both the rate of delivery and the amount of site-specific generated NO modulate a balance between cytoregulatory and cytotoxic activities. This balancing act and the very short lifetime of NO under physiological conditions pose an extreme challenge with respect to harnessing the exceptional therapeutic potential of this molecule. Over the past two decades, the race to translate the therapeutic potential of NO to the bedside has been overwhelmingly through the development of numerous NO delivery devices/vehicles. So far no one product has emerged as a clearcut winner. This review: discusses and evaluates NO-donating platforms that are available at present; attempts to enhance delivery and efficacy through encapsulation in silane-based hydrogel matrices; and discusses and evaluates the future direction of these advances.

Keywords: drug delivery, nanotechnology, nitric oxide, sol-gel

Expert Opin. Drug Deliv. (2009) 6(10):1113-1122

1. Introduction

Since being coined 'Molecule of the Year' in 1992, medical and scientific interest in nitric oxide (NO) has grown exponentially, as an ever-expanding range of physiologic impacts continue to be described and documented. Involving nearly every physiological system and relevant in an extraordinary host of pathological processes, NO, both directly and indirectly, has an enormous influence on the human body. Both the rate of delivery and the amount of site-specific generated NO modulate a balance between cytoregulatory and cytotoxic activities. This balancing act and the very short lifetime of NO under physiological conditions pose a great challenge with respect to harnessing the exceptional therapeutic potential of this molecule. Over the past two decades, the intense race to translate the therapeutic potential of NO to the bedside has been overwhelmingly through the development of numerous NO delivery devices/vehicles. However, it is first necessary to review and understand the important pathways through which NO is generated and exerts it physiologic impact in order truly to appreciate these NO delivery technologies.

1.1 Introduction to nitric oxide

Nitric oxide is generated endogenously by several distinct isoforms of the enzyme nitric oxide synthase (NOS) [1]. NOS1 and NOS3 are constitutively expressed, and are also known by the cell types from which they were cloned (respectively, endothelial NOS from endothelial cells and neuronal NOS from neuronal cells). These are

informa healthcare

10.1517/17425240903196743 © 2009 Informa UK Ltd ISSN 1742-5247

calcium-dependent, calmodulin-regulated enzymes. Interestingly, NOS2, or inducible NOS, is expressed by a wide array of cell types and generates NO in a non-calcium-dependent fashion, responding to a wide array of stimulants, such as, pro-inflammatory cytokines, bacterial polysacchrides, endotoxins and neuropeptides [2]. Unlike NOS1 and NOS3, NOS2 is not as sensitive to autoinhibition. These different isoforms are modulated by a wide range of signaling molecules. Increased concentrations of sex hormones such as estradiol, for example, result in upregulation of NOS2 and NOS3 in skeletal muscle [3], suggesting that there may be either a cytosolic or a particulate localization. Furthermore, these enzymes are also regulated by the physiologic milieu, such as changes in the availability of substrates and cofactors, presence of scavengers, and multisite phosphorylation [4]. NO can also be generated through non-NOS pathways. The most significant one appears to be by means of the conversion of the nitrite ion to NO. The proposed production of NO and longer lived nitrosolthiols from nitrite through nitrite reductase and nitrite anhydrase reactions of hemoglobin, myoglobin and other hemeproteins is a plausible mechanism for the non-NOS pathway [5,6].

With respect to physiologic impact, NO is best known for its interaction with the soluble guanylyl cyclase (sGC) pathway [4]. In this paradigm, NO binds to sGC and increases cyclic GMP levels, resulting in protein kinase G activation. Activation induces enumerable downstream events, encompassing not only the vasodilatory and neurotransmitter actions of NO, but also far-reaching consequences such as antidepressant [7] and antipyretic [8] effects. The concentrations of NO required to mediate these beneficial and even protective effects are extremely low (picomolar to nanomolar). As a free radical, NO is able to generate potent nitrosating agents capable of both signaling and cellular damage, as with peroxynitrite ($ONOO^-$) in the presence of superoxide. This highlights an important feature of NO in that its properties and cellular targets are profoundly different with varying concentrations, particularly under conditions of oxidative stress, such as the reaction with superoxides. Under these circumstances, NO is highly cytotoxic, a feature that is exploited by inflammatory cells in response to invading pathogens in concert with activation of NAD(P)H oxidase to generate NO and superoxide, forming highly cytotoxic and cytostatic $ONOO^-$. Furthermore, at these higher concentrations, extra chemical reactions occur specifically with molecular oxygen, generating nitrosating species capable of regulating protein and cell function [9]. Interestingly, high concentrations of NO and related species also mediate apoptosis in both carcinoma and inflammatory cells [10]. However, even when NO is generated in mass quantity, NO is rapidly scavenged in most physiological conditions. Several mechanisms allow for the potential of long distance NO-carrying vehicles, such as *S*-nitrosothiols, *S*-nitrosylated proteins, nitrosyl–metal complexes and nitrite [11,12]. These pathways form the basis for many of the NO delivery materials; however, the actual NO species once liberated from these 'donors' is short lived in the body.

The wide-ranging functionalities of NO in the cardiovascular, nervous and immune systems have led to many attempts over the past three decades to translate the extensive existing basic science knowledge to the bedside through various NO delivery vehicles. These quests to develop feasible means to deliver therapeutic levels of NO have been met by many obstacles. As a gas, NO is extraordinarily difficult to handle owing to problems associated with a requirement for complete exclusion of oxygen in order to limit oxidation of NO to the toxic nitrogen dioxide molecule. Despite these obstacles, the US Food and Drug Administration has approved the use of inhaled NO for the treatment of hypoxic respiratory failure and persistent pulmonary hypertension in term and near-term infants. This approval delivery modality for NO is expensive. The cost of the NO is ~ $ 6/l, bringing the cost of treating a newborn with this gas to almost $ 12,000, with a minimum charge of $ 3000 to open the tank of gas for any application [13].

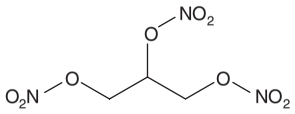
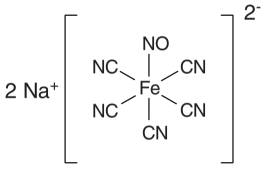
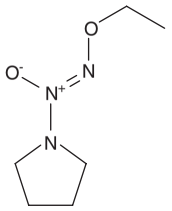
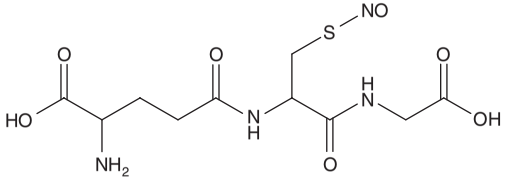
Similarly, organic nitrites and nitrates seemingly appear to be an excellent source of NO, but there are still problems arising from the difficulty in controlling the tissue-specific delivery of desired concentrations of NO. So far, most of the practical NO delivery systems have been based on NO donor molecules (**Table 1**), although more recently alternative means of generating, storing and delivering NO have been reported.

2. Organic NO donors

2.1 Nitrates/nitrites

The classic nitrovasodilators, organic nitrate and nitrite esters such as nitroglycerin, amyl nitrite, isosorbide dinitrate, isosorbide 5-mononitrate and nicorandil have been used in the treatment of cardiovascular diseases for almost a century. In fact, organic nitrates are both the oldest and still most commonly used NO donor drugs. Nitroglycerin, or Glyceryl trinitrate (GTN), has been used for decades to treat symptomatically anginal chest pain, whereas slower release therapeutics, such as isosorbide mononitrate, are used for the treatment of hypertension and chronic angina [14,15]. GTN contains 3 nitrate groups and can release 1 molar equivalent of NO from the terminal position after bioactivation [16]. The main limitation of the organic nitrates is the well-documented development of tachyphylaxis following prolonged, continuous use [17,18]. This tolerance is probably exacerbated in the setting of endothelial dysfunction and oxidative stress [19], as indicated by *in vivo* studies in animals [20-22] and humans. Reports that long-term nitrate use causes a paradoxical increase risk of cardiac events [23] support further these suggested mechanisms of adverse events with nitrates. Unfortunately, the only effective means of avoiding tolerance is to incorporate 'nitrate-vacations' in the therapeutic regimen, which can be an obvious impediment in the management of chronic conditions. More recently, a modified thiol-containing analogue of isosorbide mononitrate called LA-419 has been introduced. The drug design includes an antioxidant moiety in the drug structure

Table 1. Comparison of known NO donor molecules [30].

NO donor	Pros	Cons	Current status
Organic nitrites/nitrates (ex: GTN, NTG)  Glyceryl trinitrate (GTN)	Versatility: Rapid onset versus controlled generation Bioactivation/tissue-specific release mechanisms Resistant to extracellular NO scavengers	Tachyphylaxis Paradoxical cardiovascular damage	GTN: treatment of acute angina Isosorbide mononitrate/dinitrite: treatment of hypertension
Sodium nitroprusside (SNP)  Sodium nitroprusside (SNP)	Rapid onset Inexpensive Low toxicity at physiologic dosing	Difficult dose titration Cyanidosis Restriction to intravenous route of delivery Photolability in solution	'Gold standard' as comparative NO donor Efficacy in endothelial dysfunction No further preclinical investigations – replaced by other NO donors
Sodium nitroprusside (SNP)  V-PYYO/NO	Rapid onset Inexpensive Low toxicity at physiologic dosing	Difficult dose titration Cyanidosis Restriction to intravenous route of delivery Photolability in solution	'Gold standard' as comparative NO donor Efficacy in endothelial dysfunction No further preclinical investigations – replaced by other NO donors
S-Nitrosothiols  S-nitroso-glutathione (GSNO)	Tissue selectivity Ex: GSNO – arteries over veins Antiplatelet aggregation Direct transfer of NO^+ species to other thiols without the release of free NO Decreased susceptibility to oxidative stress Limited cytotoxicity	Photolabile Early decomposition when exposed to transition metal ions Complex/difficult synthesis Potential tissue accumulation	GSNO clinical trials Antiplatelet aggregation Neuroprotection Hepatoprotection Anti-inflammatory Wound healing

GTN: Glyceryl trinitrate; GSNO: *S*-nitroso-glutathione; V-PYRRO/NO: *O*-alkenyl/*O*-alkyl derivatives of existing NONOates.

to combat the contribution of oxidative stress, which is thought to be involved in tolerance induction. Clinical trials have demonstrated therapeutic potential for this compound as an antithrombotic agent, and a treatment for left ventricular hypertrophy and glaucoma [24].

The other clinically relevant nitrate-based NO donor is sodium nitroprusside (SNP). SNP is approved for intravenous treatment of malignant hypertension on-site in hospitals to provide rapid lowering of blood pressure. SNP is considered to be the model NO-dependent/endothelium-independent vasodilator. The mechanism of NO release from SNP in biological tissue is complex, and the complete pathway has yet to be fully elucidated. SNP is rather stable and does not release NO spontaneously in the physiologic setting. In this instance, NO production is dependent on either light or tissue-specific release mechanisms [25]. However, the greatest concern with this NO donor is the potential for release of any of the five cyanide groups incorporated in the structure [26]. There have been reported cases of cyanidosis associated with long-term use [27]. Multiple limitations have been reported, including restriction to intravenous delivery, photolability once in solution, and difficulty with dose titration [28]. The use of this family of drugs is likely to diminish given the tolerance issues, difficulty in delivery [29,30] and the emergence of alternatives without these complications.

3. Synthetic NO donors

3.1 Diazeniumdiolates

Diazeniumdiolates or NONOates, as a class, have been used for many years, although they first received attention only in the 1990s when their clinically relevant NO donor properties were first investigated [31]. These compounds consist of a diolate group [N(O$^-$)N=O] bound to a nucleophile adduct (a primary or secondary amine or polyamine) by means of a nitrogen atom [32]. Decomposition occurs spontaneously in physiologic solution, generating up to 2 molar equivalents of NO. The rate of release is dependent on the structure of the nucleophile [33], and can be from seconds to days. Multiple NONOates with varying release kinetics have been evaluated and described in the literature [34]. These compounds are favored both because their decomposition and resulting release of NO are not catalyzed by physiologic environmental factors and because the NO release follows predictable first-order kinetics [35]. This lack of a tissue-dependent requirement for NO release is probably responsible for the lack of tachyphylaxis observed for these compounds as opposed to the organic nitrites/nitrates [36].

At present, although NONOates have not approved for clinical use, there is one clinical investigation with a specific focus on cardiovascular disease. Lam *et al.* reported the results of administering aerosolized diethylenetriamine/NO to a patient with acute respiratory distress syndrome. The potential for oral preparations of NONOates has yet to be fully clarified, although transdermal preparations have already been developed [36]. The predictable release kinetics of NO from NONOates will probably stimulate further clinical investigations; however, long-term safety has yet to be established. The toxicity of by-products needs to be more fully confirmed [37], especially as subsequent reactions between decomposition products could lead to the formation of carcinogenic nitrosamines [38]. Incorporation of NONOates into polymers may represent a means of preventing the leaching of by-products [39]. At present, conjugated NONOates have a great deal of promise, especially for the treatment of certain cancers, although further characterization of these drugs is essential before they reach larger clinical trials. NONOates may also have a use in the treatment of erectile dysfunction by enhancing blood flow to the penis [40], although it remains to be determined whether these drugs can be applied in a way that would have advantages over the phosphodiesterase 5 inhibitors such as tadalafil.

To enhance efficacy, protecting groups can be added to the nucleophile adduct both to protect the terminal oxygen of the diolate moiety, thereby stabilizing the drug in solution, and to allow for targeted site delivery by relying on site-specific enzymes to cleave the modifications in order to initiate NO release. Miller and Megson [30] and Saavedra *et al.* [41] developed *O*-alkenyl/*O*-alkyl derivatives of existing NONOates (V-PYRRO/NO) to target NO release in liver cells. It was demonstrated that V-PYRRO/NO blocked tumor necrosis factor-α (TNF-α)-induced apoptosis in hepatocytes alone and protected against experimentally induced liver toxicity *in vivo*. The same group has also produced a fluorescent NONOate (GLO/NO), as well as a means to link the NONOate group to non-steroidal anti-inflammatory drugs (NSAIDs), vitamin B3 and polyethylene glycol (PEG) [30,42]. However, the clinical relevance and potential translation to the bedside at best remains theoretical at this juncture.

It is clear that NONOates have clinical potential, but this can only be maximized through an exhaustive understanding of the fundamentals behind diazeniumdiolate chemistry. These include acid–base behaviour, spectral studies, and reactivity studies, including mechanisms by which they dissociate to NO, and their redox potential. It is not surprising that there is a growing number of laboratories actively pursuing the development of new diazeniumdiolates and subsequent characterization of their properties. However, issues of toxicity are of great concern, for example, V-PYRRO/NO has the potential to be converted to *N*-nitrosopyrrolidine, an extraordinarily potent experimental hepatocarcinogen. It therefore remains up to innovative investigators to translate the growing body of knowledge on NONOnate structural and functional properties to effective and safe targeting of generated NO to specific physiologic sites.

3.2 *S*-Nitrosothiols

The *S*-nitrosothiol NO donors encompass numerous compounds that all rely on a single chemical bond between a thiol (sulfhydryl) group (R-SH) and the NO moiety for delivery.

Their biological activity is highly influenced by the molecular environment of the parent thiol. Like NONOates, *S*-nitrosothiols can release NO when exposed to physiological environments; however, this release does not occur spontaneously. Rather, they release NO by three known mechanisms: copper ion-mediated decomposition, direct reaction with ascorbate, and homeolytic cleavage by light [43-46]. As these compounds rely on complex chemistry for NO release, it can allow for multiple avenues through which the delivered NO can exert its physiologic properties [47].There are several factors capable of inducing NO release from *S*-nitrosothiols, including light, heat, transition metals, thiols, superoxide and enzymes such as superoxide dismutase [48], and various dehydrogenases [49]. Interestingly, it has been demonstrated that *S*-nitrosothiols have a low incidence of inducing tolerance with long-term use as compared with organic nitrates [50,51].

The potential clinical advantages of *S*-nitrosothiols over other classes of NO donor are several-fold, including tissue selectivity, antiplatelet aggregation [52], direct transfer of NO^+ species to a chain of other thiols without the release of free NO, decreased susceptibility to conditions of oxidative stress by effectively protecting the NO moiety from oxygen-centered free radicals, and demonstrated limited cytotoxicity at pharmacologically relevant concentrations [53]. However, there are no *S*-nitrosothiols in clinical use at present, although there are numerous animal and clinical studies demonstrating their advantageous features. Examples include: i) *S*-nitrosoglutathione (GSNO), which has been shown to decrease the occurrence of cerebral embolism after carotid endarterectomy in patients already receiving aspirin and heparin [54] and to have neuroprotective properties via regulation of antioxidant and apoptotic enzymes [55]; and ii) *S*-nitroso-*N*-acetylcysteine (SNAC) has demonstrated cardioprotective properties when infused intravenously before ischemic events [56]. More recently, a more stable analogue of GSNO, LA810, has emerged and has been demonstrated to have a marginally greater antithrombotic action than GSNO in whole blood *ex vivo* [57]. A polyethylene glycol-conjugated form of *S*-nitroso-albumin has been described with improved distribution and prolonged NO release in the circulation [58]. Incorporation of *S*-nitrosothiols into biomaterials such as films to enhance targeted delivery is being pursued, for example, GSNO encapsulated in solid polymeric films of poly(vinyl alcohol) and poly(vinyl pyrrolidone) have been found to provide a stabilization effect on the thermal decomposition of GSNO, leading to 8 – 16-fold reduction in the first-order rate constants of NO release, compared with aqueous GSNO solutions [59].

In light of the preclinical data discussed above, *S*-nitrosothiols should have a promising future. Enhanced targeted delivery is now being expanded on. For example, some groups have modified SNAP to contain lipophilic peptide components in the hope of increasing penetration into desired cells [60]. Manipulation of chemical properties, as above with respect to lipophilicity, may allow for different routes of administration, such as topical delivery. Preliminary work has investigated topical application of synthetic *S*-nitrosothiols to the skin [61].

4. NO delivery

4.1 Zeolites

A newer approach using ion-exchanged zeolites for storage and delivery of NO has recently been developed and used [30,62]. These porous insoluble materials form a framework containing metal ions that can bind gaseous NO (Ze–NO). As NO gas binds to the metal ions within the pores, it is literally 'packed' into this solid matrix. Ze–NOs of this type are very stable in the anhydrous state, but NO is displaced by water on immersion in an aqueous environment. These materials can store a high concentration of NO and the rate of release from the zeolites can be modified by altering the porosity as well as the metal ion being used [63,64]. Unlike acidified nitrite, which is known to induce an intense cutaneous inflammatory infiltrate following topical application secondary to the release of degradation products, it has recently been demonstrated that Ze–NO are in fact chemically inert, pure NO donors and therefore induce limited inflammatory response [64]. Furthermore, ruthenium complexes have been used in conjunction with zeolites, as these ions have an extraordinarily high affinity for NO while demonstrating minimal cytotoxicity [65]. Therefore, the potential flexibility with respect to pure and increased NO release allows for extensive versatility with respect translation to clinical applications, such as rapidly deployed antimicrobial wound dressings and slow-acting antithrombotic coatings for stents. However, this approach is still in its infancy and a great deal of work must still be done to capitalize on the potential benefits.

4.2 Silane-based hydrogels/sol-gels

Several synthetic NO donors including *S*-nitrosothiols, nitrosamines, diazeniumdiolates, zeolites and organic nitrates/nitrites have been incorporated into silane-based sol-gels, such as those made from tetramethoxysilane or tetraethoxysilane, in order to form polymer coatings/particles capable of slowly releasing therapeutic levels of NO. Briefly, the sol-gel process involves the transition of a system from a liquid 'sol' (generally colloidal) into a solid gel phase. In the past, sol-gels were used and continue to be used for trapping large biomolecules such as proteins, to investigate conformational structures [66,67]. These materials have the interesting property of limiting conformational dynamics of the encapsulated proteins/pharmaceuticals while still allowing for the free exchange/access of solvent and small solute molecules due to the complex porous network within the sol-gel matrix [68-70]. It is now well established that the sol-gel-derived materials provide excellent matrices for encapsulating a wide variety of organic and inorganic compounds, including many biologically relevant materials [71].

Of the NO donor species listed above and discussed previously, *N*-diazeniumdiolates have emerged as attractive

candidates for designing more biocompatible coatings because of their ability to generate NO spontaneously under physiological conditions – in fact, when NO reacts with amines, this zwitterionic stabilized structure is produced creating a complex that decomposes spontaneously in aqueous media to generate NO [71]. The rate of release depends on pH, temperature and/or the structure of the amine moiety [72-74]. The synthesis and characterization of sol-gel-derived materials with *N*-diazeniumdiolate NO donors covalently bound to the hydrogel backbone has also been reported [71,75-81]. When the sol-gel/*N*-diazeniumdiolate complex is introduced into an aqueous environment, the *N*-diazeniumdiolate decomposes to NO, thus releasing the intended product. The antimicrobial impact of these complexes has been evaluated *in vitro*, for example, the local surface flux of NO generated from these hydrogels reduced significantly the adhesion of *Pseudomonas aeruginosa* to medical implants by up to 95%. Even more recently, this group demonstrated that nanoparticles of this sol-gel-based platform overcome and are microbicidal against biofilm-fortified Gram-positive and Gram-negative bacteria as well as fungal species [82]. Given that these studies have been exclusively *in vitro*, preclinical and clinical efficacy remains to be shown.

The authors' lab recently developed a platform [83] through which the benefits of sol-gel technology is combined with features of sugar-derived glasses in order to generate, store and controllably deliver NO. The porosity of silane-based hydrogels serves as an advantage when considering utilization of sol-gels as matrices for protein-based biosensors or bioreactors, but it can present as an impediment when developing slow, sustained release drug delivery platforms. To overcome the high porosity limitation of standard sol-gels, the authors incorporated glass-forming materials such as sugars and polysaccharides (i.e., chitosan) into the sol-gel protocol with the intent of 'plugging' the pores of the sol-gel with a relatively stable (when dry) hydrogen-bonded network of glass-forming molecules [83]. This approach is derived conceptually from other studies [84] showing that polyols and PEG introduced at the initial hydrolysis/condensation phase of hydrogel formation can be incorporated into the hydrogel lattice. In these cases the properties of the hydrogel are significantly impacted (e.g., reduced ageing-related shrinkage of the sol-gel with the inclusion of PEG) through such modifications. Glassy properties also and most significantly provide a method of directly generating nitric oxide from nitrite within the solid matrices through a thermal reduction process [83,85,86]. Nitrite is directly included in the initial preparative formulation and on drying/heating it is efficiently reduced to NO by electrons from included sugars. Recent electron paramagnetic resonance measurements directly confirm that gaseous NO is contained within the dry material. This approach obviates the need to load the material with external gaseous NO or covalently attach or include NO-releasing molecules. The overall formulation consists of tetramethoxysilane (TMOS), PEG and chitosan as the primary starting materials. The resulting hydrogel/glass composite material spontaneously forms nanoparticles when the solid material is lyophilized. The resulting nanoparticle-containing powder can be suspended in solvents or incorporated into varying delivery vehicles. The dry material when stored in a sealed dry environment appears to retain NO fully for many months. The inclusion of a polarity/solvent mobility sensitive fluorophore (BADAN) in the particles reveals that the interior of the particles is both very hydrophobic and rigid, which probably accounts for the retention of the NO under dry conditions and the slow release of NO when the particles are exposed to an aqueous environment.

The clinical applicability of these 'hybrid' nanoparticles has been investigated through several preclinical studies in different specialties. Antimicrobial activity was demonstrated in an *in vivo* murine methicillin-resistant *Staphylococcus aureus*-infected wound model, through which accelerated wound closure and clearance of bacterial burden was demonstrated clinically and histologically [87]. NO nanoparticles induced erectile activity without systemic adverse events such as hypotension following topical application to the glans in retired breeder rats [88]. *In vivo* intravenous (and intraperitoneal) infusions of NO nanoparticles resulted in a several-hour sustained decrease in mean arterial pressure with associated dose-dependent increase in exhaled gaseous NO. In these studies, the particles (fluorescently labeled) were shown to remain in the circulation for hours. Intravenous infusion of the particles was also shown to decrease platelet adhesiveness, decrease inflammation and reverse acellular hemoglobin transfusion-related vasoconstriction.

5. Expert opinion

5.1 The current state of NO delivery vehicles

At present there are multiple options for NO delivery, with several showing promise for highly specific applications. Both known and to be determined issues with toxicity still require further investigation for many if not all of the current vehicles. Several of the complexes do not have general applicability and appear to be limited to topical applications. The sol-gel-based platforms appear to have the greatest potential for broad applicability owing to the flexibility of the platform with respect to the matrix and the included or attached molecular species. So far only the NO-releasing nanoparticles derived from the hydrogel/glass composite have been tested *in vivo* with respect to efficacy for topical, intravenous and intraperitoneal delivery. A key feature of this platform is the sustained release of the NO through a mechanism that does not require bioactivation or production of potentially toxic products. This platform has the flexibility probably to allow for specific manipulation of NO content, release profiles and half-life of the particles in systemic circulation. It has been shown that increasing the PEG size within the particles accelerates the release of NO, eventually leading to a burst-like release pattern [83]. It has been possible to tune the internal polarity/hydrophobicity of the particles in anticipation of

being able to fine-tune the release profiles of the NO. There are also several general advantages to the sol-gel-based approaches: i) they can be combined with other NO delivery platforms; ii) it is conceivable that the microscale-to-nanoscale zeolites can be encapsulated within sol-gel matrices and thus allow for a more biocompatible composite material; and iii) derivatizing the sol-gel allows for covalent attachment of NO-releasing molecules such as diazeniumdiolates and *S*-nitrosothiol containing proteins and peptides.

5.2 The future

The main challenges associated with moving the sol-gel-based technology forward include fully exhausting the potential toxicity issues as well as the expansion of potential clinical applications. So far, the toxicity of these materials does not appear to be a major impediment; however, the long-term adverse effects as well as the biodistribution and localization of the particles themselves *in vivo* still require further investigation. The clinical potential of NO-releasing particles is significant. The prerequisite progress to harness the full therapeutic potential of NO requires several factors apart from those mentioned previously. These include: mode of delivery; tissue targeting; controlled release; and sustainable release. At this time, the hydrogel/glass composite nanoparticles show the most promise with respect to these desired properties. The extended survival time of the particles with a concomitant sustained systemic delivery of NO could be used for treating systemic infections and malignant hypertension. The ability of circulating NO-releasing nanoparticles to increase NO levels in the exhaled breath bodes well for use of such materials to treat pulmonary infections as well as pulmonary hypertension. It has yet to be determined whether infused or aerosol-delivered particles are more effective with respect to targeting pulmonary disease. The success of the hydrogel/glass composites in treating erectile dysfunction in an animal model indicates that these particles penetrate the skin and provide local sustained release of NO. This finding indicates that these particles may prove useful for other conditions that would benefit from local delivery of NO – the potential is clear with respect to the treatment of peripheral vascular disease, chronic wounds, and other conditions associated with endothelial dysfunction and poor perfusion.

5.3 Roadmap to the future

The basic platform for the hydrogel/glass composite has the flexibility to spawn the next generation of NO delivery materials that have the properties needed to expand greatly clinical applicability. The most straightforward next steps include; i) tuning the surface properties to extend lifetime in the circulation; ii) covalent attachment of peptides or proteins that confer tissue targeting capabilities; iii) alteration of surface properties to match and maximize efficacy with respect to the specific desired mode of delivery (e.g., intravenous, aerosol, topical); iv) inclusion of synergistic therapeutics; v) attachment of imaging contrast agents; and vi) manipulation of the internal properties of the nanoparticles to create release profiles that match the therapeutic need. With these advancements now being aggressively and proactively pursued, the future is surely bright for sol-gel-based technologies with respect to the therapeutic delivery of nitric oxide.

Declaration of interest

The authors declare no conflicts of interest at this time with respect to the technology described in this paper. The authors are co-inventers on patent WO/2007/149520, with no current financial interest.

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