Functionalised solids delivering bioactive nitric oxide gas for therapeutic applications

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Abstract It is well established that nitric oxide is an effective vasodilative, antibacterial and tumoricidal agent, however its targeted delivery in a controllable manner is challenging but necessary for successful therapeutic applications. In recent years a few new methods have been developed, based on the formation of *N*-diazeniumdiolates, *S*-nitrosothiols and metal –NO coordination bonds in material structures. The typical delivery materials include nanoporous materials (such as zeolites and metal organic frameworks), silicate particles and polymers containing amine and thiol functional groups. These materials are of promising potential for delivering controllable doses of bioactive NO gas to meet the unmet therapeutic needs in the future. This review summarises these delivery materials and relevant biological assessments. Further improvement of current methods and new design of NO donors are still required in order to address the issues on NO storage and its release profile in matching with the clinical requirements.

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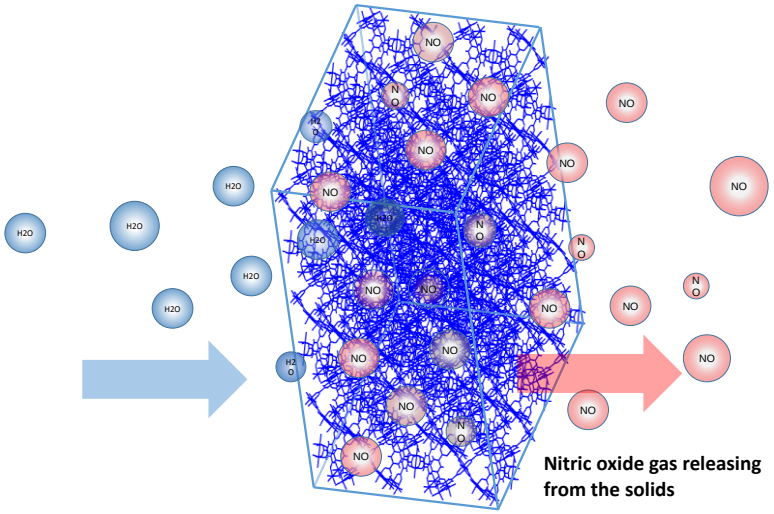
Functionalised solids deliver bioactive nitric oxide gas to meet the unmet therapeutic needs



1. Introduction

Nitric oxide (NO), as a cellular signalling molecule, plays pivotal roles in many physiological and pathological processes in the body, either by permeating into cells or binding to proteins to be carried off to all parts of the body. It is highly effective in its vasodilative, antibacterial, and tumoricidal functions, and is of growing interest for potential therapeutic applications. In the body, endogenous NO is produced either through Nitric Oxide Synthase (NOS) catalysing the metabolism of *L*-arginine or by non-enzymatic nitrite reduction. Accordingly, a series of NO donors have been developed containing *L*-Arginine, *L*-citrulline, glyceryl trinitrate, amyl nitrite, sodium nitroprusside, isosorbide mononitrate, *S*-nitrosothiols, the *N*-diazeniumdiolates and mesoionic oxatriazoles [1-3]. Alternatively, pure NO gas can also be directly delivered into the body from external sources. Currently, direct delivery of NO by inhalation has been approved for use in clinical settings for the treatment of persistent pulmonary hypertension and hypoxic respiratory failure in newborns [4]. Other important functions as an antimicrobial agent and a promoter of wound healing require further developments to progress into clinical settings. Due to nitric oxide gas nature and short half-life, it is a challenge for establishing suitable methods for delivering NO gas in a controllable manner. The design of new NO donors is thus required to address the issues existing in current donors e.g. poor storage capacity, donor stability, controllable NO release and potential toxicity of NO donors.

In recent years, the hybrid NO donors have been developed by means of (1) NO covalently bonded to substrates, i.e. *S*-nitrosothiols, *N*-diazeniumdiolates, and (2) NO chemisorption on metal active sites in materials by metal-NO complexes. The NO chemisorption stands up as a new method for bioactive NO delivery, which recently is of particular interests for topical therapeutic applications due to the direct release of pure NO gas thus avoiding potential toxic by-products [5]. The typical examples are the functionalised solids in the forms of polymers, silicate particles, metal organic frameworks (MOFs), zeolites and composites. They are advantageous over the small molecular donors in controlling NO storage and release as well as in reducing the undesirable species leaching [6], showing their potential for wound healing over the classic NO donors [7]. Since so far the research is still very limited on this new class of NO donors, this paper will focus on reporting the progress in the research of this type of materials for nitric oxide storage and release. While the controlled release of NO is a primary target for all of these materials, this review will not focus on the quantitative measurement or the various methods used to determine NO release, as this has been covered in detail in other recent reviews [8].

For functionalised porous solids delivering bioactive nitric oxide, three different strategies can be adopted, that is, (a) coordination of unsaturated metal sites present in the materials to form

metal-NO complexes, for example, in metal ion exchanged zeolites (LTA and FAU) and porous metal-organic frameworks (MOFs); (b) the amine groups in the materials to react with nitric oxide to form *N*-diazeniumdiolate (R_2N-NONOates) structures; and (c) nitroso group bonded to amine or thiol moieties i.e. $R_1N(–R_2)–N=O$ or RS-N=O in materials. These strategies can be combined in the design of materials suitable for controlling NO release. The storage capacity of nitric oxide significantly depends on the chemical composition and pore structure of materials that determine the NO-releasing kinetics. The ideal materials are not only capable of storing a high quantity of nitric oxide gas but also releasing the gas in a controllable manner to suit therapeutic requirements.

2. Functionalised nanoporous materials for NO storage and release

2.1 Zeolites as NO donors

Zeolites are porous aluminosilicate minerals, well known as effective catalysts for the reduction of nitric oxide pollutant at high temperature (~ 400 ^oC). This catalytic process is mainly composed of surface adsorption and surface reaction, different nitrogen oxide species are usually formed over the active metals (Cu, Fe, Co, Ag, Ni, Ga, Ce, Mn) catalyst surfaces with the formation of non-toxic nitrogen. For medical use such as wound healing, it requires zeolites to be able to deliver pure nitric oxide to specific locations of interest at relatively low releasing temperature without coexistence of different nitrogen oxide species. It also requires that in topical therapies the nitric oxide is discharged in a biologically friendly manner, i.e. triggered by moisture, light, and body temperature. Obviously, triggering NO release by skin moisture is the most convenient method. Previous studies have shown to some extent these requirements can be satisfied using metal-exchanged zeolites in which NO gas is stored at ambient temperature without such surface reactions at high temperature.

The concept of using zeolites as the NO donor is of great significance for practical applications of nitric oxide in the biomedical fields. The pioneering research has been led by the Morris group [9-11]. The typical zeolites, Linde type A (LTA) and Faujasite (FAU), were initially examined for storing NO. In order to store high capacity of NO, it is a prerequisite to exchange zeolites with metals, followed by a dehydration at ~573 K prior to NO loading. This features two processes: firstly, the sodium cations that balance framework negative charges and also block pores are removed through the exchange of divalent metal cations (Cu^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+} and Zn^{2+} etc) with smaller ion radii. The closed pores are thus opened. This allows NO gas more freely diffusing along the pores to access the metal sites where NO is adsorbed. This is particularly crucial for LTA zeolites because it consists of smaller size porous channels (~4.1 Å) than the zeolite FAU (~7.4 Å) [12]; secondly, the introduction of the transient metals as active adsorption sites affects the electric field gradient of cavities and the acidic or basic character of the framework [13], and thus

regulate zeolite affinity to adsorb nitric oxide and the NO-releasing rate.

Nitric oxide is commonly used as a probe molecule for the characterisation of Lewis acidic sites in zeolites [14]. It can coordinate with metals by two different modes - either nitrogen or oxygen binding with metal, exhibiting different geometries. In an ion exchanged zeolite, it prefers to form metal-NO complexes rather than metal–ON in a bent geometry. Fig. 1 (a) showed NO coordinated with extra-framework cobalt cations residing in pore channels of zeolite LTA. The angle Co-N-O in LTA is 141^{o}, whereas the Mn-N-O in zeolite FAU is 79^{o}. The difference of NO complex geometry relies on the varying coordination environments, such as the charge of NO molecule, the coordination number, the coordination geometry and the nature of the highest occupied molecular orbital [15]. The electron transfer between NO and the unsaturated metal cations equates to strong bonding of NO to zeolites, to defend against the pulling force imposed by means of reducing NO pressure. Due to the exchange of electrons and the resultant strong bonds formed, adsorption and desorption of nitric oxide on metal exchanged zeolites are irreversible (Fig. 1 (b)).

Experiments showed that at 1.0 bar loading pressure, the irreversible amount of NO adsorbed in LTA and FAU were in a ranges of ~1.2 – 2.7 mmol g^{-1} [11,16,17]. The NO-releasing was triggered by exposing these zeolites to humidified atmosphere and lasted at 50 ppb level in a time scale of more than 2 hours. The NO delivered by zeolites is high potency in the inhibition of platelet aggregation [10]. The hysteretic nature of the adsorption/desorption isotherm can be regarded as an indicator of material suitability for effectively storing and delivering NO. Similarly, the Al- and Ga-doped porous titanosilicate ETS-10 ($(Na,K)_2TiSi_5O_{13}$) materials exhibit the storage capacity in the same range of ~1.1- 1.7 mmol g^{-1}. Importantly, these materials have very low toxicity (cell viability above 87%, after 72 h) at high concentration (0.45 mg cm^{-3}) [18a]. Cytotoxicity of NO delivery materials is crucial when the materials are administrated inside the body or applied for topical therapies, which must have to be assessed before medical use.

To avoid the risk of cytotoxicity, it may be good to develop zeolite donors without the toxic metals exchanged. Zhu *et al* studied the γ-aminopropyltriethoxysilane (APTES) post modification of HZSM-5 zeolite, mesoporous zeolite, and MCM-41 mesoporous materials for NO storage and release [18b]. NO was preloaded in these materials, followed by releasing in mimic gastric juice solutions with pH 1.2. Obviously, incorporation of aminopropyl enhanced the NO loading in these materials. In the case of aminopropyl modified MCM-41, NO loading increases from 0.77 to 27.43 μmol g^{-1}. Although the quantity of NO stored or released is much less than that of metal exchanged zeolites, they do not contain toxic metals. These porous materials exhibit multifunction feature.

They can release NO, and simultaneously remove carcinogenic nitrosamines e.g. *N* – nitrosopyrrolidine and *N*-nitrosonornicotine from the gastric juice solution.

2.2 Nitric oxide releasing kinetics of zeolite biomaterials

Nitric oxide releasing kinetics can be used for the material assessment or screening. The NO release from zeolites triggered by water follows the pseudo first - order kinetics as described by the following equation;

$$M_t = M_1(1-e^{-k_1 t}) + M_2(1-e^{-k_2 t}) + \ldots,$$

where the k_1 and k_2 are the releasing rate constants for sites *I* and *II*; M_t, the releasing amount at time *t*; M_1 and M_2, the releasing amounts from sites *I* and *II*. This model has been based on the hypothesis that the NO adsorption occurs in different active sites without any interactions. In most cases, the two - term equation is used for fitting experimental data with the deviation of less than 1.0% and regression coefficient of $R^2 > 0.990$, which implies that two distinct active sites exist in the zeolite and thus the NO release can be distinguished by the different kinetic constants *k*. For example, the NO released from Mn-LTA (in Fig. 2) has smaller k_1 than k_2, indicating that the site I is in favour of discharging NO more slowly than the site II. Accordingly, when a long duration of NO release is required, more NO adsorbed on the sites I would be desirable. Otherwise, for a quick NO release, adsorption on sites II would be expected. Apparently, this classification is arbitrary because the performance of NO release from such zeolites depends on a variety of factors such as water diffusion, NO surface displacing reaction, metal location and distribution in zeolites, metal properties, framework chemical composition and structures. Homogeneous NO release has been observed in the manganese and cobalt exchanged FAU zeolites (Mn-FAU and Co-FAU). In this case, the two-term kinetics degenerates into one item, implying that the active sites are uniform and NO releases only from one uniform site without the second site ($M_2 = 0$).

The stretched exponential model $M_t/M_e = 1-\exp(-(kt)^{\beta})$ was also investigated, where M_t is the adsorbed amount at time *t*; M_e, the equilibrium uptake; *k*, the rate constant; β, exponent. This model fits the NO-releasing profile from microporous titanosilicates ETS-4 and ETS-10, containing the unsaturated pentacoordinate Ti^{4+} and hexacoordinate Ti^{4+}, respectively [18c]. Although this model has a good fit to the experimental data, the variation of exponent β made it difficult to assess the performance of materials.

To satisfy the specific medical requirements, adjusting the NO-releasing kinetics can be possibly achieved by changing the framework composition (metal cations exchanged) and modifying pore structure and surface properties. In addition, the zeolites can be formulated into polymer composites with tunable hydrophilic and hydrophobic properties. For instance, zeolite A

was embedded within porous polylactic acid fibres of a nonwoven mat generated by electrospinning. As a result, the NO release was effectively regulated through the molecule diffusion restriction [19]. NO-releasing kinetic study will provide an important tool for the evaluation of material performance.

2.3 Nanoporous MOFs as NO donors

As a new type of porous materials, nanoporous metal-organic frameworks (MOFs) have demonstrated the advantages in the fields of gas adsorption and drug delivery over other materials. This is ascribed to MOFs' diverse structures, constructed through the assembly of organic ligands and metals, with tunable pore structures, chemical composition and functionalities [20-23]. These properties make nanoporous MOFs becoming a promising candidate for the NO delivery.

2.3.1 Open metal site role in MOFs

Nitric oxide has a strong affinity to interact with coordinatively unsaturated metals (also called open metal sites (OMS) in MOFs) in materials even at elevated temperature; similarly, this should be the same in the case of MOFs containing the OMSs. The proof-of-concept of NO interaction with the OMSs of MOFs has been approved through the examination of a copper benzenetricarboxylate MOF (HKUST-1: $Cu_3(BTC)_2(H_2O)_3$; BTC:1,3,5-benzenetricarboxylate) as the NO storage material [24]. HKUST-1 contains Cu_2 units coordinated by four carboxylate groups. There are two cages present in HKUST-1: one smaller pocket cage and one bigger cage with coordinated water pointing to it. The smaller pocket cage inside an octahedral unit (called secondary building unit SBU) is built of Cu_2 dimers at its six vertices coordinated by four BTC ions. The bigger cage is built by eight such SBUs; ~ 1.0 nm size channel crosses through these cages [25]. The water or other solvents can be removed from the cages and copper sites after a proper activation approach is taken (such as activation at ~ 120 ^oC under vacuum). The activation allows NO to be chemically adsorbed on the OMS and physically adsorbed in cages. The capacity of NO adsorbed in MOFs reaches to ~ 5.0 mmol g^{-1} of the dehydrated sample, ~ 5 - 7 times of that of zeolites and other materials [10,26]. Biological studies showed that the NO released is potent for the inhibition of platelet aggregation.

The infrared (IR) spectroscopy investigation elucidates the mechanism of NO adsorption in MOFs (HKUST-1) related with the OMS. The formation of a Cu(II)-NO adducts reveals the crucial role of OMS for storing high capacity of NO [24]. In a contrast, the MOFs without OMS adsorb much less NO, although they are porous. A hysteresis was observed between NO adsorption and desorption, attributed to the stronger coordination bonds of NO with copper OMS, which is similar to that of the metal-exchanged zeolites. From these results, it can be seen that the MOFs suitable for high NO storage should contain a high fraction of OMS in frameworks.

The high NO storage of up to ~7.0 mmol g^{-1} has been observed over the nanoporous MOFs (CPO-27) $[M_2(C_8H_2O_6)(H_2O)_2]\cdot 8H_2O$ (M: Co and Ni). This type of MOFs adsorbs NO ~7 times than the zeolites [27a]. Taking the metal fraction of ~ 7.0 – 8.0 mmol g^{-1} in MOF into account, it looks likely that all the metal sites (Co or Ni) are accessible to the NO at a low NO equilibrium pressure of < 1.0 bar. The NO adsorption isotherm has a Langmuir adsorption characteristic. The stored NO can be fully released using a moisture triggering approach. Such unprecedented result is ascribed to the unique one-dimensional honeycomb pore structure and the framework surface chemistry. The cobalt CPO-27 stores slightly less NO, and releases NO more slowly than the analogous Ni-MOF. Obviously, in this case, the metal property determines NO-releasing kinetics. This type of MOFs has a long time of NO release (> 14 hrs), which is an advantage when it is used for topical therapy for chronic wound healing.

The adsorption capacity is one of the key parameters to determine material efficacy in gas adsorption. A big reservoir of gas stored in the materials will provide more opportunities for tailoring the NO-releasing flux and rate in order to meet medical needs. At low adsorption temperatures (e.g. 196 K, dry ice), the NO adsorption includes a portion of NO physisorbed in the pores after the OMSs become saturated, which can be simply discharged by reducing pressure.

The whole scenario of adsorption-storage-delivery was described by Morris *et al* (in Fig. 4) [27a]. Every step in the cycle determines whether or not NO is delivered effectively. This cycle is derived based on a judicious examination of crystallographic structure variation with NO loading and release. An effective material must be able to provide a considerable number of adsorption centres. After proper activation, the metal sites previously blocked by the coordination of small molecules (water or other solvents) will be opened to allow NO molecules to attack. Consequently, a maximum adsorption is allowed to be achieved (that is, the metal sites are saturated by NO molecules). No catalytic conversion of the NO bound to metals occurs; this requires a material catalytically inactive at room or body temperature, thereby a considerable amount of pure NO is delivered. There are a few approaches to trigger NO release, including UV and microwave irradiation, thermal treatment and water displacement, among which water discharging NO is the simplest and most biologically-friendly method.

2.3.2 Other effects

In addition to the fraction of open metal sites in MOFs, the nature of the connection between metals and organic ligands, the pore and cage properties (size, shape and dimensionality), and framework flexibility and functionality also impact NO adsorption and release characteristics. If MOF particle size is an issue for medical uses, now small size MOF particles such as HKUST-1

can be conveniently obtained [27b]. Pinto et al studied the Co- and Ni-MOFs built from vitamin B3 (nicotinic acid) for NO storage and release [27c]. They carried out the density functional theory analysis of NO interaction with MOF frameworks. The result showed that besides the metal site coordinating with NO the neighbour uncoordinated carboxylic oxygen atom make a bond with the N atom of the NO (d = 2.11 Å). Such interaction between NO and functionality of organic ligand stabilises the NO molecule bonded to the metal centre and hence influences the NO- releasing.

In the case of MOF Cu-SIP ($Cu_2(OH)(C_8H_3O_7S)(H_2O)$), it contains a copper cluster $Cu_4(OH)_2$ coordinated by four -COO groups from ligand 5-sulfoisophthalate in a butterfly-like structure with the head and tail coppers coordinated with water only (Fig. 3(a)) [28]. Accompanying with the hydration-dehydration-rehydration (loss of the water at $370 < T < 405K$) process, a reversible single-crystal to single-crystal transformation occurs. This has been demonstrated by in situ diffractions at a variable temperature (in Fig. 3 (a) and (b)) [29]. Such a chemical transformation occurs with respect to the rearrangement of the weaker sulfonate-metal bonds. Its structural flexibility in response to NO induction led to a gating adsorption effect; when NO pressure is beyond the threshold, MOF framework is opened and starts to adsorb NO (in Fig. 3 (c)). This phenomenon was also observed by Kitagawa *et al* on the porous coordination polymer built from zinc and tetracyanoquinodimethane (TCNQ), involving the charge-transfer interaction between TCNQ and NO guests accommodated in pores [30]. This framework property attributes some MOFs with exceptional high adsorption selectivity to NO.

2.3.3 N-Diazeniumdiolate functionalised MOFs

Although the coordinatively unsaturated metals in MOFs (or zeolites) play a key role in enhancing NO storage and regulating NO release rate, the incorporation of a diversity of functional groups (–Br, $–NH_2$ and $–OC_3H_7$ etc) in the MOFs, that are either inherited from organic ligands or a result of post-synthetic modification, provides an increased chance of achieving the objective [31-33]. Over the last few decades, *N*-diazeniumdiolate compounds have been widely investigated due to their capability of releasing NO [34]. The scheme for this type of compound to store and release NO are as follows: exposing the free amines in a compound to NO gas that will generate an *N*-diazeniumdiolate structure, $2R_2NH + 2NO \rightarrow R_2N[N(O)NO]^- + R_2NH_2^+$, in which NO is stored; releasing NO is achieved through allowing the *N*-diazeniumdiolates contacting with water or other protonated media to decompose the *N*-diazeniumdiolate, $R_2N[N(O)NO]^- + H^+ \rightarrow R_2NH + 2NO$. According to this scheme, it looks likely to functionalise MOFs with *N*-diazeniumdiolate groups to enhance NO storage and release.

Preparation of MOFs with amine functionalities is the prerequisite condition for this type of NO donor. The pore size and volume in the MOFs must have to be big enough to accommodate

amine functionalities, which to the most extent allows NO to access amines to form *N*-diazeniumdiolates. For this purpose, three approaches can be used: (a) adsorption of amine compounds in pores; (b) binding amine compounds to the metal centres in MOFs; (c) using organic ligands containing amines to build MOFs. For examples, to take advantage of *N*-diazeniumdiolates in NO delivery, it is the first to incorporate amines into MOF, such as through the pyridyl-N of 4-(methylamino)-pyridine (4-map) to coordinate with the Cu site in HKUST-1 using vapor-phase diffusion method (Fig. 5 section A) [35]. Subsequently, the MOFs are exposed to NO gas (at pressure 2 bar) to form *N*-diazeniumdiolates. When exposed to moisturised air, the *N*-diazeniumdiolated HKUST-1 continuously release NO for 5 days. A concern was about the *N*-diazeniumdiolate group facilitated 4-map leaching in water, which may restrict the applications of materials in the aqueous environment. This needs to be considered when using a similar method for fabricating NO donors. Another method is through post- modification [36a], that is, the amine ligands in MOFs are converted to *N*-diazeniumdiolate structures. For instance, the parent MOFs (IRMOF-3 and UMCM-1) were built from 2-amino-1,4-benzenedicarboxylic acid and Zn. They contain pendant amine groups. Since the zinc is coordinatively saturated by carboxylic oxygen atoms from the strut ligands, there are no open metal sites within the framework. Upon exposure to NO gas (~100 mbar) at room temperature, the amine groups in the ligands were converted into *N*-diazeniumdiolates (Fig. 5 sections B and C). This is a crucial step to convert the parent MOFs into the NO donors. Distinct from the amine-functionalised HKUST-1 that is non-porous due to the 4-map groups converging in the pores, this material to some extent still preserves porosity. However, upon soaking in aqueous solution, they have the same issue of amine-leaching similar to the *N*-diazeniumdiolate functionalised HKUST-1. Peikert et al synthesised the HKUST-1 analogous MOFs from amino functionalized trimesic acid ligands (H_3RNHbtc, R=Me, Et, ^{n}Pr, and ^{i}Pr). This is different from the post modification, the secondary amines directly inherit from the ligand building blocks. To keep the structure integration, the thermal activation was controlled at 120^{o}C and NO loading was operated in the gas-solid reaction without a basic medium [36b].

2.4 Polymer composites as *NO donors*

In addition to nanoporous zeolites and MOFs, in recent years, development of other polymeric NO donors have also been investigated. These NO donors contain the moieties of *S*-nitrosothiols, nitrosamines, NO−metal complexes, organic nitrites, nitrates and the compounds containing *N*-diazeniumdiolates. The common practice is to disperse thiol or amine containing compounds in polymers, followed by exposure to NO to convert parent materials into NO donors.

To achieve this, three approaches are usually used through the incorporation of *N*-diazeniumdiolate type NO donors into polymer composites as shown in Fig. 6: (a) blending *N*-

diazeniumdiolate compounds within polymer matrices; (b) covalently binding *N*-diazeniumdiolate groups to pendant polymer chains; (c) *N*-diazeniumdiolate groups covalently bound directly to the polymer backbone [37,38]. These approaches have a distinct impact on NO storage and release kinetics. In a typical example, disodium 1-[2-(carboxylato)-pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (PROLI/NO) was doped in electrospun polymer microfibers, which increased the half-life of NO-releasing by 2 - 200 times longer than that of PROLI/NO alone [39a]. Since the NO release follows a water substitution mechanism, the hydrophobic or hydrophilic property of polymer substrates will strongly affect water permeating into polymer matrices to contact NONOate or RSNO segments to trigger NO-releasing. This was also elucidated by a comparison between poly(vinyl chloride) (PVC) and polyurethane (PU) polymers as substrates. PVC was the hydrophobic polymer, whereas PU was the hydrophilic polymer. The NO release half-life of the PVC polymer was prolonged much more than that of the PU polymer [39a]. In addition to the direct method using polymers or composites for delivering NO, the indirect method has been studied by Neufeld et al [39b]. In this case the water-stable copper-based MOF $H_3[(Cu_4Cl)_3(BTTri)_8]$(H_3BTTri = 1,3,5-tris(1H-1,2,3-triazol-5-yl)benzene) or Cu-BTTri was incorporated within the polysaccharide chitosan to form membranes. Such MOF/polymer plays a catalyst role in promoting the NO generation from the RSNO S-nitrosoglutathione (GSNO). They claimed that MOF structure was retained during exposed in aqueous solution; the formulated materials can be reused without significant degradation of its activity. This result supports the development of future MOF-based biomaterials.

In-depth reviews covering polymer composites for the release of NO have been published previously [6,40], these provide a more detailed analysis of promising macromolecular materials and nanoparticle technology for future biomedical applications. As mentioned before, grafting the NO donors on different carriers will create new NO delivery materials having varied behaviours of NO storage and release. For example, the RS-NO and *N*-NONOate type NO donor groups were grafted on nanoparticle surfaces. Various silica particles are within the scope of the NO donor precursors [26, 37, 41-45], among which the *N*-diazeniumdiolate and thiol functionalised particles are of particular interests in the development of potential medical devices with various biological functions, e.g. inhibition of platelet aggregation, locally vasodilating blood vessels, enhancement of wound healing and anti-bacterial.

2.5 Functionalised particles as NO donors

2.5.1 N-diazeniumdiolate Functionalised particles

The *N*-diazeniumdiolate functionality is a characteristic structure formed in many NO donor materials. In aqueous media, it spontaneously dissociates into NO and the parent amine. The

recovered amine present in aqueous media (such as blood) to some degree is the setback for small molecule *N*-diazeniumdiolate compounds to deliver NO because it is an undesirable by-product. An improvement is to disperse *N*-diazeniumdiolate compounds into polymers [37]. However, this is not an ideal solution, since some *N*-diazeniumdiolate compounds leaching out from the polymer matrix is difficult to be avoided. This raises a potential toxicity issue because it could be oxidised in blood into a carcinogenic *N*-nitrosamine. The concern of toxicity could be significantly reduced if parent amine compounds are covalently anchored to polymer chains or backbone, thus reducing the risk of labile amines leaching into the blood [26,37].

A strategy is to graft *N*-diazeniumdiolates on the surface of fumed silica particles that are then embedded within the given polymer matrices [41]. In a special case, the amine-containing silanes were grafted and then converted into *N*-diazeniumdiolate structures after exposure to NO gas (at ~ 5.5 bars). The advantage of this approach is to create a variety of NO-releasing polymers with reduced toxicity risk through embedding the *N*-diazeniumdiolate-functionalised, fumed silica particles within polymer matrices. Thereafter, the NO donor polymer can be adapted for various specific medical applications. For example, to devise a polymer film able to prevent the adhesion of platelets; topical creams for wound healing; or some products that can locally vasodilate blood vessels [41, 44].

In order to enhance the NO storage of silica particles and improve control over the NO release kinetics, Schoenfisch *et al* investigated a new class of amine-functionalized silica nanoparticle for NO release [43, 45-47]. The silica nanoparticles contain amine groups, created by the sol-gel process [48] through co-condensation of tetraethoxy- or tetramethoxysilane (TEOS or TMOS) and aminoalkoxysilane with an alcohol solvent and an ammonia catalyst. Subsequent exposure of the derived particles to NO gas (~ 5.0 bars) transforms the amine groups into *N*-NONOates, completing the conversion of silica particles into *N*-diazeniumdiolate NO donors (Fig. 7). The chemistry and amount of aminoalkoxysilane in reactants gave the final NO storage in a wide range of 0.05 –1.78 mmol g^{-1}. The best result is equivalent to that of metal-exchanged zeolites. The maximum amount of NO released are 10 − 5500 ppb·mg^{-1} with half-lives 0.1 − 12 hours and release durations up to 30 hrs. As with the other *N*-diazeniumdiolate NO donors, the release is triggered by exposure to moisture at biologically relevant pH and temperature (pH 7.4, 37°C), hence its advantage for biomedical applications. This type of material favours storing large quantities of NO and modulating NO release kinetics over large macromolecular donors. Now, various silica nanoparticles have been widely studied for medical uses, if the amine or diazeniumdiolate leaching can be well restricted, this would be one of the promising methods. Analogously, this method can be used for the formulation of zeolite particles. To get rid of amine

leaching issue, another strategy could be adopted such as the photolysis of nitro to produce NO. Taladriz-Blanco et al have demonstrated a Layer-by-layer assembled gold nanoparticles that were achieved by alternate coating of the Au core with the cationic polyelectrolyte PAH and the tailored anionic polyelectrolyte PAA-NO. The NO released is regulated by the NO photoprecursor layers [39].

2.5.2 Thiol-functionalised silica particles

In a similar process as mentioned above, the thiol-functionalized silica particles have been fabricated from mercaptosilane and alkoxysilane [49]. After reaction with nitrous acid nitrosates, the thiols in the particles were converted into *S*-NO groups i.e. RS-H + HNO_2 → RS-NO + H_2O. The formation of the red colour particles after nitrosation is an indicator of a successful reaction. Similar to the low molecular weight *S*-nitrosothiol NO donors, the derived RSNO- functionalised silica particle is sensitive to light. Under the light excitation at visible band ~ 550 nm, the S–N bond is cleaved to release NO (Fig. 8) [50]. The *S*-nitrosothiol silica particles mixed with polymers to form the polymer composites has been used as NO photo-donors for bioactive NO delivery [51a], for example, Chang et al prepared NO-releasing core/shell nanoparticles possessing silica cores and S-nitrosothiol-modified chitosan shells [51b]. The thiol-functionalised silica particle overcomes the shortages observed in the low molecular weight RSNOs (such as *S*-nitroso-glutathione (GSNO), *S*-nitroso-*N*-acetylcysteine (SNAC), and *S*-nitroso-*N*-acetyl-penicillamine (SNAP)) that have a low NO storage capacity and uncontrollable NO release [52a]. The particles made by the sol-gel process store more NO within the network (~4.40 mmol NO g^{-1}) than the fumed silica particles that have the *S*-NO groups grafted on the exterior surfaces only, and they can release NO in a more controllable manner. For other interesting nitric oxide-releasing polymeric nanoparticles, dendrimers and micelles, for biomedical applications, see the recent review [51c].

3. Biological tests of NO-releasing solids

Biological tests have proved the pivotal roles of the NO released from functionalised solids in such roles as inhibiting platelet aggregation and anti-adhesion of platelets on surfaces and defence against microbial infection. The following are the typical results reported in recent publications.

3.1 Relaxing smooth muscle of blood vessels

As a signal messenger, NO plays a key role in vasodilatation in the body by causing smooth muscle of blood vessels to relax, widening blood vessels and hence easing the blood flow and reducing pressure. Correspondingly, the medicines used for the treatment of heart disease and angina are capable of producing NO in the body through enzyme conversion to affect the relaxation

of smooth muscle and dilate blood vessels, increasing their diameter and thereby increasing blood flux. An interesting experiment, using MOF NO donor demonstrated this biological function of NO, in which a pressed pellet MOF CPO-27 (Ni) was used [27]. Under physiological conditions, the MOF pellet placed a distance of 2 mm from the pig coronary arteries in an organ bath released NO, rapidly resulting in relaxation of the precontracted vessel. Subsequent removal of the pellet from the organ bath gradually recovers muscle tension.

In a recent study of novel metal-NONOates as NO-donors, the relaxation of precontracted rat coronary aortas has been observed. The binding of the NONOate derived from N-aminoethylpiperazine *N*-diazeniumdiolate to the metal affects the NO release rate. The nickel-based complex [Ni(PipNONO)Cl] proved more effective than DETA/NO, a widely used and commercially available *N*-diazeniumdiolate, at inducing relaxation of the precontracted rat aortic rings. The effect of the Ni complex was concentration dependent. The copper analogue as compared demonstrated a slower release rate than the Ni complex and the unbound *N*-diazeniumdiolate, however, it was ineffective at inducing vasorelaxation [53].

In addition to the beneficial effects of NO delivered in inducing vasodilation in the cardiac system, there has been a recent study published demonstrating the potential of NO-delivering nanoparticles for the treatment of liver fibrosis and portal hypertension, using both *in vitro* and *in vivo* experiments [54]. Non-cytotoxic polymeric nanoparticles were designed in the studies to deliver NO into hepatic stellate cells (HSCs) through the *S*-nitrosoglutathione NO-donor compound. These nanoparticles targeted at HSCs in order to limit their impact on surrounding systems. Results demonstrated the uptake by primary rat HSCs and human HSC cell lines in the liver. When vitamin A coated nanoparticles reach the liver cells and release NO, inhibition of collagen I and α-smooth muscle actin production is observed, indicating a reduction in the progression of liver fibrosis. A reduction in portal pressure of around 20 % was also observed, which demonstrates the potential of the NO-releasing nanoparticles to alleviate portal hypertension as well as liver fibrosis.

3.2 Inhibition of cancer cell growth

At a low-level nitric oxide acts as a cellular signalling molecule and vasodilator, with other beneficial biological roles such as its involvement in wound healing and immune defence. However, when the concentration is increased NO can cause damage to mitochondria and DNA by nitrosation or oxidation [55]. It is such effect that has led to research into the anti-cancer potential of NO-releasing materials. Due to the damaging nature of high NO concentration, it is of particular importance to have targeted delivery of the payload.

The antitumor efficacy of NO delivered by silica particles against human ovarian tumour was evaluated by Schoenfisch *et al* [46]. They showed the high antitumor activity of nanoparticle-derived NO against ovarian tumour or transformed cell lines than that of NO released from a small molecule NO donor, pyrrolidine (PYRRO)/NO. Silica nanoparticles deliver NO to permeate into the cytosol of the treated cells and remain localised to late endosomes and lysosomes. The different *N*-diazeniumdiolate-functionalized silica particles prepared from 3-methylaminopropyl-trimethoxysilane (MAP3), *N*-(6-Aminohexyl) aminopropyltrimethoxysilane (AHAP3) and 3-aminopropyltrimethoxysilane (APTMS), respectively, via sol-gel polymerization, have the particle size in the range of 90 - 350 nm controlled by varying aminoalkoxysilane molar percentage (10 - 75 mol %, balance TEOS). Particle size-dependent tumour cell cytotoxicity was observed; larger nanoparticles demonstrated the enhanced destabilisation of mitochondrial function and caspase-induced apoptosis in tumour versus non-tumor cells. This selectivity makes it possible to develop NO-based chemotherapies with increased antitumor efficacy and decreased toxicity to non-cancerous cells. For future applications, modification of the solid surface with polyethylene glycol (PEG) is of interests in increasing biocompatibility of this type of particles.

N-diazeniumdiolates have shown potential as NO donors in cancer treatments due to the spontaneous dissociation of NO upon exposure to physiological conditions. The recent studies demonstrated the possibility to manipulate NO release by selecting the protecting groups that are able to stabilise the *N*-diazeniumdiolate functionalities until exposure to an enzyme when the *N*-diazeniumdiolate anion was cleaved resulting in the physiological triggering of NO dissociation [56]. *Escherichia coli* nitroreductase, an enzyme not usually found in normal human cells, triggers the metabolism of the protected *N*-diazeniumdiolate (NTR), 1-(2-methylpiperidin-1-yl)diazen-1-ium-1,2-diolate which is an inactive NO prodrug to produce the reactive *N*-diazeniumdiolate anion. Subsequently, the *N*-diazeniumdiolate anion releases NO upon exposure to physiological liquid at pH = 7.4. The NTR activated approach with the enhanced selective toxicity towards cancer cells illustrates potential future applications.

In addition to the tumoricidal potential of high concentration of NO, low dose of NO has also demonstrated efficacy in cancer treatments by reducing anti-cancer drug resistance when used alongside chemotherapy [55]. Multi-drug resistance is a common problem with anti-cancer drug therapies, which is frequently linked to the presence of membrane proteins called P-glycoprotein (P-gp). These proteins draw the chemotherapy drugs out from the tumour cells, reducing the intracellular concentration and thereby reducing the efficacy as anti-tumor agents. The increased efficacy of anti-cancer drugs has been observed when they were used in conjunction with NO-releasing drugs. The study used MCF-7 human breast cancer cell lines treated with doxorubicin

(DOX) chemotherapeutic drug. Upon release of NO from NO-donor modified nanoparticles, P-gp expression was reduced, leading to higher concentrations of DOX within the cancer cell lines and improved anti-tumor efficacy. This demonstrates that the NO/DOX combination is able to overcome multi-drug resistance [55].

3.3 Inhibition of platelet aggregation and adhesion

The NO released from various classes of functionalised solids e.g. metal-exchanged zeolites [9, 10, 24], MOFs [24, 30, 57, 58] and silica nanoparticles [41, 44] playing an important role in the inhibition of platelet aggregation and adhesion has been observed [9]. Fig. 9a illustrates results of a platelet aggregation test using Co-exchanged zeolite LTA, in which platelet aggregation was initiated using the thromboxane A2 analogue, U46619 (8 íM). The cobalt exchanged zeolite without loaded NO (control) and LTA zeolite (blank) did not prevent platelet aggregation, only an NO-loaded Co-exchanged zeolite-A/PTFE (polytetrafluoroethylene) sample completely inhibits platelet aggregation in platelet-rich plasma (PRP). The central role of NO released from the sample was confirmed by the addition of the NO scavenger, oxyhaemoglobin (40 íM), which prevents the inhibitory effect. The NO loaded MOF (HKUST-1) also demonstrated the ability to inhibit platelet aggregation [24]. To examine the potential biomedical application of new NO-releasing solids, NO-releasing fumed silica particles were dispersed into polyurethane layer of Tygon medical tubing device [41]. It was found that the PU/Sil-2N[6]-N_2O_2Na coating on the inner wall of the Tygon tubing generated NO-flux levels to effectively prevent platelet aggregation and adhesion on inner wall of tubing (Fig. 9c), whereas the PU/silica surfaces that did not release NO had no inhibitory effect (Fig. 9b).

Prevention of platelet aggregation and adhesion is of particular importance following surgical procedures, as the interaction of foreign materials with blood causes haemostasis disruption, which can lead to the formation of thrombus. Medical implants such as stents and catheters have a tendency to cause thrombus formation; this is problematic as it can result in failure of the implanted device. Research into localised delivery of NO to inhibit platelet aggregation and adhesion has resulted in two primary approaches: a) coating implants with novel NO-releasing materials; b) surface modification to incorporate NO-releasing materials. *N*-diazeniumdiolate and *S*-nitrosothiol NO donors have been the focus of research due to their controllable release kinetics and facile NO dissociation under physiological conditions, that makes them ideal NO donors for incorporation into modified surface materials and thin film coatings [59].

3.4 Topical promotion of wound healing activity

The NO-releasing zeolite (Ze-NO) that contains Mn exchanged was compared with acidified NO_2^- when being applied for topical therapy [60]. It was found that Ze-NO had the same role as

acidified NO_2^- in increasing dermal blood flow. However, Ze-NO did not cause any side effects such as marked erythema, edema, and ulceration observed in the acidified NO_2^- treatment. It neither causes infiltration of macrophages and neutrophils into the epidermis and dermis nor reduces Langerhans cells in the epidermis like acidified NO_2^- did. Both acidified NO_2^- and NO-releasing zeolite resulted in a moderate increase in dermal T cells. Pure NO released from Ze-NO can diffuse across the epidermis to the dermis to exert its biological activity and induces a moderate Th1-cell response. It concluded that Ze–NO is an effective NO donor without the dramatic pro-inflammatory effects found in an ascorbic acidified NO_2^-.

In another study by Neidrauer *et al* [61], a topical ointment containing NO-loaded zinc-exchanged zeolite A (33% w/w) have antimicrobial, anti-inflammatory and wound healing effects. NO-loaded zinc-exchanged zeolite A powder was mixed with an emulsifying ointment. The effect on wound closure was examined by application of ointment to the cutaneous wound on obese male Zucker rats, three times per week for twenty days, commencing the day after wounding. Wound area was calculated at various stages using image analysis and MATLAB software. For wounds treated with NO loaded zeolite, the average rates of healing were 15.1 % per day, compared with the control zeolite without NO loaded, 11.7 %. It thus demonstrates the statistically significant benefit of the NO loaded zeolite. Combining the NO loaded zeolite with the hydrophobic ointment reduces the NO release rate by at least one-third due to the reduced rate of water transport to the zeolite particles. Antimicrobial and anti-inflammatory effects were also observed from the NO-loaded zeolite; these combined actions improve the efficacy of this material for topical treatment of wound healing.

3.5 Antimicrobial activity

Gaseous NO at a level ~200 ppm is effective to kill bacteria without damaging human dermal fibroblasts [4]. At this level, NO kills both gram-positive and gram-negative bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). The use of nanoparticles as NO delivery vehicle was found to significantly enhance the efficacy of NO to kill bacteria beyond that of small molecule bactericidal alone. The *N*-diazeniumdiolate functionalised silica nanoparticles were capable of delivering NO [43] and killed *P. aeruginosa* cells more effectively than a small *N*-diazeniumdiolate (i.e. PROLI/NO) [3]. The amount of NO required from PROLI/NO to completely kill *P. aeruginosa* was approximately one order of magnitude greater than that required from the *N*-diazeniumdiolate functionalized nanoparticles. Obviously, this is attributed to a low local concentration of NO yielded from the PROLI/NO when dispersed in both PBS and TSB solutions that did not diffuse into bacterial cells effectively. It is interesting to find that the NO released from nanoparticles at certain levels is cytotoxic only to *P. aeruginosa* cells but not human and mouse

dermal fibroblasts. More studies showed that the NO released from these particles also killed a series of biofilm-based microbial cells including *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Candida albicans*, in particular, gram-negative *P. aeruginosa* and *E. coli* biofilms [2]. Bear in mind, these bacteria plague a high percentage of all leg and foot ulcers resulting in chronic wounds with impaired healing and are common causes of burn wound infections leading to significant morbidity and mortality in burn wound victims. To address unmet medical need, the development of high-efficiency NO-releasing nanoparticles to kill bacteria is particularly expected to enhance wound healing. In addition to wound healing, the *N*-diazeniumdiolate functionalised nanoparticles can also be formulated into an oral care composite with additional antimicrobial agents for oral care applications, allowing NO to be released from particles to diffuse through biofilms and kill oral bacteria preventing infection [62].

In a recent study by Brisbois *et al* [63], the efficacy of an *N*-diazeniumdiolate NO-donor was assessed for antimicrobial actions in a mouse burn model. The delivery of NO was via polymer film doped with DBHD/N_2O_2 (*N*-diazeniumdiolate NO donor) and poly(lactic-co-glycolic) acid (PLGA), which promoted and prolonged NO release. The wound was exposed to *Acinetobacter baumannii* and allowed to incubate for twenty-four hours prior to application of NO-releasing patch, control patch or no patch. Following twenty-four hours of patch treatment, the sample was excised, homogenised and grown on agar plates. A significant reduction (~ 4 logs) in bacteria present in NO-treated samples was observed when compared to control samples, thus illustrating the potential antimicrobial benefits of NO-releasing materials.

4. In summary

So far a diversity of NO delivery materials, e.g zeolites, MOFs and silica particles have been developed, which demonstrate a wide spectrum of characteristics in NO storage and releasing. Distinct from the endogenous NO delivery, the current material-based technologies for exogenous NO delivery mostly rely on the active centres present in materials e.g. the amine and thiol functionalities, and the uncoordinated metal sites. The pore structures of materials influence the accessibility of these centres to NO molecules. Undoubtedly, the concentration of these centres or the material porosity determines the NO storage capacity and controls the deliverable amount of NO. Meanwhile, the interaction between NO and the storage material determines the storage stability and controls the NO release profile. Consequently, the knowledge of material characteristics in relation to NO storage and release is necessary for the intelligent NO donor design. To meet the reuirements of material cytotoxicity to health cells, new NO delivery solids should avoid the use of toxic metals and restrict their leaching, so does the amine leaching if amines

are incorporated. Formulation of polymer composite containing the materials as metioned above will be attractive for practical use.

Currently, many different NO delivery materials have been designed to suit different therapeutic applications as it is a challenge for one type of NO delivery method to meet all practical requirements. For example, the requirements of an NO-releasing material proposed for the destruction of cancer cells will be vastly different from that intended to promote wound healing and antimicrobial activity. The dosage of NO required for these two actions differs greatly. Exact doses of NO required to activate particular biological responses is unknown yet. This is an area where research is still required before progression into a clinical setting will be possible, but trends can be observed which show that much higher doses are required for cytotoxicity (nanomolar range) than for cellular signaling (picomolar range) to induce the regulation of blood flow and promotion of wound healing. Materials exhibiting a high capacity for NO loading are essential for introducing anti-tumor effects than for many of the other functions, however, with the provision of being able to introduce controllable NO release, a high capacity donor will allow for prolonged release times thus reducing the number of dose administrations required. For the development of optimal therapeutic NO delivery materials, the focus must remain on tuning the NO release rate or flux. In order to know targets for each application, further biological studies are required to quantitatively establish NO concentration ranges where the desired effects are observed. So far the information about the toxicology of the NO delivery materials reviewed as above is much limited. The corresponding investigation is required in order to clarify the material feasibility for medical uses.

Acknowledgements
The authors gratefully appreciate the supports from EPSRC (EP/M027295/1), the initial research funding from Queen's University Belfast and Aston University, and the DEL PhD studentship.

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Figures

Fig. 1. (a) Structure of the cobalt-NO complex in zeolite LTA; (b) hysteric adsorption and desorption of nitric oxide on cobalt exchanged zeolite LTA (Modified from ref. [4] with ACS permission).

Fig. 2. The integrated NO release from zeolite LTA exchanged with Mn might be divided into two parts kinetically. One has high NO-releasing rate constant k2 that tends to release NO faster than the other with lower k1. This indicates the non-uniform of NO interacting with the framework.

Fig. 3. Structural transformation of Cu-SIP MOF and NO gating adsorption. (a) left: hydrated structure; right: dehydrated structure. (b) left: low-temperature diffraction spots observed in relation to the hydrated structure (at 300K); middle: no diffraction spots observed (390 K); right: high-temperature diffraction spots observed in the dehydrated structure (465 K). (c) Gating effect induced by structural transformation leads NO adsorption and desorption to a big hysteresis. Beyond a threshold (~270mbar), the framework was opened allowing NO to be adsorbed. (Modified from ref. [29] with ACS permission)

Fig. 4. Activation removes the coordinated water bound to metal sites and water adsorbed in channels. Loading NO allows these opened metal sites to bind NO. When the material is exposure to a controlled humidity atmosphere such as human skin surface, the water substitutes NO bound to metal sites, discharges NO to meet medical needs. The material is H_2O-solvatedly regenerated for recharging NO. (Modified from ref. [27a] with ACS permission).

Fig. 5. Embedment of *N*-diazeniumdiolate (NONOate) within MOFs. Section A: 4-(methylamino)-pyridine (4-map) was bound to the open Cu sites in the MOF HKUST-1, followed by exposure to NO to form the NONOates; section B and C: converting the inherited amine groups into the NONOates in the IRMOF-3 and UMCM-1 MOFs, respectively. (Modified from ref. [35] and [36a] with RSC and ACS permission).

Fig. 6. Three approaches to convert polymers into *N*-diazeniumdiolate type NO donors (Reproduced from ref. [37] with permission of Elsevier publisher).

Fig. 7. Silica nanoparticles were produced by a co-condensation reaction TEOS or TMOS and aminoalkoxysilane. Under the basic condition, the amine groups incorporated in particles were converted into *N*-diazeniumdiolate that decomposes to release NO in the PBS solution (pH=7.4, at a body temperature) (Modified from ref. [43, 45, 46] with ACS permission).

Fig. 8. Schematic photoinitiated NO release from the RSNO functionalised silica particles.

Fig. 9. (a) Nitric oxide loaded Co- exchanged zeolite LTA completely inhibited platelet aggregation in platelet-rich plasma (PRP); (b) platelet aggregation and adhesion on the PU/silica surface (without NO released); (c) no platelet aggregation and adhesion occurs on the NO-releasing coating (PU/Sil-2N[6]-N2O2Na) surfaces. (Modified from ref. [10] and [44] with ACS and NPG permissions).

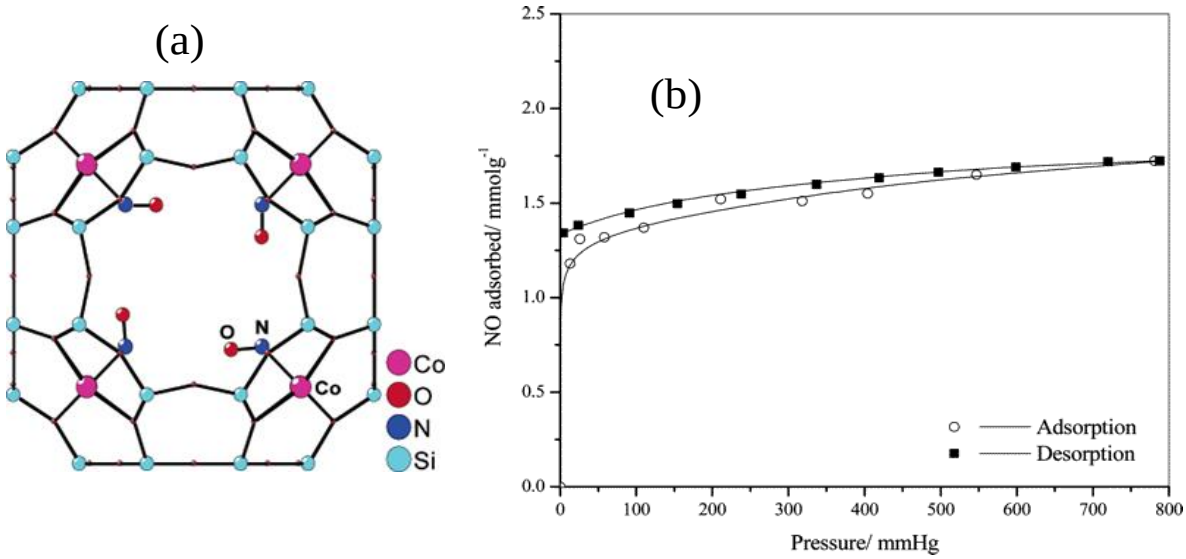


Fig. 1. (a) Structure of the cobalt-NO complex in zeolite LTA; (b) hysteric adsorption and desorption of nitric oxide on cobalt exchanged zeolite LTA (Modified from ref. [4] with ACS permission).

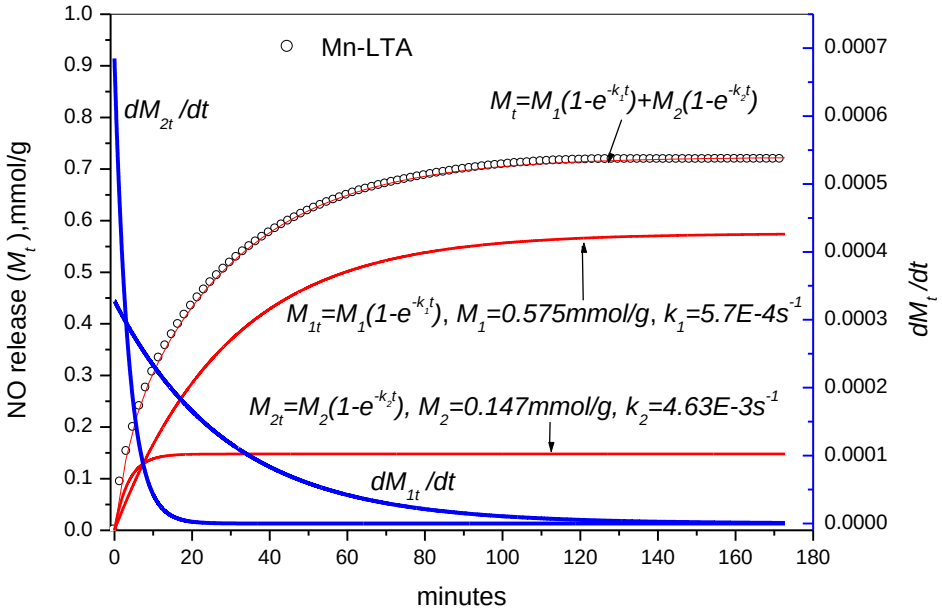


Fig. 2. The integrated NO release from zeolite LTA exchanged with Mn might be divided into two parts kinetically. One has high NO-releasing rate constant k_2 that tends to release NO faster than the other with lower k_1. This indicates the non-uniform of NO interacting with the framework.

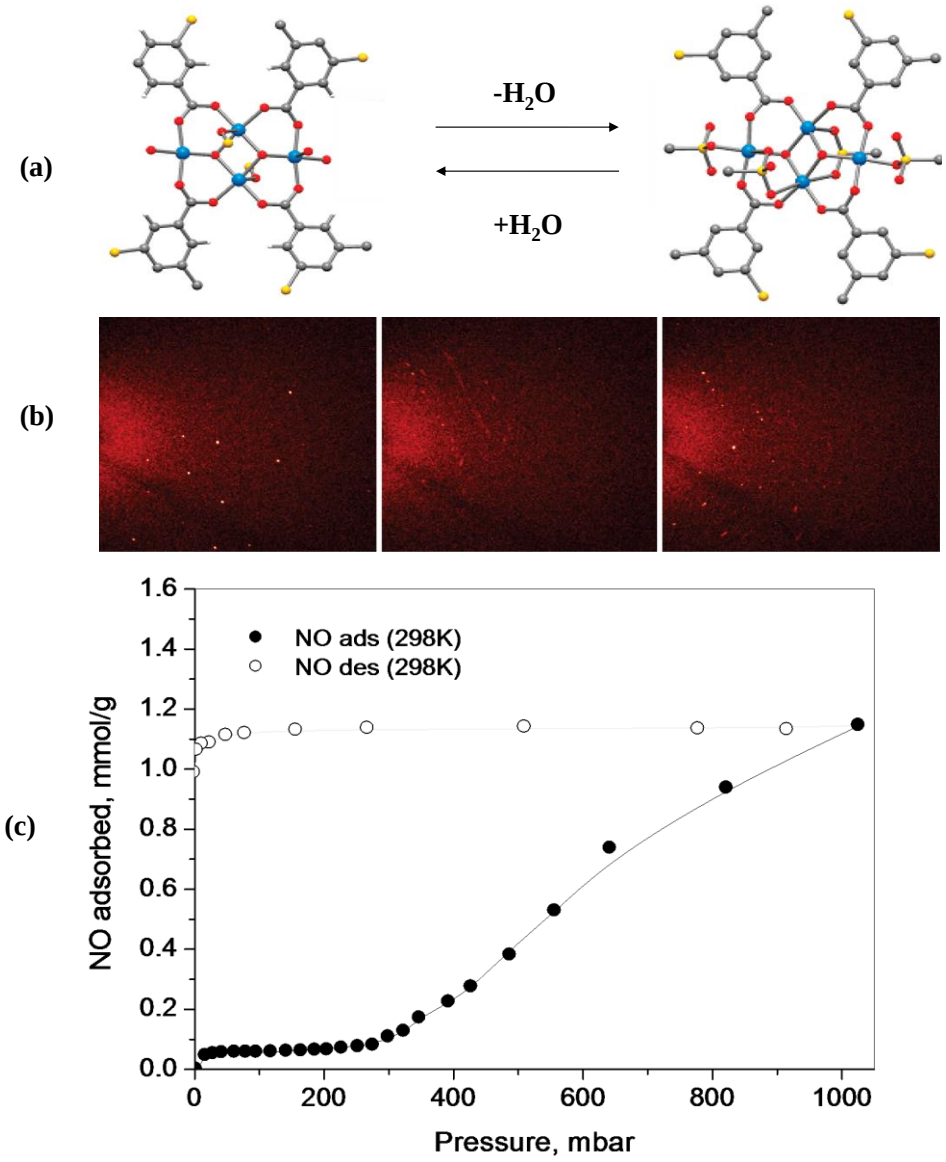


Fig. 3. Structural transformation of Cu-SIP MOF and NO gating adsorption. (a) left: hydrated structure; right: dehydrated structure. (b) left: low-temperature diffraction spots observed in relation to the hydrated structure (at 300 K); middle: no diffraction spots observed (390 K); right: high-temperature diffraction spots observed in the dehydrated structure (465 K). (c) Gating effect induced by structural transformation leads NO adsorption and desorption to a big hysteresis. Beyond a threshold (~270 mbar), the framework was opened allowing NO to be adsorbed. (Modified from ref. [29] with ACS permission)

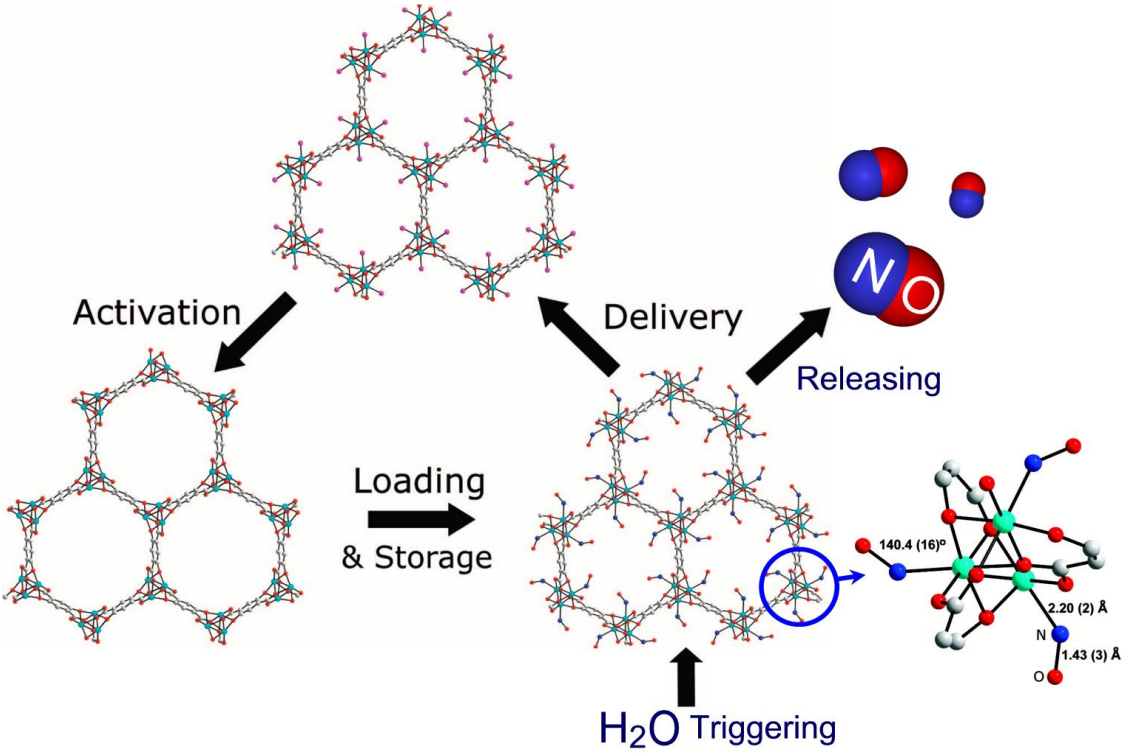


Fig. 4. Activation removes the coordinated water bound to metal sites and water adsorbed in channels. Loading NO allows these opened metal sites to bind NO. When the material is exposure to a controlled humidity atmosphere such as human skin surface, the water substitutes NO bound to metal sites, discharges NO to meet medical needs. The material is H_2O-solvatedly regenerated for recharging NO. (Modified from ref. [27] with ACS permission).

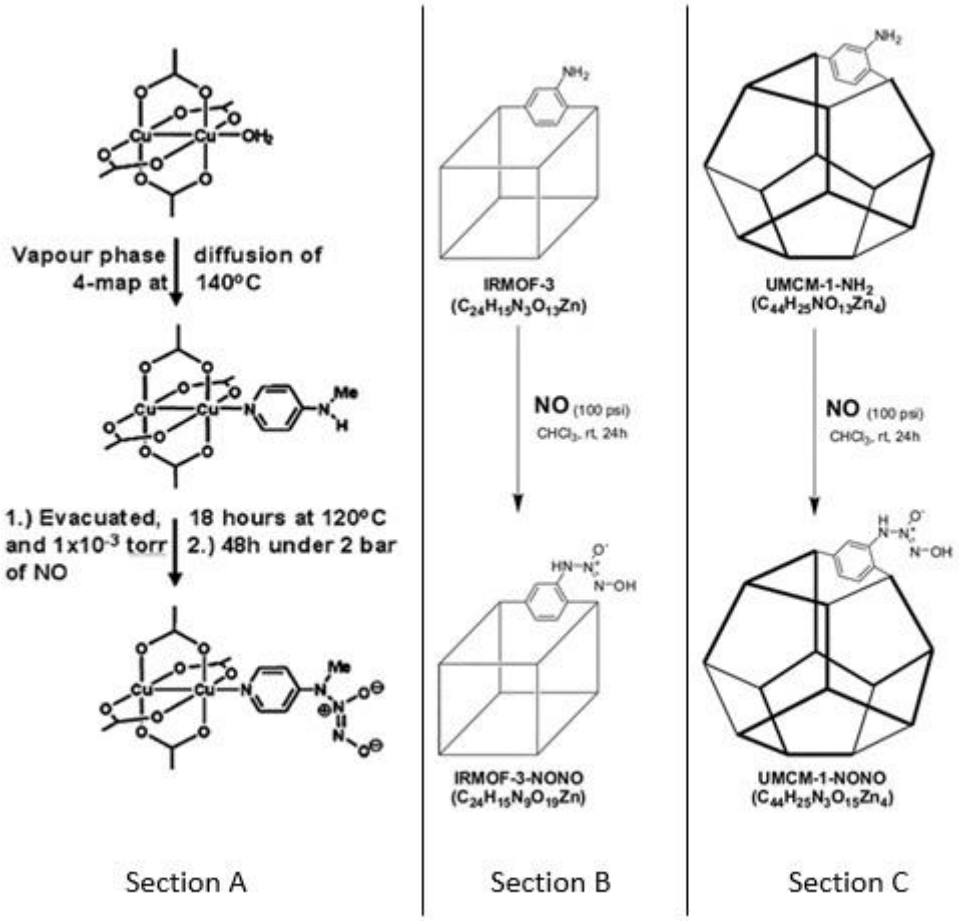


Fig. 5. Embedment of *N*-diazeniumdiolate (NONOate) within MOFs. Section A: 4-(methylamino)-pyridine (4-map) was bound to the open Cu sites in the MOF HKUST-1, followed by exposure to NO to form the NONOates; section B and C: converting the inherited amine groups into the NONOates in the IRMOF-3 and UMCM-1 MOFs, respectively. (Modified from ref. [35] and [36a] with RSC and ACS permission).

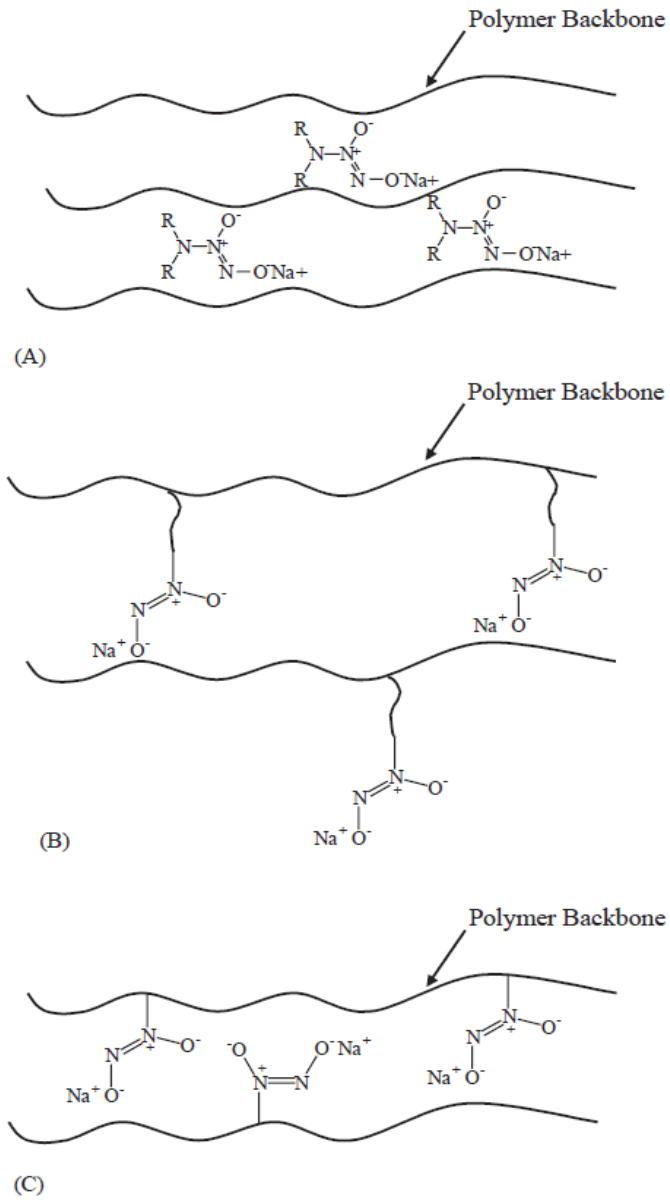


Fig. 6. Three approaches to convert polymers into *N*-diazeniumdiolate type NO donors (Reproduced from Ref. [37] with permission of Elsevier publisher).

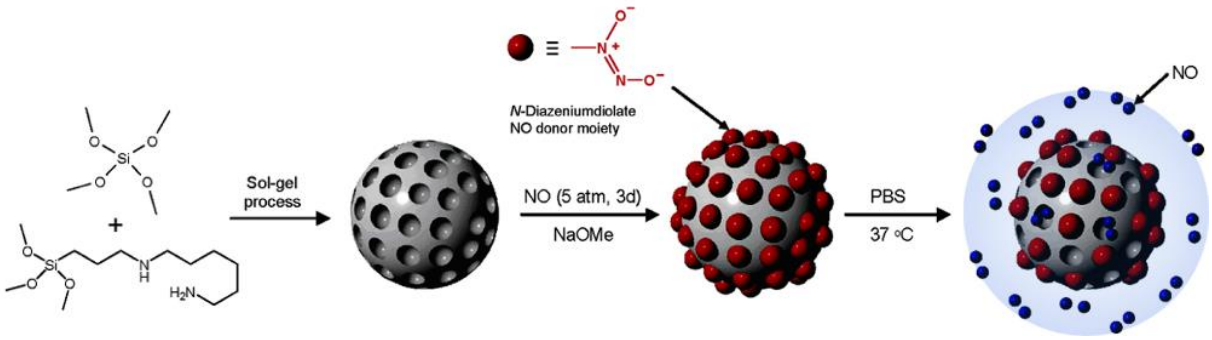


Fig. 7. Silica nanoparticles were produced by a co-condensation reaction TEOS or TMOS and aminoalkoxysilane. Under the basic condition, the amine groups incorporated in particles were converted into *N*-diazeniumdiolate that decomposes to release NO in the PBS solution (pH=7.4, at a body temperature) (Modified from ref. [43, 45, 46] with ACS permission).

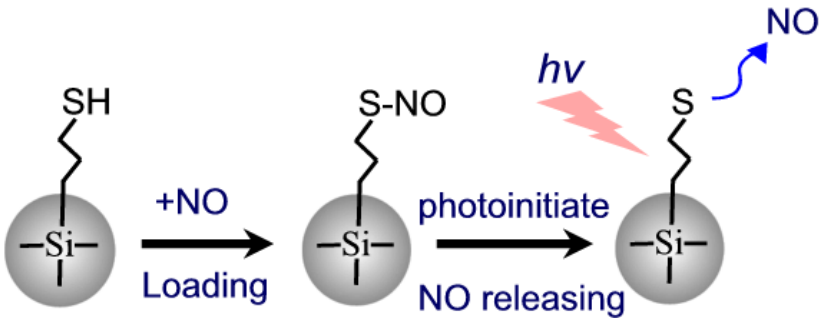
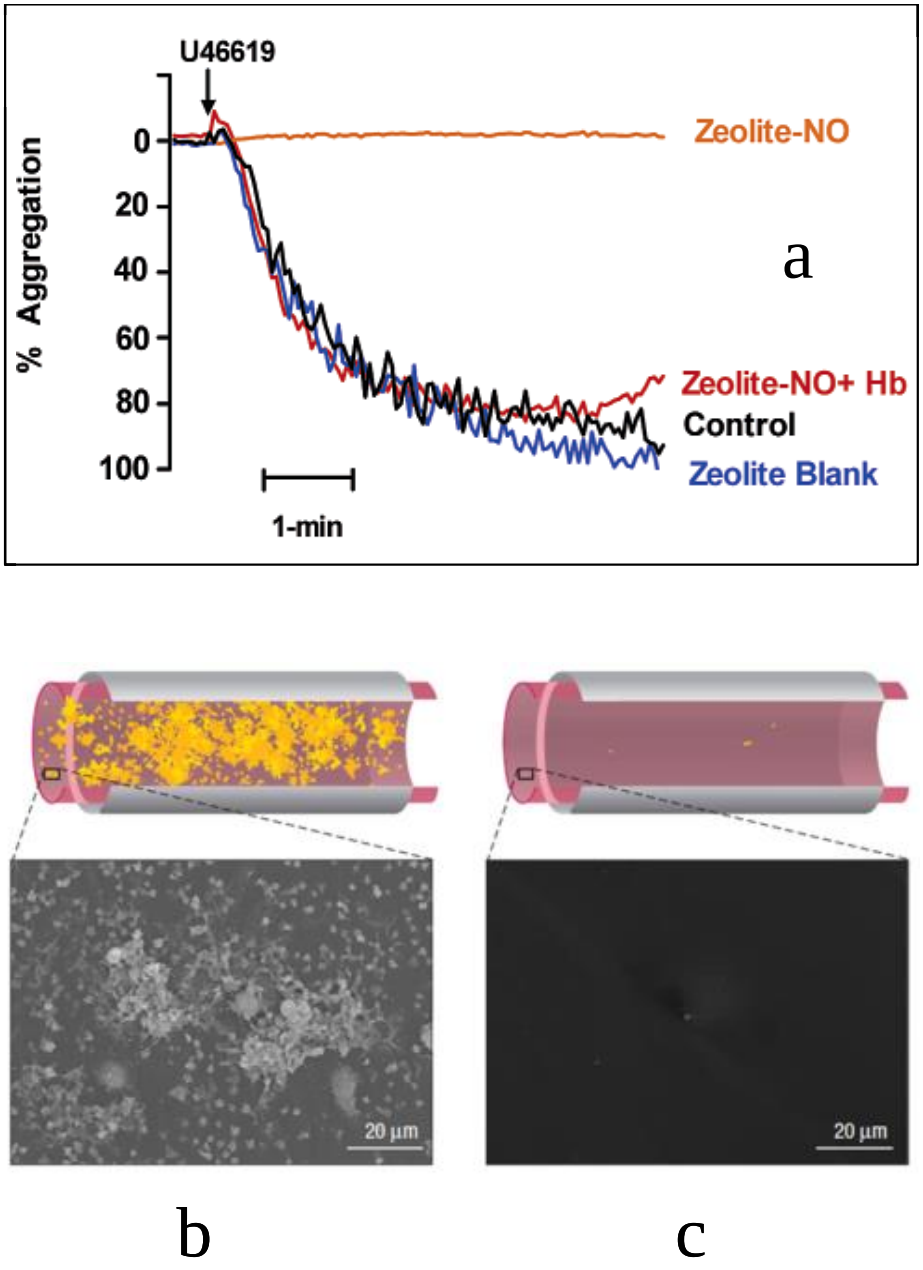


Fig. 8. Schematic photoinitiated NO release from the RSNO functionalised silica particles.



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