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Review article

# Use of gasotransmitters for the controlled release of polymer-based nitric oxide carriers in medical applications

Chungmo Yang[a,1], Soohyun Jeong[a,1], Seul Ku[b], Kangwon Lee[a,c,*], Min Hee Park[a,**]

[a] Department of Transdisciplinary Studies, Graduate School of Convergence Science and Technology, Seoul National University, Seoul 08826, Republic of Korea
[b] School of Medicine, Stanford University, 291 Campus Drive, Stanford, CA 94305, USA
[c] Advanced Institutes of Convergence Technology, Gyeonggi-do 16229, Republic of Korea

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ABSTRACT

Nitric Oxide (NO) is a small molecule gasotransmitter synthesized by nitric oxide synthase in almost all types of mammalian cells. NO is synthesized by NO synthase by conversion of L-arginine to L-citrulline in the human body. NO then stimulates soluble guanylate cyclase, from which various physiological functions are mediated in a concentration-dependent manner. High concentrations of NO induce apoptosis or antibacterial responses whereas low NO circulation leads to angiogenesis. The bidirectional effect of NO has attracted considerable attention, and efforts to deliver NO in a controlled manner, especially through polymeric carriers, has been the topic of much research. This naturally produced signaling molecule has stood out as a potentially more potent therapeutic agent compared to exogenously synthesized drugs. In this review, we will focus on past efforts of using the controlled release of NO via polymer-based materials to derive specific therapeutic results. We have also added studies and our future suggestions on co-delivery methods with other gasotransmitters as a step towards developing multifunctional carriers.

## 1. Introduction

Nitric oxide (NO) is a chemical compound, one of the several oxides of nitrogen, regarded as an industrial air pollutant until the 1987 discovery by Louis J. Ignarro shed new light on its function as a bio-active signaling molecule and physiological modulator [1]. The study initially aimed to elucidate on the similar properties of NO and endothelium-derived relaxing factor (EDRF) [1]. However, studies suggested that both were identical, and it was from this report that NO began to be investigated as a physiological modulator, a signaling molecule that can also be applied in clinics [2–5]. This gaseous signaling molecule or gasotransmitter works along the events of vascular tones, immune responses, angiogenesis, apoptosis, wound healing and tissue repair, neurotransmission, and sleep control [3,6,7]. Such findings of NO as a comprehensive physiological modulator were granted the honor of a Nobel prize in 1998. Ever since the discovery of NO as signaling molecule, other gaseous signaling molecules have been reported: carbon monoxide (CO) (1991) [8] and hydrogen sulfide ($H_2S$) (1996) [9]. These two gasotransmitters regulate various functions through signaling pathways either with or without NO [10]. Altogether, NO, $H_2S$ and CO have gained new potential as prospective therapeutic tools.

NO is biologically synthesized by nitric oxide synthase (NOS), which exists as three isoforms: endothelial (eNOS), neuronal (nNOS) and inducible (iNOS) nitric oxide synthase [11–14]. The family of NOS produces NO catalyzed from the conversion of L-arginine to L-citrulline, and each NOS can be activated by different conditions. The resulting NO stimulates soluble guanylate cyclase (sGC), subsequently increasing the conversion of guanosine triphosphate (GTP) to 3′,5′-cyclic guanosine monophosphate (cGMP) [15]. This signal transduction activates various protein kinases such as protein kinase G (PKG). (Fig. 1.) These cascade reactions (NO-cGMP-kinase activation) amplify NO signal and lead to various functional outcomes in human nervous, cardiovascular, immune, respiratory, endocrine, urogenital and even excretory systems [16]. Additionally, NO mediates various physiological, biochemical and pathological functions based on its concentration (Fig. 2.). High concentrations of NO can induce cell apoptosis, DNA base deamination, nitrosylation of enzymes, and mitochondrial damage with nitrosative stress [17], whereas low concentrations of NO can promote angiogenesis, cell proliferation, growth, and nutrient delivery [18–20]. Additional reports describe the induction of anti-thrombus activity at NO

* Correspondence to: K. Lee, Department of Transdisciplinary Studies, Graduate School of Convergence Science and Technology, Seoul National University, Seoul 08826, Republic of Korea.
** Corresponding author.
*E-mail addresses:* kangwonlee@snu.ac.kr (K. Lee), minheepark@snu.ac.kr (M.H. Park).
[1] These authors contributed equally to this work.

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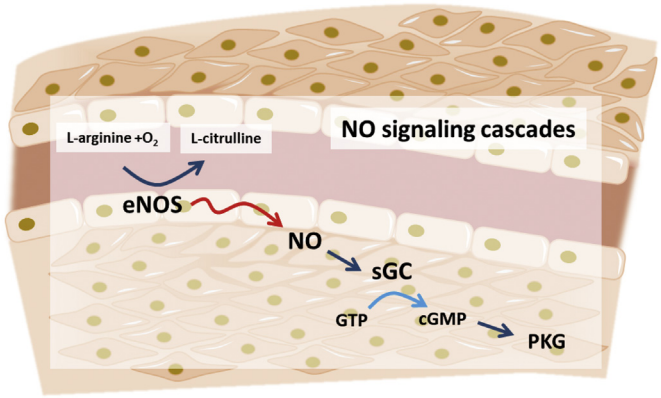


**Fig. 1.** Schematic illustration of endogenous nitric oxide synthesis by eNOS and the subsequent signaling cascade reactions (NO-cGMP-kinase activation) in endothelial cells and smooth muscle cells.

concentrations of 0.3–0.6 fmol $s^{-1}$ $cm^{-2}$ [21] and the inhibition of bacterial adhesion at 1–20 pmol $s^{-1}$ $cm^{-2}$ [22].

Different activities arising from NO delivery have been studied for clinical use in the form of inhaled gas and injected medicines [2,23,24]. For example, BiDil® is a FDA-approved drug used for treating heart failure in certain patients using isosorbide dinitrate, the precursor of NO [25,26]. However, given the limited clinical applications of BiDil® and the side effects that often follow, additional research took place, including the development of various NO donors that release NO upon different triggers like pH change, light, and heat [27–30]. Nitrate/nitrite/nitroso compounds [31,32], *N*-diazeniumdiolate (NONOate) [33,34] and *S*-nitrosothiol (RSNO) [35,36] are the most widely studied donors and are considered for clinical applications (Fig. 3). NONOate generates the stoichiometric product 2NO with first-order release kinetics, leading to the development of 1-(hydroxy-NNO-azoxy)-L-proline (PROLI NONOate), 1-[(ethenyloxy)-NNO-azoxy]-pyrrolidine (PYRRO NONOate), 1-[N-(3-aminopropyl)-N-(3-ammoniopropyl)amino]diazen-1-ium-1,2-diolate (DPTA NONOate), and 1-[N-(2-aminoethyl)-N-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate (DETA NONOate) [27]. RSNOs are adducts of R-SH and can generate NO upon various triggers, such as the contact with metal ions, reducing agents and several enzymes [37]. There are also endogenous donors such as RSNO and *S*-nitrosoglutathione (GSNO) distributed in red blood cells, plasma, and tissue [38]. However, all aforementioned NO donors face two major challenges in clinical applications: the burst release profile and the short half-life.

One of the methods to overcome these two hurdles is by using polymeric vehicles. They can both protect NO leak from trigger sources and prolong the release and circulation time. Polymeric nano- or microparticles, self-assembly micelles and star-shaped polymers are various examples that have been studied [39–41]. Selecting an appropriate polymer design is undoubtably an important matter, and much work has also been done in experimenting with various polymeric delivery systems as excellently reviewed elsewhere [42]. Polymeric carriers for NO delivery requires for the system to release NO in a controlled manner, as the physiological function in response to NO is concentration-dependent. Although clinical trials on polymeric delivery of gasotransmitters has been lacking, there are ample studies demonstrating their therapeutic potential, which we will discuss in our review.

## 2. NO-related therapeutic studies

Use of gasotransmitters is a novel approach in the field of regenerative medicine and drug delivery studies. Among all, NO has been the most widely studied as its action includes but is not limited to applications in wound healing, hypertension, cancer targeting and infection. However, as mentioned earlier, NO's reactive property makes it prone to a short circulation time and an early burst release profile, making it cumbersome for disease-specific applications. Thus, many previous studies have focused on delivering NO in a more sustained manner through delivery systems ranging from low molecular weight (LMW) NO donors [27,43] to polymer-encapsulation methods [44,45]. The design of polymeric delivery systems of NO is especially important in creating specific release profiles differing in time span and load capacity, therefore, studying the various fabrication methods is vital. However, we believe our review must let our readers, not only with those with backgrounds in nanoparticle research but also with backgrounds in other various scientific fields, grasp what physiological effect NO can bring before delving into the specific chemistry. This flow of context would help people to better understand the concept of co-delivery of gasotransmitters, which we are stressing in the latter sections of this review. Accordingly, we will first go through prior studies that aimed for clinical benefits through the delivery of NO, focusing on their efforts to safeguard the delivery of NO. How they overcame the challenges of burst release and a short half-life for specific disease models is another focus discussed later in this chapter.

### 2.1. Cardiovascular disease and wound healing

Blood vessels are crucial in repairing and healing damaged tissues, and it is not surprising to see studies that aimed to promote the formation of blood vessels in overcoming various diseases. Cardiovascular disease is one of them [46], and applications include treating circulatory dysfunction, ischemia-reperfusion injury, thrombosis, and restenosis. Angiogenesis is the formation of new vessels from pre-existing vessels, which consists of several steps to create complete perfusable blood vessels. Once pro-angiogenic conditions such as hypoxia have been met, angiogenic factors including NO, angiopoietin, and vascular endothelial growth factor (VEGF) are expressed and act as up-regulators for angiogenesis. Each factor is necessary in different stages of this process, and NO is involved in the early stages of angiogenesis [47]. Vasodilation refers to the expanding of blood vessels through the relaxation of smooth muscle cells that are adjacent to blood vessels.

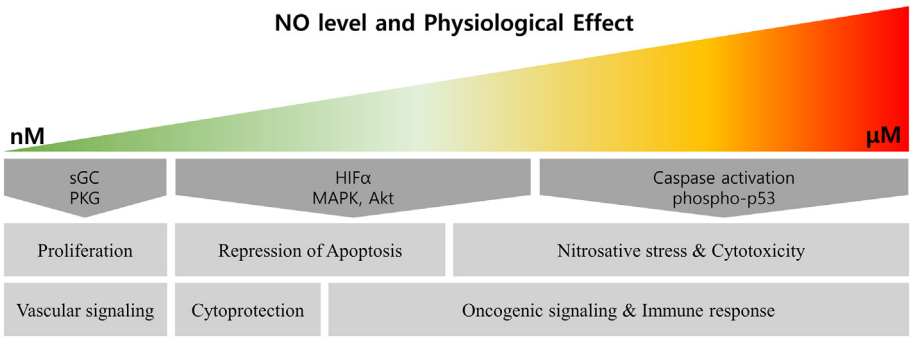


**Fig. 2.** Overview of biological functions of nitric oxide in various physiological, biochemical and pathological systems.

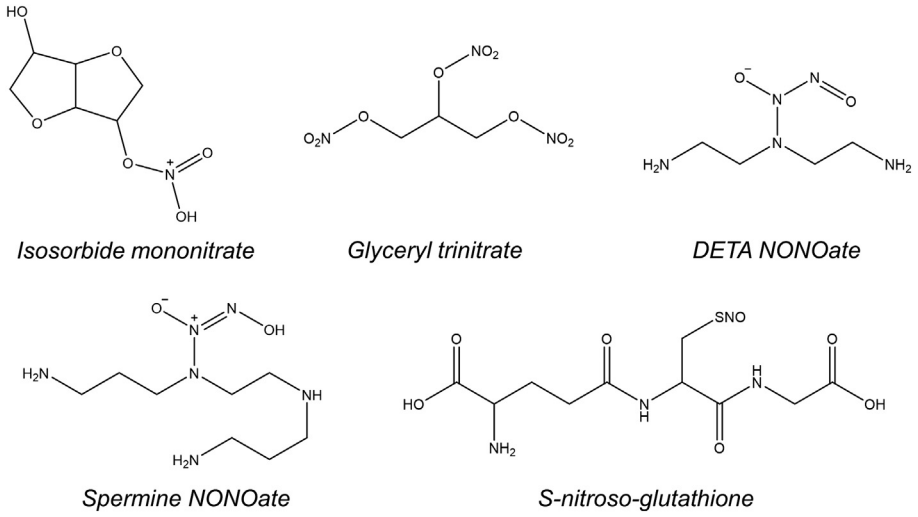


**Fig. 3.** Representative NO donors

Vasodilation starts when either VEGF or acetylcholine bind to its receptor on the endothelial cells. Then, eNOS produces NO radical species that penetrate the smooth muscle cell. The produced NO radicals result in an increase in intracellular calcium ions (through the L-type $Ca^{2+}$ channel), decreasing potassium ion levels (through ATP-sensitive potassium channel and voltage and $Ca^{2+}$ activated $K^+$ (*BK*) channels), ultimately resulting in the elevation of cell polarity enough to cause vasorelaxation [10,48]. Angiogenesis and vasodilation can be both stimulated by NO through shared ion channel-mediated pathways.

Since low concentrations of NO promote and sustain angiogenesis, the controlled release of NO is vital. As a result, LMW NO donors or NO pro-drugs were considered as cardiovascular therapeutic agents. However, their fast release of NO, hemoglobin scavenging properties and toxicity in humans limited their use in clinical settings. Polymer-based materials are alternative delivery platforms to treat cardiovascular disease. After surgical interventions such as angioplasty, stenting, or bypass grafting, restenosis is a major limitation for clinical success [49]. Following arterial restenosis, neointimal hyperplasia can narrow the arterial lumen. Given NO's known vasodilatory effect and its potential use in clinical applications, Do et al. prepared biodegradable microspheres encapsulating an NO donor using PEG/PLGA [50,51]. They loaded NO-microspheres in the channeled stent and showed that NO release led to the increase of cGMP levels, reducing levels of neointima. Johnson et al. reported the use of *S*-nitroso-*N*-acetyl penicillamine-derived generation-4 polyamidoamine dendrimers (G4-SNAP) as an ischemia-reperfusion injury treating agent [52]. These dendrimers have been shown to release NO following triggering by glutathione (GSH) (t[NO] = 1.28 μM NO/mg) with optimal concentrations of G4-SNAP being 230 pM.

### 2.2. Cancer therapy

NO plays an important role in cancer cell genotoxic mechanisms, anti-apoptotic effects, angiogenesis, limiting of host immune response against the tumor and promotion of metastasis [53]. Concentrated NO can induce apoptosis by suppressing cellular respiration and DNA synthesis, shifting iron metabolism, activating caspase family proteases, upregulating p53 and altering the expression of apoptosis-associated proteins, which can inhibit tumor metastasis and regression [54]. NO works as an antitumor agent in the human body and is a potential cancer chemo-treatment agent with or without the anti-cancer drug. Kumar et al. developed PEG-PLA block copolymer nanoparticles containing NO prodrugs, including PABA/NO ($O^2$-{2,4-dinitro-5-[4-(*N*-methylamino)benzoyloxy]phenyl} 1-(*N*,*N*-dimethylamino)diazen-1-ium-1,2-diolate) and Double JS-K (1,5-bis-{1-[(4-ethoxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diol-2-ato}-2,4-dinitrobenzene), as potential anticancer agents [55]. The PEGylated polymeric nanoparticle protects itself from glutathione, a NO prodrug activator. In particular, anticancer activities were enhanced in both human U937 myelomonocytic leukemia cells and H1703 non-small cell lung cancer cells upon NO treatment.

Triggering NO release by external sources such as changes in pH, heat, and NIR has been shown to also facilitate effective cancer treatments. By using a photosensitive NO donor, NO release materials can be designed for specific release at the site of interest. J. Garcia et al. developed and designed NIR-triggered NO release materials using upconverting nanoparticles (UCNPs) coated with a silica shell and the activated NO precursor [56]. pH-responsive injectable hollow microspheres (HM) have also been developed by M.F. Chung *et al.* (Fig. 5a) [57]. They encapsulated CPT-11 (the anticancer agent irinotecan) and DETA NONOate in PLGA HMs. The PLGA HMs generated NO bubbles for localized drug delivery and generated a P-glycoprotein(Pgp) mediated multidrug resistance (MDR) effect in cancer cells. At below pH 6.6, the MDR cells that were treated with NO-HMs had significantly lower levels of viability and had reduced Pgp expression levels following the large accumulation of intracellular CPT-11.

### 2.3. Antibacterial efficacy

Bacterial infections are major concerns arising from wounds and various diseases [58–60]. Bacterial infections impair the wound healing process as seen in chronic wounds resulting from diabetic foot ulcers [61]. NO plays a crucial role as an antibacterial agent against several types of bacteria and as participants of the intrinsic immune response [22]. NO exerts an antibacterial effect in two ways, depending on the concentration. At low concentrations, NO promotes the growth and activity of immune cells. At high concentrations, NO inhibits or kills target pathogens via the respiratory burst of a neutrophil and via the covalent bonding to DNA, proteins, and lipids [62]. To date, several NO-releasing materials made of silica, gold, liposomes, and dendrimers have been designed for antibacterial activity [63,64]. The Schoenfisch lab showed the use of NONOate functionalized-silica-nanorods to release 2000–14,000 ppb of NO per mg of particles for strong antibacterial efficacy against Gram-positive *Staphylococcus aureus* (*S. aureus*) and Gram-negative *Pseudomonas aeruginosa* (*P. aeruginosa*) [65]. When the same group evaluated the antibacterial efficacy of three NO-releasing silica nanoparticles, they revealed that the efficacy increased as the particle size decreased [66]. NO-releasing PLGA-PEI

nanoparticles from biodegradable polymers were also reported by H. Nurhasni et al. [64]. They developed NO-releasing particles using a combination of PLGA and PEI/diazeniumdiolate that showed a NO-releasing profile over six days in a sustained manner. The nanoparticles were also shown to be effective materials for killing against both methicillin-resistant (MRSA) and methicillin-sensitive (MSSA) *S. aureus*. Martinez et al. reported NO-releasing nanoparticles composed of tetramethylorthosilicate, polyethylene glycol, chitosan, glucose, and sodium nitrite for treating skin infections with efficacy against MRSA and MSSA [67].

## 3. Controlled release of nitric oxide via polymeric vehicles

Since NO-mediated action depends on its concentration, controlled NO-releasing materials are an essential consideration to make in developing clinically applicable materials. Sustained release is required for angiogenesis and healing (from pico- to nano-molar concentrations), antibacterial activity (from nano- to micro-molar concentrations), and cancer therapy (above micro-molar conditions). To date, small molecule NO donors have been developed and employed for each purpose. However, NO donors show a fast release profile and have been found to be incompatible for clinical applications. The polymeric carriers can compensate for such limitations and even provide better practicality since chemical modifications are readily possible [68,69]. Like existing drug delivery systems, biodegradable and biocompatible polymers have been applied as NO-releasing polymeric carriers (Table 1). Moreover, carriers can differentiate their shape, size, and labeling and loading strategies. In this section, we will discuss particles, micelles, vesicles with amphiphilic copolymers, star-shaped dendrimers and polymers of other various shapes, each with distinct advantages (Fig. 4). Polymeric carriers can also form a protective layer from early release trigger sources, conjugate the targeting moiety, and prolong circulation times and release periods. In the interest of public health, it is essential to treat specific diseases without side effects. Therefore, in drug research, stability issues and reducing side effects are prime considerations. To date, several stimuli-responsive polymeric carriers have been developed to overcome the limitations. Later in this section, stimuli-responsive carriers triggered exogenously (light and temperature) and endogenously (especially pH variations) will be discussed.

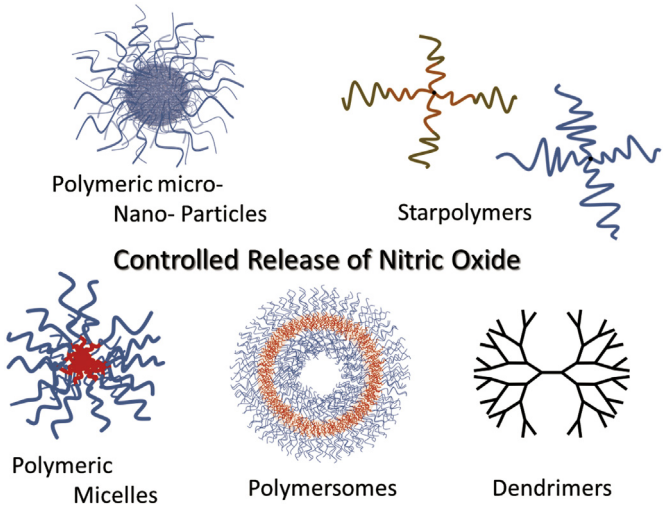


**Fig. 4.** Polymer-based vehicles for exogenous delivery of nitric oxide.

### 3.1. Polymeric nano- or microparticles, micelles and self-assembled vehicles

Polymeric carriers are strong candidates for NO-releasing vehicles since they are simple to prepare. Single- and double-emulsion methods are widely used for drug loading [70]. NO-releasing polymeric vehicles have two main kinds of NO loading strategies. One is by direct encapsulation of NO donors by polymer networks, and the other is by hydrophilic and -phobic interactions. Another production method of NO-releasing moiety in polymer chains includes the use of secondary amines via exposure to NO gas pressured at 5 atm. This owes to the fact that *N*-diazeniumdiolates binds to nucleophile adducts such as secondary amines [33]. In the case of RSNO, it is made from nitrosation of thiols in chemical reactions [71]. Thiol-containing materials can generate SNO moieties using $N_2O_4$, HNO, RONO, $NO_2$, $HNO_2$ in an aqueous phase (typically reacting with $NO_2^-$ generated from $NaNO_3$ in pH 2.0 of HCl solution) and tert-butyl nitrite (*t*-BuONO) in a non-aqueous phase [72,73].

The direct encapsulation of NO donors is an effective strategy given its simplicity, and there having been many studies exploring this topic.

**Table 1**
Summary of polymer-based NO-releasing vehicles.

| Materials | NO donating moiety | Formulation | Target model | Other considerations | Ref |
|---|---|---|---|---|---|
| PEG/PLGA | DETA NONOate | Microparticle | Restenosis | Increased cgmp levels<br>Intima-to-media ratio reduced | [51] |
| PAMAM | *S*-nitrosothiol modified | Dendrimer | Ischemia-reperfusion injury | GSH-initiated release | [92] |
| PEG-PLA | PABA NONOate and JS-K | Nanoparticle | Cancer treatment | Anti-cancer activities against human u937 and h1703 | [55] |
| PLGA | DETA NONOate | Hollow microsphere | Pgp mediated MDR | Injectable<br>Co-delivery of CPT-1 | [57] |
| PLGA-PEI | Diazeniumdiolate modified | Nanoparticle | Antibacterial agents | Effective against MRSA and MSSA | [64] |
| PLGA | DETA NONOate | Microparticle | FSAD | Target pH environment in vagina | [75] |
| PLGA | SNAP | Microparticle | – | Release period varied by changing capping functional groups | [76] |
| PAM-PAZd | Diazeniumdiolate modified | Micelle | – | Alteration of no-releasing polymer chain hydrophobicity | [79] |
| Oligoethylene glycol-methacrylate | GSNO | Micelle | – | Stability improvement | [80] |
| Phospholipids and cholesterol | NO gas | Liposome | Intimal hyperplasia | – | [86] |
| Dipalmitoylphosphatidylcholine | SPER NONOate and DPTA NONOate | Liposome | Cancer treatment | – | [87] |
| PPI | Diazeniumdiolate modified | Dendrimer | Antibacterial agents | Greatest biocidal activity (≥99.999% killing) | [89] |
| PEG-lysine | Diazeniumdiolate modified | Dendrimer | Vascular cell proliferation and inhibit platelet adhesion | Ligand-specific targeting of inflamed endothelium | [95] |
| P(OEGA)-based polymer | Diazeniumdiolate modified | Star polymer | Antibacterial agents | Enhanced dispersal of biofilms with non-toxicity to cell | [39] |
| POEGMA-PVBA | Diazeniumdiolate modified | Star polymer | Antibacterial agents | Synergistic effect with gentamicin | [40] |

Jeh et al. prepared biodegradable microparticles with PROLI NONOate encapsulated in it [74]. They used hydrophilic polymers PLGA and PELA to encapsulate the hydrophilic NO donor PROLI NONOate by double emulsion. The addition of gelatin enhanced the hydrophilic binding moiety between PLGA and PROLI NONOate. In another study, Yoo et al. developed NO-releasing PLGA microparticles for treatment of female sexual arousal disorder (FSAD) [75]. They encapsulated DETA NONOate in the PLGA microparticles by w/o/w and w/o/o double emulsion solvent evaporation that showed increased intracellular cGMP level in vaginal cells. Lautner et al. also reported PLGA microparticles with the NO donor, *S*-nitroso-*N*-acetyl-D-penicillamine (SNAP), encapsulated in the microparticles [76]. They used a solid-in-oil-in-water emulsion solvent evaporation method to prepare the PLGA microparticles. The PLGA microparticles showed a NO release profile of 10 days to 4 weeks dependent on the capping functional groups.

In addition to the direct encapsulation of NO donors, there has been much focus on using amphiphilic polymers to form concentration-dependent structures such as micelles, sheets, cylindrical micelles and vesicles through hydrophilic or hydrophobic interactions [77,78]. Although polymeric micelles efficiently produce hydrophilic drugs, it is difficult to encapsulate the hydrophobic core because of the aqueous bloodstream and the low encapsulation efficiency even when using the double emulsion method. In order to overcome these challenges, conjugating NO-donating moieties on polymer chains or encapsulating hydrophilic cores using vesicle structures has been investigated.

H. Nurhasni et al. prepared a NONOate moiety on a PEI secondary amine by charging the compound with NO gas at 80 psi for three days [64]. Then the NONOate modified PEI was mixed with PLGA to form nanoparticles through probe sonication. Jo et al. also reported a diazeniumdiolate-embedded block copolymer micelle [79]. The study designed pro-amphiphilic and amphiphilic block copolymers polymerized with *N*-acryloylmorpholine and *N*-acryloyl-2,5-dimethylpiperazine. The hydrophilic poly (*N*-acryloyl-2,5-dimethylpiperazine) (PAZd) changed into a hydrophobic poly (sodium-1-(*N*-acryloyl-2,5-dimethylpiperazin-1-yl) diazenium -diolate) (PAZd/NONOate) modified secondary amine by NO gas. The block copolymer, PAM-PAZd (poly (*N*-acryloyl morpholine)-b-poly (*N*-acryloyl-2,5-dimethylpiperazine), created well-formed micellar structures with a diameter of 50 nm. NO release from the micelle resulted in a 7-day half-life, supporting the hypothesis that the hydrophobic core protects NONOate from protons in the aqueous phase. Even though the copolymer micelles had a remarkable delayed half-life, it was not fully demonstrated that the conjugation of the hydrophilic diazaniumdiaolate could change its hydrophobicity. Duong et al. reported conjugation of GSNO into the hydrophobic core of micelle to enhance its stability (Fig. 5b) [80]. GSNO-conjugated micelles were prepared by copolymerizing oligoethylene glycol (OEG) and 2-vinyl-4,4-dimethyl-5-oxazolone (VDM) monomers, after which GSNO was then conjugated to a VDM segment. The micelles showed a NO release profile over 14 days, a significant improvement in stability. Similarly, Gao et al. developed a micelle platform for the sustained release of NO [81]. This work estabilished a relationship between polymer architecture and NO release kinetics. They grafted on amphiphilic copolymers, either mPEG-PLA or -D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), followed by conjugation of nitrate as a NO donor in the PHEMA backbone. Although TGPS-modified polymer micelles showed a delayed onset, they also showed a faster steady-state NO release than the counterpart.

Finally, polymers with hydrophilic and hydrophobic characteristics can form a vesicular structure in the shape of bilayers or interdigitated formations [82,83]. Polymersomes, a class of artificial vesicles formed by synthetic amphiphilic block copolymers, are a type of drug delivery system that shows much promise [84]. Vesicular vehicles are potentially handy materials for the exogenous delivery of NO, because they can quickly pass through cell membranes, are significantly stable and enable the dual loading of cargo [85]. In a study conducted by Huang et al., NO loaded echogenic liposomes were developed for the inhibition of intimal hyperplasia [86]. They prepared liposomes composed of phospholipids and cholesterols co-encapsulated with NO and Ar gas. The liposomes co-encapsulated with NO and Ar attenuated intimal hyperplasia in a balloon-injured artery. Suchyta et al. similarly reported the use of dipalmitoylphosphatidylcholine-based liposomes to enhance NO donor stability and delivery (Fig. 5c) [87]. NO release from liposomes was prolonged by the adjacent microenvironment formed by the human pancreatic cancer cells. The liposomes induced apoptosis on the human PANC-1 cell *in vitro*.

### 3.2. Dendrimers

Dendrimers are 3-dimensional architectures that consist of hyperbranched globular nanopolymers. Attractive features of such structures include their nanoscopic size, narrow polydisperse index and room for multiple functional groups at the periphery and within cavities [88]. *N*-diazeniumdiolate moiety dendrimers synthesized by the direct exposure of polymers containing secondary amines to NO gas have been investigated for NO release and delivery. Stasko et al., who were the first to report NO-releasing polypropyleneimine (PPI) dendrimers, prepared a generation of 3 and 5 PPI dendrimers (DAB-Am-16 and DAB-Am-64) modified with *N*-diazeniumdiolate [89]. The dendrimers exhibited NO storage up to 5.6 μmol NO/mg and NO release over 16 hours. Similarly, Lu et al. reported PPI dendrimers as NO releasing vehicles [90]. They synthesized structurally diverse NO-releasing- PPI dendrimers using a one-step manner using ring opening or conjugate addition reactions with one of following compounds: propylene oxide (PO), styrene oxide (SO), acrylonitrile (ACN), poly(ethylene glycol) methyl ether acrylate or 1,2-epoxy-9-decane (ED). PPI dendrimers G2-5 were converted to *N*-diazeniumdiolate by reaction with NO gas. Changing the exterior conjugation diversified release kinetics and storage capacity, strengthening the potential use of PPI dendrimers as antibacterial agents.

Sun et al. reported the use of NO-releasing PPI dendrimers as antibacterial agents against both Gram-positive and -negative pathogenic bacteria such as methicillin-resistant *Staphylococcus aureus* [91]. PPI dendrimers were functionalized with PEG or SO and a NO-donating moiety. PEG functionalized dendrimers exhibited various NO release kinetics and storage properties. NO functionalized PPI dendrimers showed enhanced biocidal action and reduced cytotoxicity against mammalian fibroblasts.

Another functionalized type of dendrimers, poly (amidoamine) (PAMAM) dendrimers, is widely studied for its feasibility as a NO-releasing *N*-diazeniumdiolate modification. Lu et al. reported the successful synthesis of NO-releasing PAMAM dendrimers with different exterior functionalities by reacting primary amines to either PO or 1,2-epoxy-9-decane (ED) [92]. Secondary amine species underwent modification to *N*-diazeniumdiolate following exposure to NO gas at 10 atm. Varying the ratio of exterior species could tune the hydrophilicity of dendrimers that inevitably changed the resulting NO release kinetics and total storage capacity. The resulting dendrimers exhibited significant antibacterial activity against Gram-negative *Pseudomonas aeruginosa* and low cytotoxicity on L929 mouse fibroblast cells. They concluded that the optimal exterior PO to ED ratio for biocidal and L929 mouse fibroblast cells were 7:3 and 5:5, respectively. They later revealed that tuning the alkyl chain and PAMAM dendrimers modified with *N*-diazeniumdiolate resulted in varying levels of antibacterial activity (Fig. 5d) [93,94]. PEG-based dendrimers can also be used as NO releasing carriers when their functional groups are modified [95]. Taite et al. synthesized dendrimers that had a bound lysine on the PEG central core, followed by forming of a NO donor moiety on their ends. The dendrimers released NO for a notably significant time (up to 60 days) under physiological conditions. The authors added that their dendrimers could be used as therapeutic agents by targeting the ligands of inflamed endothelium.

Dendrimers modified with NO-donating moieties other than *N*-diazeniumdiolate, such as *S*-nitrosothiol, nitrosyl, have also been

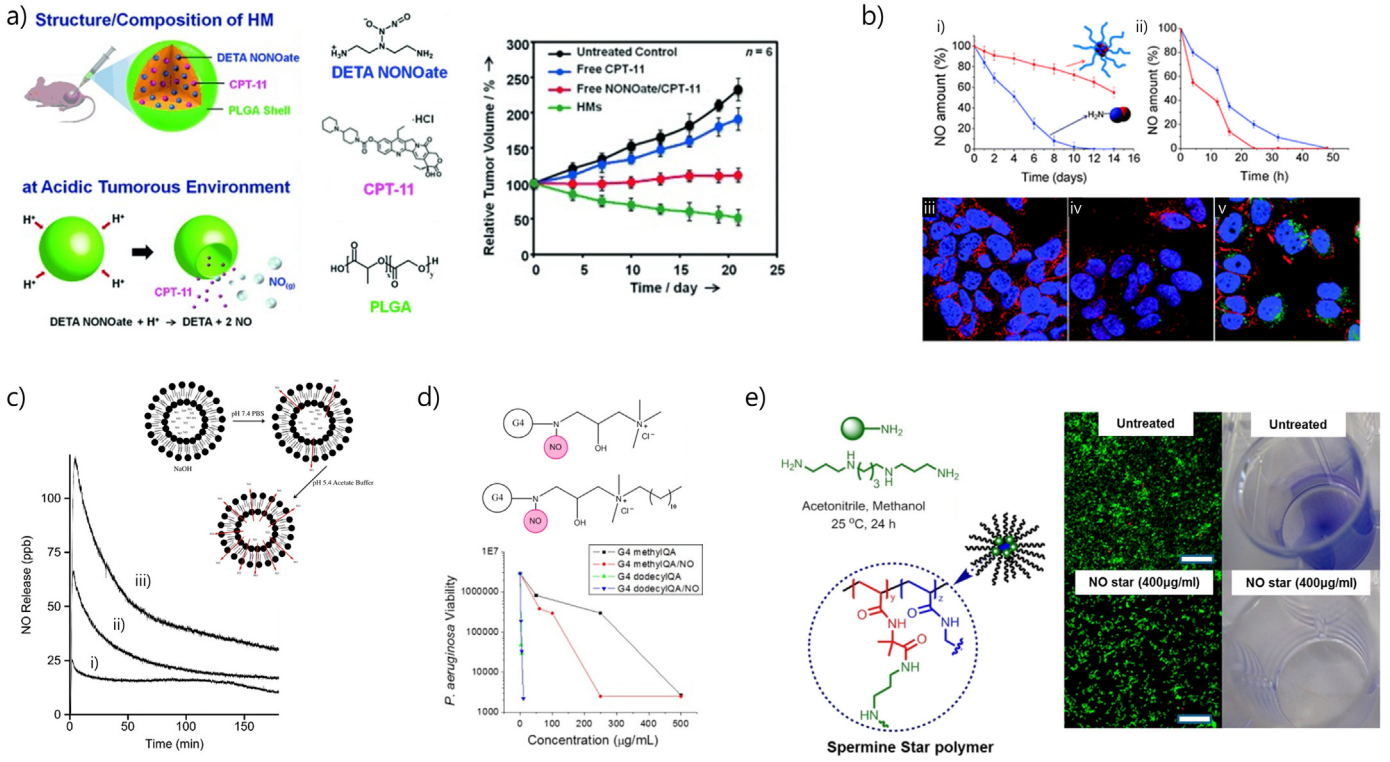


**Fig. 5.** NO release profiles and physiological effects for each NO carrier type. a) Schematic structure/composition of HMs and changes in relative tumor volume of mice with MCF-7/ADR tumors in response to HM treatments. b) Release profile of NO (red line) with NO-nanoparticles and (blue line) GSNO in different media: (i) in water and (ii) in water in the presence of ascorbic acid and Confocal microscopy of BE(2)-C: (iii) non-treated cells, (iv) treated cells with DAF-FM and (v) treated cells with NO-nanoparticles and DAF-FM. c) NO release profiles of various encapsulated NO donors. (i) DPTA/NO, (ii) DPTA/NO:SPER/NO and (iii) SPER/NO. d) Schematic chemical structure of NO-releasing PAMAM dendrimer and bactericidal effect. e) Illustration of NO-star polymer and confocal images live bacterial cells stained with SYTO 9. (Representative images reproduced with permission from Ref. a) [57] Wiley, b) [80] The Royal Society of Chemistry, c) [87], d) [94] and e) [39] American Chemical Society.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

researched [96–98]. G4 PAMAM dendrimers with *S*-nitroso-*N*-acetylpenicillamine (SNAP) were prepared by the cyclized reaction of *N*-acetyl-D,L-penicillamine with an exterior primary amine as reported by Johnson et al. [52]. The dendrimers were shown to reduce ischemia-reperfusion injury in a rat model. The release kinetics of NO triggered by 500 μM of GSH was t[NO] = 1.28 μM NO/mg. Similarly, Stasko et al. prepared generation 4 PAMAM dendrimers functionalized with *S*-nitrosothiol [99]. The PAMAM dendrimers were modified with either NAP or *N*-acetyl-L-cysteine (NACys). The release of NO was triggered by exposure to either light or copper. They demonstrated that the structure of tertiary or primary nitrosothiol renders a significant effect on NO release kinetics. Another NO-releasing carrier was reported by Roveda *et al.* via the functionalization of dendrimers using nytrosyl, another NO-donating group [100]. The release of NO for ruthenium nitrosyl complexes functionalized on PAMAM dendrimers was shown to be triggered by UV light (wavelength = 355 nm) or by chemical reactions. They prepared PAMAM dendrimers with ruthenium nitrosyl complexes in a one-step synthesis via peptide bonding between the carboxyl group of the isonicotinic acid ligand and the amine species on dendrimers. The PAMAM dendrimer G0, G2, and G3 released up to 1.43 μmol NO/mg of dendrimers.

### 3.3. Star-shaped polymers

Star-shaped polymers are composed of at least three linear polymeric chains bound by a single multi-branched core structure [101]. They are more stable than micelles and easier to produce than dendrimers. Duong et al. reported NO-releasing core-cross-linked star polymers containing *N*-diazeniumdiolate moieties (Fig. 5e) [39]. They prepared star polymers using reversible addition−fragmentation chain transfer (RAFT) polymerization via an arm first approach. The arms were extended in the presence of 2-vinyl-4,4-dimethyl-5-oxazolone monomer (VDM) with *N*, *N*-methylene-bis(acrylamide) as a crosslinker, from which spermine was attached to the core to yield core cross-linked star polymers. The *N*-diazeniumdiolate moieties were functionalized by reaction between the secondary amine group and NO gas. The resulting star polymers displayed a slow and controlled release of NO and antibacterial efficacy, which was demonstrated by the prevention of biofilm formation in *Pseudomonas aeruginosa*. The same group prepared NO and gentamicin co-delivery star polymers to reduce the formation of a *Pseudomonas aeruginosa* biofilm through a similar approach [40]. Gentamicin was conjugated to star polymers by reacting primary amines with the aldehyde on 3-vinylbenzylaldehyde (VBA). Since several amine groups exist in gentamicin, *N*-diazeniumdiolate moieties were readily formed with highly pressured NO gas. NO release from GEN-NO star followed first-order kinetics that exhibited a half-life time around 1 hour under physiological condition. They also confirmed the synergetic effects of both NO and gentamicin against *Pseudomonas aeruginosa* using a planktonic viability test. The NO-GEN star showed a strong killing effect compared to both spermine and free gentamicin.

Duan et al. also developed a multiarm poly (acrylic acid) star polymer with the purpose of delivering cisplatin and NO [102]. They synthesized a 4-arm star polymer using RAFT and macromolecular design via the interchange of xanthates (MADIX) polymerization [103]. The poly (acrylic acid) star polymer was water soluble and extremely fluid, suggesting it would be suitable for use as a targeted delivery system. Hydrophilicity and fluidity was also maintained from the conjugation of the star polymer using cisplatin, a hydrophilic first-line chemotherapy drug for specific cancers, and the NO prodrug, O2-(2,4-dinitrophenyl) 1-[4-(2-hydroxy) ethyl]-3-methyl piperazin-1-yl]

diazen-1-inum-1,2-diolate. Whereas the cisplatin exhibited zero-order kinetics, NO released from NO-star polymers showed first order kinetics, displaying a half-life of approximately 3.2 h. From the results, they implied the feasibility of cancer chemotherapy using the synergism of the fast release of NO and the slow release of cisplatin. They later reported that NO release multiarm star polymers were also considerably effective in anti-cancer chemotherapy [104]. They prepared 4-arm star sugar poly-(6-O-methacryloyl-D-galactose) as described above, with JS-K based NO analogs. The star polymer was water soluble, had a low PDI and had a viscosity suitable to be used as a chemotherapeutic agent. The multiarm star displayed superiority in inhibiting human head and neck squamous cell carcinoma (HNSCC) size growth *in vivo* compared to free JS-K and control.

### 3.4. Development of stimuli-responsive vehicles ranging from small molecule donors to polymers

At the forefront of tissue engineering and regenerative medicine, recent progress in drug delivery vehicles has had significant focus on stimuli-responsive materials chemistry. Stimuli-responsive vehicles can provide drug delivery and on-demand release at specific sites in a spatial-, temporal- and dosage- controlled manner [105]. Various stimuli can be used for switching the release of drugs on and off, such as pH, temperature, light, magnetic field, ultrasound or electric pulses [106–109]. These attractive points led to further research in the development of advanced polymers via smart chemistry, in hybrid polymer-proteins and in polymer-liposomes [110–111]. For the release of NO and other signaling molecules, stimuli-responsive carriers are ideal because of the importance of varying concentrations in biological contexts [112]. As a result, research has focused on specific triggers when tuning LMW NO donors or using stimuli-responsive materials.

For non-invasive and remote spatiotemporal control, the photo-response system is attractive in achieving on-demand drug release and therapy where the drugs or drug carriers respond to a specific wavelength (ultraviolet, visible or near-infrared). Either a one-time release or an on-off drug release system can be controlled by photosensitive modifications of nanocarriers. One example of designing NO donors for photosensitive NO release has been developed by Ullrich et al., where they developed linsidomine (3-morpholinosydnonimine, SIN-1) to release NO in response to light [113]. Linsidomine is an anti-anginal drug that acts as a vasodilator. They irradiated the samples with polychromic visible light for 9 hours, resulting in a 61% increase in nitrite formation compared to samples left in the dark. The concentration of oxygen also decreased by 2% after the 9-hour irradiation. These results show that the SIN-1 release of NO is oxygen-dependent and enhanced by visible light.

Additional visible light-triggered NO donors include studies by Sortino et al., *who* reported the development of light-controlled NO-release materials using flutamide (FM), an anticancer drug [114]. They found that FM led to the controlled release of NO upon light excitation (wavelength = 380 nm). Karaki et al. also developed visible-light-triggered organic caged-NOs using *N*-pyramidalized bicyclic nitrosamine derivatives [115]. Since the nitrosamine derivatives can absorb visible light, nitrosamines functioned as organic caged-NOs. They presented the stable bicyclic nitrosamine as a spatiotemporal NO releasing donor at room temperature and under regular sunlight because the N-NO bond can be cleaved by antibonding under visible light. Kanayama et al. reported PEGylated polymer micelles with a photo NO-donating moiety (Fig. 6c) [116]. They prepared PEG-*b*-PNTP copolymers and micelles by self-assembly. The report covered PEGylated polymer micelles containing 4-nitro-3-trifluoro-methylphenyl units that could generate NO following a photo-trigger.

In addition to photo-responsive drug delivery vehicles, drug delivery vehicles composed of thermo-responsive polymers can also provide local therapy using an exogenous heat source. The method has advantages using body temperature as a stimuli and has the added

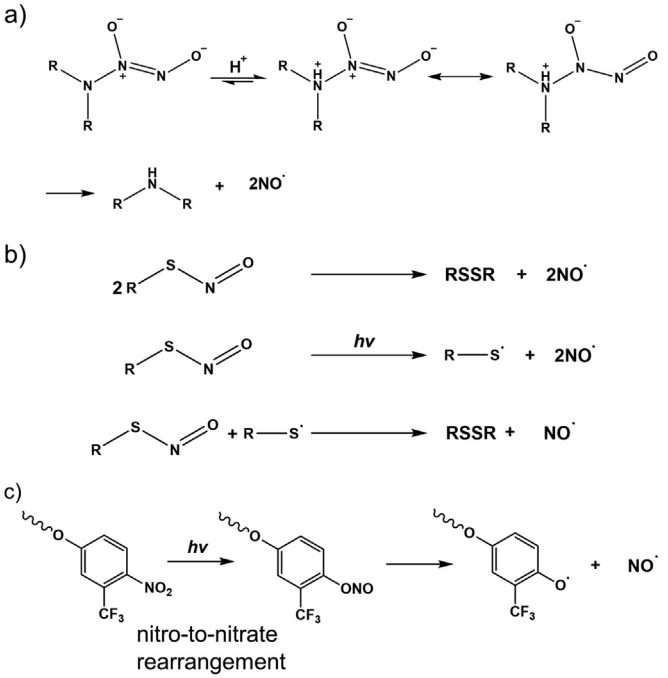


**Fig. 6.** Release mechanisms of representative NO donors. a) NONOate, hydrolysis by protonation, b) Decomposition of RSNO and c) Light triggered NO release by nitro-to-nitrate rearrangement.

possibility of localized treatment. Thermo-responsive systems range from poly (*N*-isopropyl acrylamide) (PNIPAM), which exhibits a lower critical solution temperature at 32 °C at which point the polymer transitions into its hydrophilic state; Poly (*N*-vinyl caprolactam); Poly (*N*-ethyl oxazoline); and thermo-sensitive peptides [106,117,118]. Usually, high temperatures damage and kill cancer cells with minimal injury to healthy tissue. Heat stress effects chemotherapy and ionizing radiation treatment by increasing intracellular drug uptake and intratumor drug concentrations, enhancing DNA damage and improving oxygenation due to increased blood flow [119]. Enhanced local NO production during hyperthermia treatments has been revealed by the previous report [120]. For this reason, researchers have endeavored to apply NO-release materials as an additive tool for enhancing the effect of hyperthermia, anticipating synergisms. Some studies have also revealed that supplementing NO alleviates hyperthermia when using NO donors such as molsidomine, Sodium nitroprusside (SNP), etc. [121,122].

The pH environment varies in organs, such as the gastrointestinal tract or the vagina, and within intracellular compartments, such as endosomes or lysosomes. Cancer microenvironments also display a relatively low pH in a phenomenon known as the Warburg effect [123,124]. Moreover, nanoparticles confront lower intracellular pH's compared to the extra-environment of cells when endocytosed. As a result, polymers with weakly acidic or basic residues can be utilized as pH-responsive features [125]. Weakly acidic and basic pendant groups accept protons and release them at low or high pH values. Poly acids or bases have been implemented as drug carriers, including in anti-cancer drugs such as doxorubicin, paclitaxel, and docetaxel [126]. Thus, pH-sensitive polymeric vehicles have been considered as useful tools for therapeutic applications. For the pH-dependent release of NO in various environments, research has focused on either tuning LMW NO donors or designing carriers containing pH-sensitive polymers. Most of NONOate-based NO donors such as PROLI-, PYRRO-, MAHMA-, DEA-, PAPA-, DPTA-, SPER-, DETA NONOates depend on the pH (< 8). These primary NO donors based on NONOates have a brief half-life even under physiological conditions. Protonation triggers the decomposition of $[NN(O)NO]^-$ group of NONOates with the apparent $pK_a$. Although the

O atoms in the functional group are the main site of protonation, only a few derivatives undergo heterotic cleavage of the N-N bond creating an amine and NO (Fig.6). For example, PROLI- and PYRRO NONOates exhibit a mere 2 and 3-second-long half-life, respectively. Although a high concentration of NO is required for cancer therapy, the molecules have to be specifically delivered at the desired site to prevent side effects and to optimize concentrated payload delivery. Therefore, the carriers should be designed to fulfill the necessary conditions including a long circulation time under physiological condition, as well as the selective and sensitive release of molecules at specific pH environments. An example includes the use of nitrophorins, which are hemeproteins containing NO found in saliva. The proteins bind to NO tightly at low pH and release NO at high pH. Swails et al. reported the pH-dependent mechanism of NO release from nitrophorin 2 and 4 [127]. They revealed a slower release of NO from nitrophorin 2 when compared to nitrophorin 4. The difference in release patterns has not been fully understood yet.

Additionally, the alteration of pH by NO donor or polymers should be considered when designing pH-sensitive carriers. Pravdic et al. reported effects of SNAP, SPER NONOate and propylamine NONOate on the intracellular pH of cardiomyocytes [128]. They investigated the effect of NO donors and NO-releasing polymers on intracellular pH when following the release of NO or degradation of the polymers. All NO donors decreased intracellular pH in cardiomyocytes. SNAP decreased basal intracellular pH from 7.09 ± 0.07 to 6.98 ± 0.08, and in the case of spermine NONOate from 7.11 ± 0.09 to 7.01 ± 0.08.

Most of the NO donors release NO directly upon encountering its trigger source, such as protons, light, heat and other biomolecules. However, these direct sources cannot guarantee target specificity nor adequate control of the amount of NO released. Using a double trigger to induce NO release has been proposed as a solution which could be realized by generating acid or heat, followed by a subsequent transfer of energy by light or heat. A few studies reported sequential stimuli-driven NO-release by using acid generating organic compounds (2-nitrobenzaldehyde) and NIR fluorescent materials [129,130]. Most RSNO compounds are thermally unstable, and their S-NO bond can be cleaved by thermal-, photo- or metal ion- initiation leading to NO release. For example, light can trigger homolytic S—N bond cleavage, generating NO and the corresponding thiyl radical. This property has facilitated the design of materials capable of NO release onto specific tissues *in situ* as photochemotherapeutic agents (Fig.6) [131]. S. M. Shishido et al. reported thermal and photochemical NO-releasing carriers containing S-nitrosothiols incorporated in Pluronic F127 gel [132]. The release of NO from GSNO and SNAC (*S*-nitroso-*N*-acetylcysteine) was activated by irradiation via UV/Vis light (wavelength = 336 nm and 545 nm). Due to the thermal gelation properties, NO was released in a controlled manner.

Recently, pH- and thermo- responsive *N*-diazeniumdiolated double-layered hollow P(AmEMA-*co*-EGDMA)/P(NIPAM-*co*-DAEMA-*co*-EGDMA) microspheres were reported by Liu *et al.* [133]. NO-releasing moieties were conjugated on P(bocAmEMA-*co*-EGDMA) hollow microspheres with a loading capacity of NO of 3.0 μmol/mg. The half-life of *N*-diazeniumdiolated microspheres ranged between 10 min to 50 min and 10 min to 400 min with varying temperature (20 °C–60 °C) and pH (4–11), respectively. The same group also reported on hollow nanoparticles functionalized with *S*-nitrosothiol [134]. The thiolated hollow polymeric nanoparticles are composed of ethyleneglycol dimethacylate (EGDMA) and 2-hydroxyl methacrylate (HEMA) and were prepared via distillation precipitation polymerization. The hydroxyl group was esterified using acryloyl chloride, and the surface thiol group was functionalized via nitrosation using acidified nitrite. The hollow polymer nanoparticles had a storage capacity of 1.55 μmol/mg and a decomposition ratio of 77% (1.2 μmol/mg) in PBS buffer, and the release profiles were performed using copper cation as a trigger source. Interestingly, these nanoparticles were less swollen in real bovine serum due to the simultaneous formation of *S*-nitrosothiol intermediates with endogenous thiols in proteins and amino acids. These studies provide a glimpse into the efforts to develop stimuli-responsive polymers that can locally release NO on demand for use in various clinical applications.

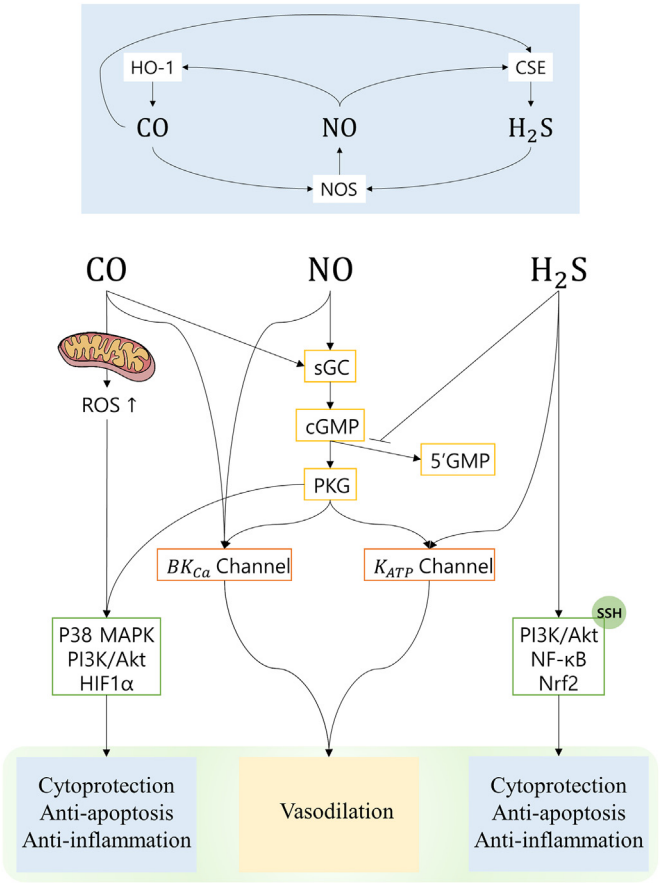


**Fig. 7.** The interplay of NO, CO and $H_2S$. Each Gasotransmitter regulates another gasotransmitter by up or down regulating its synthesizing enzyme (top). Key signaling pathways shared by NO, CO, and $H_2S$ are shown (bottom). CO can directly activate the BKca Channel or partake in the PKG pathway for vasodilation. A sudden increase in ROS production from mitochondria upregulates the signaling pathway via MAPK, Akt, or HIF1α. It can also activate the signaling pathway by HIF1α protein stabilization. NO mainly promotes vasodilation by the sGC/cGMP/PKG pathway. Like CO, NO can also directly activate the BKca channel for vasodilatory effect. $H_2S$ acts synergistically with NO by taking sGC into more NO-responsive form [135] or by inhibiting PDE that degrades cGMP. $H_2S$ activates the $K_{ATP}$ channel directly by sulfhydration, the same method it activates another signaling pathway to induce the cytoprotective effect.

## 4. Future perspectives on interactions of gasotransmitters

NO is not the only gasotransmitter that researchers have tried to deliver for therapeutic applications. Two other gasotransmitters, $H_2S$, and CO, have been discovered to have potential therapeutic applications in vasorelaxation and cytoprotection. Remarkably, $H_2S$ and CO share signaling pathways from which their physiological effects are derived (Fig. 7). This strongly suggests that the co-delivery of gasotransmitters would produce a synergistic effect; however, there is a lack of research in realizing this potential effect. In the following section, we briefly discuss recent works done in delivering each gasotransmitter, examine the shared signaling pathway of NO, $H_2S$ and CO, and provide insight on recent studies on the delivery of multiple gasotransmitters and its future direction.

### 4.1. Co-delivery system

NO, CO and $H_2S$ are intimately connected. Their synthesizing enzyme is regulated by the presence of other gasotransmitters and their signaling pathway in vasorelaxation and in anti-apoptotic and anti-inflammatory events is shared. However, studies in delivering more than a single gaseous molecule are scant; even the delivery of CO or $H_2S$ by polymeric vehicle is in its nascent state. Since NO has been spotlighted as a promising therapeutic agent, we believe that the two other gasotransmitters would greatly benefit the way clinical treatment is conducted. Here, we briefly summarize each background molecular mechanism for physiological action which is illustrated in Fig. 5. Then, we cover the recent advances in CO and $H_2S$ donor materials as well as their respective polymeric carrier designs and effects. Most importantly, we suggest the use of gasotransmitter co-delivery systems for various disease treatment methods as a topic for future scientific studies.

#### 4.1.1. Carbon monoxide

The use of carbon monoxide (CO) would not have been clinically tested without John Haldane's discovery of the binding of CO to hemoglobin [136]. CO is an endogenously generated gas which works in the formation of iron(II) and biliverdin. CO is rendered by haem oxygenase(HO); there exists three forms, two of which, the inducible form (HO-1) and one of the constitutive form(HO-2), have been studied [137–142], and the last (HO-3), which is still poorly understood. Once CO diffuses in the cell, it can increase cGMP by binding to guanylyl cyclase [143], ultimately leading to muscle cell relaxation [144,145]. This coincides with the mechanism that NO follows in triggering vasorelaxation, the sGC/GMP/PKG pathway. Like NO, CO administration results in the amelioration of vascular diseases, as shown in the work on pulmonary arterial hypertension [146,147]. Other pathways seem to account for the CO action in rendering an anti-apoptotic or anti-inflammatory effect [148]. This is accomplished by a partial increase in ROS in mitochondria, ultimately leading to the activation of various key signaling pathways such as the p38 MAPK pathway. The anti-inflammatory action is mediated by MAPK, Akt or c-Jun N-terminal kinase(JNK) pathways [149,150]. However, a slight increase in ROS by CO should be understood as the mean for initiating signal transduction. The cytoprotective effect, especially in renal transplants, against induced ischemia/reperfusion(I/R) injury is reported to be realized by CO's action upon cytochrome P450 by inhibiting toxic ROS formation [151–153]. Other than signaling pathways induced by ROS increase, CO also interacts with and stabilizes HIF1α, rendering the cytoprotective effect [154].

CO delivery by CO-releasing molecules (CORM) gained attention since direct CO administration was found to not be enough to gain stable and specific physiological effects (Fig. 8a). CO-bound metal carbonyl compounds have been highlighted the most since it showed the most promising effect in use for future clinical agents [155]. The Motterlini group developed CORM2, which is most widely used in clinical studies today, since unlike iron and manganese bound CO, CORM2 has been found to release CO spontaneously [156]. Later the same group prepared CORM3 by reacting CORM2 with glycine [157]. Like CORM2, CORM3 exerted physiological outcomes comparable to the direct inhalation of CO, including but not limited to vasodilatory, anti-inflammatory, anti-ischemic, and anti-apoptotic effects [157–160]. There are other diverse types of CORMs including photoCORM that require photolysis for CO release. This was again first introduced by Motterlini et al. [156], but several other groups and especially Schatzschneider's and Rimmer's groups introduced improved photoCORMs, $[Mn(CO)_3(tpm)]^+$ (tpm = tris(1-pyrazolyl)methane) and $[W(CO)_5(tppts)]^{3-}$ (tppts = tris(sulfonatophenyl)phosphine), respectively [161,162], although these photoCORMs require further toxicity studies. Recently, photoCORMs within the phototherapeutic window of 620nm

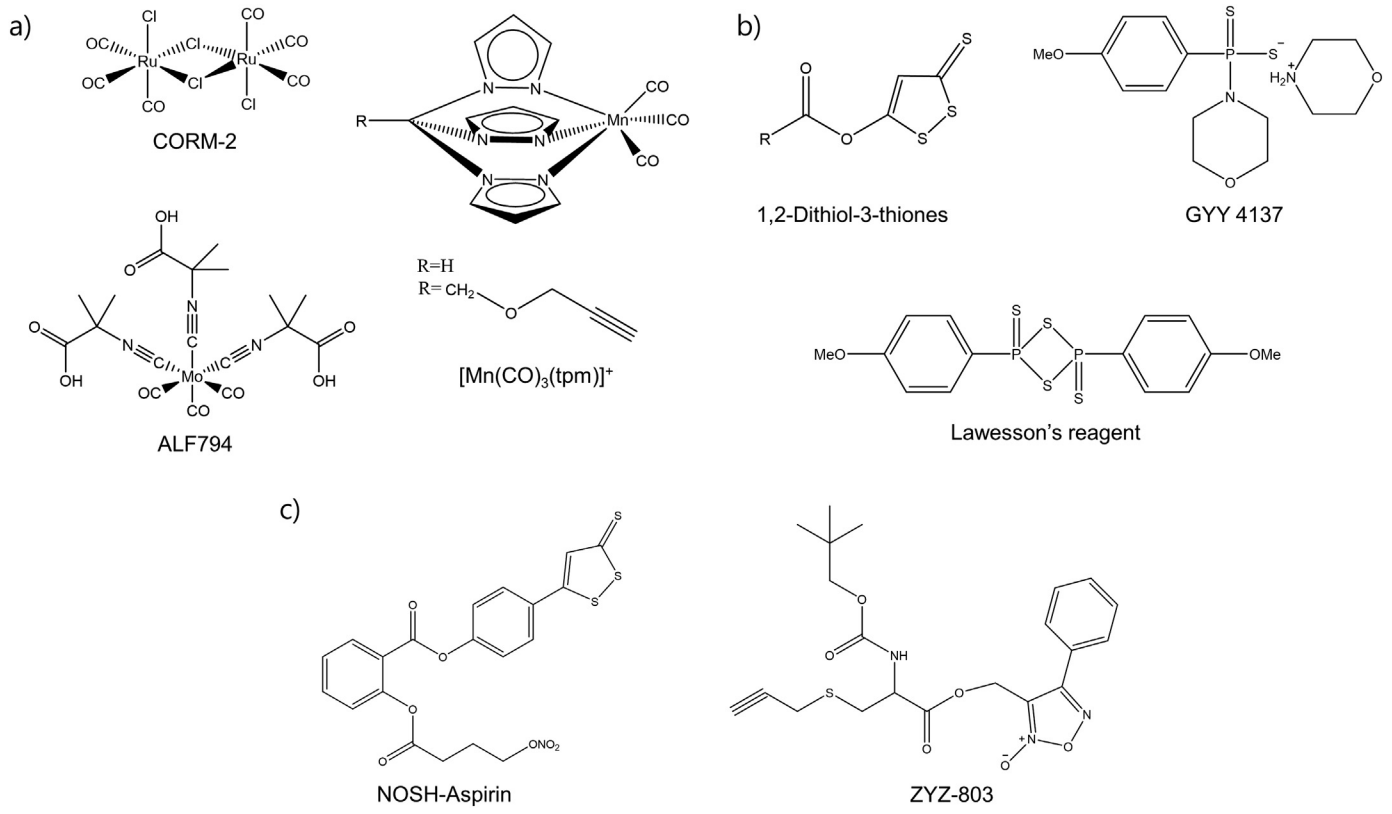


**Fig. 8.** Chemical structures of several reported CO or $H_2S$ releasing molecules. a) CO, b) $H_2S$ donors and c) NO and $H_2S$ releasing molecules reported by [201,202].

to 850nm with minimum toxicity have been introduced [163,164]. $(OC)_3Re(bby)(thp)^+$ (thp = tris(hydroxymethylphosphine, bby = bipyridine) [165], ALF794 [166] and $B_{12}$-MnCORM-1 [167] are such examples.

CORMs hold great potential for medical applications, however, they are limited by the restricted release control mechanisms and their low CO holding capacity [168], which has led to further studies in the polymeric delivery of CO to address such problems. Among several methods investigated, polymeric nanocarriers are the most promising. Kunz et al. were the first to develop CORM-copolymers in which *N*-(2-hydroxyproply) methacrylamide poly[(HPMA-co-bis(2-pyridylmethyl)-4-vinylbenzylamine) copolymers hold $Re(CO)_3$ via refluxing [169]. The CO-releasing profile was not provided in the results, but it became the basis for the study of new CO delivery methods, after which Brückmann et al. reported HPMA based, manganese tricarboyl moiety-attached copolymers that released CO upon photoactivation [170].

Micellar CO-releasing nanoparticles are the most potent polymeric carriers. Hesegawa et al. were the first to report micellar encapsulation of CORM in the triblock copolymer, poly[PEG-b-OrnRu-b-nBu], that releases CO only upon addition of thiol-compounds at a rate slower than CORM3 [171]. Other groups reported improved micellar CO-releasing polymers. Yin et al. reported poly(styrene-alt-maleic acid) copolymer (SMA) with CORM2 that provided specific anti-inflammatory effects and superior circulation times [172]. Pierri et al. went further by adding the temporal control of CO-releasing micelles by recently reporting on photoCORMs comprised of micelles that can trigger release upon NIR light. Other than micelles, the use of inorganic compounds such as silica nanoparticles as well as proteins has been investigated for effective CO release [173]. However, since the issue of biodegradability and fast clearance are still left to be overcome, these alternatives are less likely to be used in clinics. Nonetheless, the field of CO delivery for therapeutic applications, namely in anti-inflammation, is the new challenge. However, not much of the studies have been made in CO-releasing nanoparticles. Thus, much progress is necessary for the effective delivery of CO in clinical interventions.

#### 4.1.2. Hydrogen sulfide

Hydrogen sulfide ($H_2S$) most recently emerged as a gasotransmitter of interest among the trio mentioned in this review following the 1996 work by Kimura [9] that identified $H_2S$ as a neurological modulator. $H_2S$ is endogenously synthesized by at least the following compounds: cystaoninine β synthetase(CBS), cystathionine γ lyases (CSE) and cysteine aminotransferase (CAT) followed by 3-mercaptopyruvate sulfurtransferase(3-MST). $H_2S$ also shows vasorelaxative, anti-inflammatory, proliferative and cytoprotecting effects as seen in NO and CO [174]. $H_2S$ derived vasorelaxation and anti-inflammatory effects come from activating $K_{ATP,}$ possibly by interacting with its extracellular cysteine residues or by sulfhydration [175–178]. Such activations or inhibitions of ion channels stimulate signaling pathways leading to different physiological effects. Activation of the PI3K/Akt and MAPK pathway promotes proliferative effect, whereas NF-κB activation promotes an anti-inflammatory effect [179]. Other than in ion channel regulation, $H_2S$ has also been implicated as an antioxidant, increasing glutathioine levels and inducing Nrf2 expression [180,181].

Inorganic $H_2S$ donors, such as $Na_2S$ or NaHS, were the first $H_2S$ releasing agents studied (Fig. 8b) [182–185]. However, since they rapidly increase $H_2S$ levels and are short-lived, they invite the dangers of potential adverse side effects, since $H_2S$, like NO and CO, acts dependently on its concentration. The most widely used $H_2S$ donors are Lawessons' reagent derivatives, morpholin-4ium 4 methoxyphenyl (morpholino) phosphinodithiaoate (GYY4137) and 1,2-dithiole-3-thiones(DTT) [186]. They both lack temporal control in that they release $H_2S$ upon hydrolysis, but they have been proven to be effective in slowing the release profile and elongating the circulation time [186]. For GYY4137, low-level $H_2S$ was released for almost seven days in physiological conditions [187]. Temporal control by trigger agents can be derived by thiol-activated $H_2S$ donors [188–191] or photo-induced $H_2S$ donors [192], but cytotoxicity concerns prevent their use in versatile applications.

### 4.2. Beyond single actions through the crosstalk of gasotransmitters

There have been a few reports on polymeric agents releasing the combination of NO, CO and $H_2S$ when they share the biological signaling background of their actions. As mentioned before, NO's action mainly depends upon the activation of sGC thereby promoting cGMP production and activating PKG [193,194]. In the case of CO, its anti-inflammatory and vasorelaxation effect also comes from a cGMP-dependent pathway [136]. Although $H_2S$'s proliferative effect possibly entails the inhibition of the sGC/cGMP pathway, it inhibits phosphodiesterase-5 (PDE) which degrades existing cGMP, promoting the forward reaction of NO/CO rendered cGMP production [195–197]. Also, $H_2S$ can stimulate NOS by increasing phosphoactive form of NOS, eNOS-$P^{Ser1177}$, ultimately promoting NO production [198].

Additionally, reciprocal stimulation of $H_2S$ by NO has been reported where the NO donor increases CSE expression level [199]. There has also been a report that NOS deficiency inhibits $H_2S$'s angiogenic potential [200]. Although some studies report that $H_2S$'s pro-angiogenic effects are also regulated by NO-independent pathways, it seems clear that the polymeric release of both gasotransmitters will bolster angiogenesis or other physiological effects necessary for clinical applications [200].

Research progress has been made in the development of materials or polymers that can be used to release two or more gasotransmitters. R. Kodela et al. showed the use of hybrid NOSH compounds (aspirin bearing NO and $H_2S$ releasing moieties) as anti-inflammatory pharmaceuticals (Fig. 8c) [201]. The NOSH compounds were tested against human cancer cell lines (adenomatous, epithelial and lymphocytic) of six different tissue origins, and the compounds displayed comparable anti-inflammatory properties to aspirin. Likewise, Q. Hu et al. presented angiogenic activities induced by $H_2S$ and NO released from ZYZ-803, a slow-releasing $H_2S$-NO hybrid molecule (Fig. 8c) [202]. The molecule increased angiogenesis in *in vitro* rat aortic rings and in *in vivo* murine ischemic hindlimb models. From the results, two gasotransmitters can stimulate CSE expression and eNOS activity to produce $H_2S$ and NO. These reports mark the potential of cooperatively regulating the cell signaling pathway.

## 5. Conclusion

Research on gasotransmitters has undoubtedly broadened up our perspective by adding a new layer of knowledge in understanding complex biological systems. The most studied gasotransmitter, NO, acts by activating sGC and subsequently targeting protein kinases, rendering therapeutically desirable physiological effects including vasodilation and angiogenesis. Delivery of exogenous NO by donors and polymeric vehicles has also gained significant attention and accomplished notable feats. However, it is unfortunate that not much has been reported in delivering NO alongside with other gasotransmitters. Like any other critical molecules, NO, CO and $H_2S$ regulate physiological conditions through crosstalk, and the delivery of NO with either CO or $H_2S$ or with both could provide valuable insight into the mechanisms in which agents work together and also possibly create a magnified effect, such as the increased vasodilation by NO and $H_2S$ co-delivery. (Fig. 9). Delicate and precise measurements of gasotransmitter release from polymeric carriers is one of the many challenges that should be overcome. However, we are hopeful that the scientific progress and studies in the coming years will bring burgeoned insight onto gaseous signaling molecules. Ceaseless efforts to exhume mysteries of gasotransmitters, methods for better delivery, and optimal control would also improve human health through therapeutic applications.

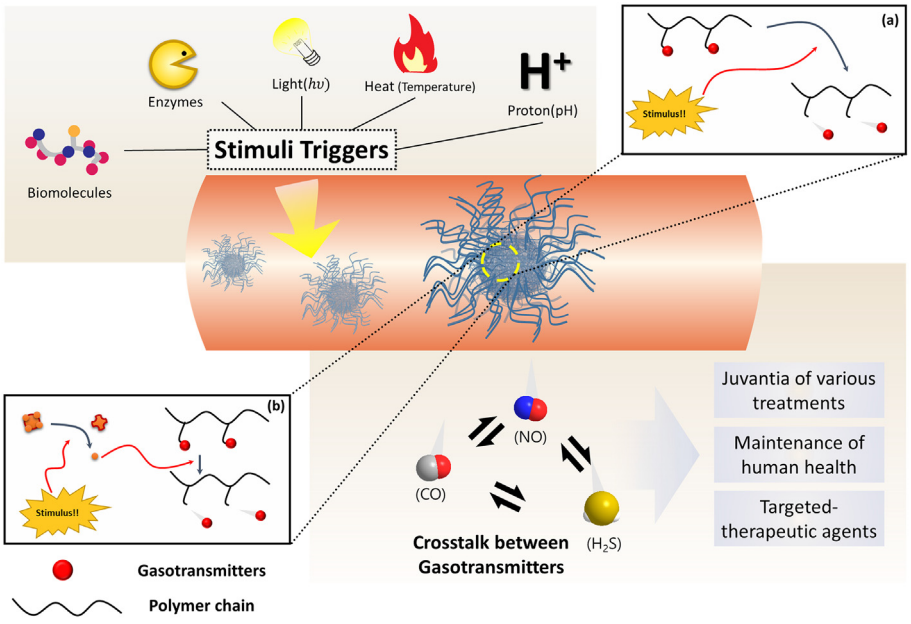


**Fig. 9.** Representation of multi-gasotransmitter releasing polymeric carriers. Nitric oxide, hydrogen sulfide, and carbon monoxide can be released from stimuli-responsive polymeric carriers triggered by external (light) or internal (biomolecules, enzymes, heat, and pH) stimuli. The triggering mechanisms of gasotransmitters are (a) direct and (b) indirect cleavage of gasotransmitter-polymer or donor bond.

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## References

[1] L.J. Ignarro, R.E. Byrns, G.M. Buga, K.S. Wood, Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical, Circ. Res. 61 (1987) 866–879.
[2] A.W. Carpenter, M.H. Schoenfisch, Nitric oxide release: Part II. Therapeutic applications, Chem. Soc. Rev. 41 (2012) 3742–3752.
[3] J.R. Steinert, T. Chernova, I.D. Forsythe, Nitric oxide signaling in brain function, dysfunction, and dementia, Neuroscientist 16 (2010) 435–452.
[4] M.T. Gewaltig, G. Kojda, Vasoprotection by nitric oxide: mechanisms and therapeutic potential, Cardiovasc. Res. 55 (2002) 250–260.
[5] R.M.J. Palmer, A.G. Ferrige, S. Moncada, Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor, Nature 327 (1987) 524–526.
[6] J.S. Stamler, D.J. Singel, J. Loscalzo, Biochemistry of nitric-oxide and its redox-activated forms, Science 258 (1992) 1898–1902.
[7] V. Bauer, R. Sotnikova, Nitric oxide - the endothelium-derived relaxing factor and its role in endothelial functions, Gen. Physiol. Biophys. 29 (2010) 319–340.
[8] G.S. Marks, J.F. Brien, K. Nakatsu, B.E. McLaughlin, Does carbon monoxide have a physiological function? Trends Pharmacol. Sci. 12 (1991) 185–188.
[9] K. Abe, H. Kimura, The possible role of hydrogen sulfide as an endogenous neuromodulator, J. Neurosci. 16 (1996) 1066–1071.
[10] C. Coletta, A. Papapetropoulos, K. Erdelyi, G. Olah, K. Módis, P. Panopoulos, A. Asimakopoulou, D. Gerö, I. Sharina, E. Martin, C. Szabo, Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation, Proc. Natl. Acad. Sci. 109 (2012) 9161–9166.
[11] S. Moncada, R.M.J. Palmer, E.A. Higgs, Biosynthesis of nitric oxide from l-arginine, Biochem. Pharmacol. 38 (1989) 1709–1715.
[12] S. Moncada, R.M.J. Palmer, E.A. Higgs, Nitric-oxide - physiology, Pathophysiol. Pharmacol. Pharmacol. Rev. 43 (1991) 109–142.
[13] R.M.J. Palmer, D.S. Ashton, S. Moncada, Vascular endothelial-cells synthesize nitric-oxide from L-arginine, Nature 333 (1988) 664–666.
[14] R.G. Knowles, S. Moncada, Nitric-oxide synthases in mammals, Biochem. J. 298 (1994) 249–258.
[15] W.P. Arnold, C.K. Mittal, S. Katsuki, F. Murad, Nitric-oxide activates guanylate cyclase and increases guanosine 3'-5'-cyclic monophosphate levels in various tissue preparations, Proc. Natl Acad. Sci. USA 74 (1977) 3203–3207.
[16] S.P.L. Cary, J.A. Winger, E.R. Derbyshire, M.A. Marletta, Nitric oxide signaling: no longer simply on or off, Trends Biochem. Sci. 31 (2006) 231–239.
[17] D.A. Wink, Y. Vodovotz, J. Laval, F. Laval, M.W. Dewhirst, J.B. Mitchell, The multifaceted roles of nitric oxide in cancer, Carcinogenesis 19 (1998) 711–721.
[18] A. Villalobo, Nitric oxide and cell proliferation, FEBS J. 273 (2006) 2329–2344.
[19] D. Hirst, T. Robson, Nitric oxide in cancer therapeutics: interaction with cytotoxic chemotherapy, Curr. Pharm. Design. 16 (2010) 411–420.
[20] B. Kuzin, I. Roberts, N. Peunova, G. Enikolopov, Nitric oxide regulates cell proliferation during Drosophila development, Cell 87 (1996) 639–649.
[21] A. Ramamurthi, R.S. Lewis, Nitric oxide inhibition of platelet deposition on biomaterials, Biomed. Sci. Instrum. 35 (1999) 333–338.
[22] E.M. Hetrick, M.H. Schoenfisch, Reducing implant-related infections: active release strategies, Chem. Soc. Rev. 35 (2006) 780–789.
[23] E. Troncy, M. Francœur, G. Blaise, Inhaled nitric oxide: clinical applications, indications, and toxicology, Can. J. Anaesth. 44 (1997) 973–988.
[24] Y. Qian, J.B. Matson, Gasotransmitter delivery via self-assembling peptides: treating diseases with natural signaling gases, Adv. Drug Deliv. Rev. 110 (2017) 137–156.
[25] R. Temple, N.L. Stockbridge, Bidil for heart failure in black patients: the u.s. food and drug administration perspective, Ann. Intern. Med. 146 (2007) 57–62.
[26] H. Brody, L.M. Hunt, BiDil: assessing a race-based pharmaceutical, Ann. Fam. Med. 4 (2006) 556–560.
[27] L.K. Keefer, Fifty years of diazeniumdiolate research. From laboratory curiosity to broad-spectrum biomedical advances, ACS Chem. Biol. 6 (2011) 1147–1155.
[28] N. Naghavi, A. de Mel, O.S. Alavijeh, B.G. Cousins, A.M. Seifalian, Nitric oxide donors for cardiovascular implant applications, Small 9 (2013) 22–35.
[29] P.G. Parzuchowski, M.C. Frost, M.E. Meyerhoff, Synthesis and characterization of polymethacrylate-based nitric oxide donors, J. Am. Chem. Soc. 124 (2002) 12182–12191.
[30] P.G. Wang, M. Xian, X. Tang, X. Wu, Z. Wen, T. Cai, A.J. Janczuk, Nitric oxide donors: chemical activities and biological applications, Chem. Rev. 102 (2002) 1091–1134.
[31] J.N. Cohn, G.T. McInnes, A.M. Shepherd, Direct-acting vasodilators, J. Clin. Hypertens. 13 (2011) 690–692.
[32] M.R. Miller, I.L. Megson, Review - recent developments in nitric oxide donor drugs, Br. J. Pharmacol. 151 (2007) 305–321.
[33] J.A. Hrabie, L.K. Keefer, Chemistry of the nitric oxide-releasing diazeniumdiolate ("nitrosohydroxylamine") functional group and its oxygen-substituted derivatives, Chem. Rev. 102 (2002) 1135–1154.
[34] E. Rivera-Tirado, M. Lopez-Casillas, C. Wesdemiotis, Characterization of diazeniumdiolate nitric oxide donors (NONOates) by electrospray ionization mass spectrometry, Rapid Commun. Mass. Sp. 25 (2011) 3581–3586.
[35] R.J. Singh, N. Hogg, J. Joseph, B. Kalyanaraman, Mechanism of nitric oxide release from *S*-nitrosothiols, J. Biol. Chem. 271 (1996) 18596–18603.
[36] K.A. Broniowska, N. Hogg, The chemical biology of *S*-nitrosothiols, Antioxid. Redox Signal. 17 (2012) 969–980.
[37] G. Richardson, N. Benjamin, Potential therapeutic uses for *S*-nitrosothiols, Clin. Sci. 102 (2002) 99–105.
[38] N.S. Bryan, T. Rassaf, J. Rodriguez, M. Feelisch, Bound NO in human red blood cells: fact or artifact? Nitric Oxide 10 (2004) 221–228.
[39] H.T.T. Duong, K. Jung, S.K. Kutty, S. Agustina, N.N.M. Adnan, J.S. Basuki, N. Kumar, T.P. Davis, N. Barraud, C. Boyer, Nanoparticle (star polymer) delivery of nitric oxide effectively negates *Pseudomonas aeruginosa* biofilm formation, Biomacromolecules 15 (2014) 2583–2589.
[40] T.K. Nguyen, R. Selvanayagam, K.K.K. Ho, R.X. Chen, S.K. Kutty, S.A. Rice, N. Kumar, N. Barraud, H.T.T. Duong, C. Boyer, Co-delivery of nitric oxide and antibiotic using polymeric nanoparticles, Chem. Sci. 7 (2016) 1016–1027.

[41] D.A. Riccio, M.H. Schoenfisch, Nitric oxide release: Part I. Macromolecular scaffolds, Chem. Soc. Rev. 41 (2012) 3731–3741.
[42] T. Ramasamy, H.B. Ruttala, B. Gupta, B.K. Poudel, H.-G. Choi, C.S. Yong, J.O. Kim, Smart chemistry-based nanosized drug delivery systems for systemic applications: a comprehensive review, J. Control. Release 258 (2017) 226–253.
[43] A.B. Seabra, G.Z. Justo, P.S. Haddad, State of the art, challenges and perspectives in the design of nitric oxide-releasing polymeric nanomaterials for biomedical applications, Biotechnol. Adv. 33 (2015) 1370–1379.
[44] M.C. Jen, M.C. Serrano, R. van Lith, G.A. Ameer, Polymer-based nitric oxide therapies: recent insights for biomedical applications, Adv. Funct. Mater. 22 (2012) 239–260.
[45] J.F. Quinn, M.R. Whittaker, T.P. Davis, Delivering nitric oxide with nanoparticles, J. Control. Release 205 (2015) 190–205.
[46] M. Simons, J.A. Ware, Therapeutic angiogenesis in cardiovascular disease, Nat. Rev. Drug Discov. 2 (2003) 863–872.
[47] C. Clapp, S. Thebault, M.C. Jeziorski, G.M. De la Escalera, Peptide hormone regulation of angiogenesis, Physiol. Rev. 89 (2009) 1177–1215.
[48] M. Parent, A. Boudier, I. Fries, A. Gostyńska, M. Rychter, J. Lulek, P. Leroy, C. Gaucher, Nitric oxide-eluting scaffolds and their interaction with smooth muscle cells in vitro, J. Biomed. Mater. Res. A 103 (2015) 3303–3311.
[49] A.W. Clowes, M.M. Clowes, Kinetics of cellular proliferation after arterial injury. 2. Inhibition of smooth-muscle growth by heparin, Lab. Investig. 52 (1985) 611–616.
[50] R.F. Furchgott, J.V. Zawadzki, The obligatory role of endothelial-cells in the relaxation of arterial smooth-muscle by acetylcholine, Nature 288 (1980) 373–376.
[51] Y.S. Do, E.Y. Kao, F. Ganaha, H. Minamiguchi, K. Sugimoto, J. Lee, C.J. Elkins, P.G. Amabile, M.D. Kuo, D.S. Wang, J.M. Waugh, M.D. Dake, In-stent restenosis limitation with stent-based controlled-release nitric oxide: Initial results in rabbits, Radiology 230 (2004) 377–382.
[52] T.A. Johnson, N.A. Stasko, J.L. Matthews, W.E. Cascio, E.L. Holmuhamedov, C.B. Johnson, M.H. Schoenfisch, Reduced ischemia/reperfusion injury via glutathione-initiated nitric oxide-releasing dendrimers, Nitric Oxide 22 (2010) 30–36.
[53] S.K. Choudhari, M. Chaudhary, S. Bagde, A.R. Gadbail, V. Joshi, Nitric oxide and cancer: a review, World J. Surg. Oncol. 11 (2013) 118.
[54] D. Fukumura, S. Kashiwagi, R.K. Jain, The role of nitric oxide in tumour progression, Nat. Rev. Cancer 6 (2006) 521–534.
[55] V. Kumar, S.Y. Hong, A.E. Maciag, J.E. Saavedra, D.H. Adamson, R.K. Prud'homme, L.K. Keefer, H. Chakrapani, Stabilization of the nitric oxide (NO) prodrugs and anticancer leads, PABA/NO and Double JS-K, through incorporation into PEG-protected nanoparticles, Mol. Pharm. 7 (2010) 291–298.
[56] J.V. Garcia, J.P. Yang, D.K. Shen, C. Yao, X.M. Li, R. Wang, G.D. Stucky, D.Y. Zhao, P.C. Ford, F. Zhang, NIR-triggered release of caged nitric oxide using upconverting nanostructured materials, Small 8 (2012) 3800–3805.
[57] M.F. Chung, H.Y. Liu, K.J. Lin, W.T. Chia, H.W. Sung, A pH-responsive carrier system that generates NO bubbles to trigger drug release and reverse P-glycoprotein-mediated multidrug resistance, Angew. Chem. Int. Ed. 54 (2015) 9890–9893.
[58] D.M. Morens, G.K. Folkers, A.S. Fauci, The challenge of emerging and re-emerging infectious diseases, Nature 430 (2004) 242–249.
[59] M.J. Hajipour, K.M. Fromm, A. Akbar Ashkarran, D. Jimenez de Aberasturi, I.R.d. Larramendi, T. Rojo, V. Serpooshan, W.J. Parak, M. Mahmoudi, Antibacterial properties of nanoparticles, Trends Biotechnol. 30 (2012) 499–511.
[60] M. Qasim, D.J. Lim, H. Park, D. Na, Nanotechnology for diagnosis and treatment of infectious diseases, J. Nanosci. Nanotechnol. 14 (2014) 7374–7387.
[61] V. Falanga, Wound healing and its impairment in the diabetic foot, Lancet 366 (2005) 1736–1743.
[62] P. Tripathi, P. Tripathi, L. Kashyap, V. Singh, C. Bogdan, The role of nitric oxide in inflammatory reactions (Retraction of vol 51, pg 443, 2007), FEMS Immunol. Med. Microbiol. 66 (2012) 449.
[63] W. Cai, J. Wu, C. Xi, M.E. Meyerhoff, Diazeniumdiolate-doped poly(lactic-co-glycolic acid)-based nitric oxide releasing films as antibiofilm coatings, Biomaterials 33 (2012) 7933–7944.
[64] H. Nurhasni, J.F. Cao, M. Choi, I. Kim, B.L. Lee, Y.J. Jung, J.W. Yoo, Nitric oxide-releasing poly(lactic-co-glycolic acid)-polyethylenimine nanoparticles for prolonged nitric oxide release, antibacterial efficacy, and in vivo wound healing activity, Int. J. Nanomedicine 10 (2015) 3065–3080.
[65] Y. Lu, D.L. Slomberg, B. Sun, M.H. Schoenfisch, Shape- and nitric oxide flux-dependent bactericidal activity of nitric oxide-releasing silica nanorods, Small 9 (2013) 2189–2198.
[66] D.L. Slomberg, Y. Lu, A.D. Broadnax, R.A. Hunter, A.W. Carpenter, M.H. Schoenfisch, Role of size and shape on biofilm eradication for nitric oxide-releasing silica nanoparticles, ACS Appl. Mater. Interfaces 5 (2013) 9322–9329.
[67] L.R. Martinez, G. Han, M. Chacko, M.R. Mihu, M. Jacobson, P. Gialanella, A.J. Friedman, J.D. Nosanchuk, J.M. Friedman, Antimicrobial and healing efficacy of sustained release nitric oxide nanoparticles against *Staphylococcus aureus* skin infection, J. Investig. Dermatol. 129 (2009) 2463–2469.
[68] M. Komiyama, K. Yoshimoto, M. Sisido, K. Ariga, Chemistry can make strict and fuzzy controls for bio-systems: DNA nanoarchitectonics and cell-macromolecular nanoarchitectonics, Bull. Chem. Soc. Jpn. 90 (2017) 967–1004.
[69] A. Mandal, R. Bisht, I.D. Rupenthal, A.K. Mitra, Polymeric micelles for ocular drug delivery: from structural frameworks to recent preclinical studies, J. Control. Release 248 (2017) 96–116.
[70] J.A. Hanson, C.B. Chang, S.M. Graves, Z.B. Li, T.G. Mason, T.J. Deming, Nanoscale double emulsions stabilized by single-component block copolypeptides, Nature 455 (2008) (85-U54).
[71] D.L.H. Williams, The chemistry of *S*-nitrosothiols, Acc. Chem. Res. 32 (1999) 869–876.
[72] K. Goto, Y. Hino, T. Kawashima, M. Kaminaga, E. Yano, G. Yamamoto, N. Takagi, S. Nagase, Synthesis and crystal structure of a stable *S*-nitrosothiol bearing a novel steric protection group and of the corresponding *S*-nitrothiol, Tetrahedron Lett. 41 (2000) 8479–8483.
[73] M.D. Bartberger, K.N. Houk, S.C. Powell, J.D. Mannion, K.Y. Lo, J.S. Stamler, E.J. Toone, Theory, spectroscopy, and crystallographic analysis of *S*-nitrosothiols: conformational distribution dictates spectroscopic behavior, J. Am. Chem. Soc. 122 (2000) 5889–5890.
[74] H.S. Jeh, S. Lu, S.C. George, Encapsulation of PROLI/NO in biodegradable microparticles, J. Microencapsul. 21 (2004) 3–13.
[75] J.W. Yoo, J.S. Lee, C.H. Lee, Characterization of nitric oxide-releasing microparticles for the mucosal delivery, J. Biomed. Mater. Res. A 92a (2010) 1233–1243.
[76] G. Lautner, M.E. Meyerhoff, S.P. Schwendeman, Biodegradable poly(lactic-co-glycolic acid) microspheres loaded with *S*-nitroso-*N*-acetyl-D-penicillamine for controlled nitric oxide delivery, J. Control. Release 225 (2016) 133–139.
[77] S.M. Shishido, A.B. Seabra, W. Loh, M.G. de Oliveira, Thermal and photochemical nitric oxide release from *S*-nitrosothiols incorporated in pluronic F127 gel: potential uses for local and controlled nitric oxide release, Biomaterials 24 (2003) 3543–3553.
[78] J. Kim, G. Saravanakumar, H.W. Choi, D. Park, W.J. Kim, A platform for nitric oxide delivery, J. Mater. Chem. B 2 (2014) 341–356.
[79] Y.S. Jo, A.J. van der Vlies, J. Gantz, T.N. Thacher, S. Antonijevic, S. Cavadini, D. Demurtas, N. Stergiopulos, J.A. Hubbell, Micelles for delivery of nitric oxide, J. Am. Chem. Soc. 131 (2009) 14413–14418.
[80] H.T.T. Duong, Z.M. Kamarudin, R.B. Erlich, Y. Li, M.W. Jones, M. Kavallaris, C. Boyer, T.P. Davis, Intracellular nitric oxide delivery from stable NO-polymeric nanoparticle carriers, Chem. Commun. 49 (2013) 4190–4192.
[81] M. Gao, S.H. Liu, A.P. Fan, Z. Wang, Y.J. Zhao, Nitric oxide-releasing graft polymer micelles with distinct pendant amphiphiles, RSC Adv. 5 (2015) 67041–67048.
[82] D.E. Discher, A. Eisenberg, Polymer vesicles, Science 297 (2002) 967.
[83] B.M. Discher, Y.-Y. Won, D.S. Ege, J.C.M. Lee, F.S. Bates, D.E. Discher, D.A. Hammer, Polymersomes: tough vesicles made from diblock copolymers, Science 284 (1999) 1143.
[84] J.S. Lee, J. Feijen, Polymersomes for drug delivery: design, formation and characterization, J. Control. Release 161 (2012) 473–483.
[85] R. Bleul, R. Thiermann, M. Maskos, Techniques to control polymersome size, Macromolecules 48 (2015) 7396–7409.
[86] S.-L. Huang, P.H. Kee, H. Kim, M.R. Moody, S.M. Chrzanowski, R.C. MacDonald, D.D. McPherson, Nitric oxide-loaded echogenic liposomes for nitric oxide delivery and inhibition of intimal hyperplasia, J. Am. Coll. Cardiol. 54 (2009) 652–659.
[87] D.J. Suchyta, M.H. Schoenfisch, Encapsulation of N-diazeniumdiolates within liposomes for enhanced nitric oxide donor stability and delivery, Mol. Pharm. 12 (2015) 3569–3574.
[88] P. Kesharwani, K. Jain, N.K. Jain, Dendrimer as nanocarrier for drug delivery, Prog. Polym. Sci. 39 (2014) 268–307.
[89] N.A. Stasko, M.H. Schoenfisch, Dendrimers as a scaffold for nitric oxide release, J. Am. Chem. Soc. 128 (2006) 8265–8271.
[90] Y. Lu, B. Sun, C.H. Li, M.H. Schoenfisch, Structurally diverse nitric oxide-releasing poly(propylene imine) dendrimers, Chem. Mater. 23 (2011) 4227–4233.
[91] B. Sun, D.L. Slomberg, S.L. Chudasama, Y. Lu, M.H. Schoenfisch, Nitric oxide-releasing dendrimers as antibacterial agents, Biomacromolecules 13 (2012) 3343–3354.
[92] Y. Lu, D.L. Slomberg, A. Shah, M.H. Schoenfisch, Nitric oxide-releasing amphiphilic poly(amidoamine) (PAMAM) dendrimers as antibacterial agents, Biomacromolecules 14 (2013) 3589–3598.
[93] B.V. Worley, K.M. Schilly, M.H. Schoenfisch, Anti-biofilm efficacy of dual-action nitric oxide-releasing alkyl chain modified poly(amidoamine) dendrimers, Mol. Pharm. 12 (2015) 1573–1583.
[94] B.V. Worley, D.L. Slomberg, M.H. Schoenfisch, Nitric oxide-releasing quaternary ammonium-modified poly(amidoamine) dendrimers as dual action antibacterial agents, Bioconjug. Chem. 25 (2014) 918–927.
[95] L.J. Taite, J.L. West, Poly(ethylene glycol)-lysine dendrimers for targeted delivery of nitric oxide, J. Biomat. Sci.-Polym. E 17 (2006) 1159–1172.
[96] P.G.Z. Benini, B.R. McGarvey, D.W. Franco, Functionalization of PAMAM dendrimers with [RuIII(edta)(H2O)] −, Nitric Oxide 19 (2008) 245–251.
[97] A.B. Seabra, N. Duran, Nitric oxide-releasing vehicles for biomedical applications, J. Mater. Chem. 20 (2010) 1624–1637.
[98] A.C. Roveda, D.W. Franco, Nitric oxide releasing-dendrimers: an overview, Braz. J. Pharm. 49 (Sci) (2013) 1–14.
[99] N.A. Stasko, T.H. Fischer, M.H. Schoenfisch, *S*-nitrosothiol-modified dendrimers as nitric oxide delivery vehicles, Biomacromolecules 9 (2008) 834–841.
[100] A.C. Roveda Jr., T.B.R. Papa, E.E. Castellano, D.W. Franco, PAMAM dendrimers functionalized with ruthenium nitrosyl as nitric oxide carriers, Inorg. Chim. Acta 409 (Part A) (2014) 147–155.
[101] S. Bywater, Preparation and properties of star-branched polymers, Physical Chemistry, Springer Berlin Heidelberg, Berlin, Heidelberg, 1979, pp. 89–116.
[102] S.F. Duan, S. Cai, Y.M. Xie, T. Bagby, S.Q. Ren, M.L. Forrest, Synthesis and characterization of a multiarm poly(acrylic acid) star polymer for application in sustained delivery of cisplatin and a nitric oxide prodrug, J. Polym. Sci. A Polym. Chem. 50 (2012) 2715–2724.
[103] M.H. Stenzel, T.P. Davis, C. Barner-Kowollik, Poly(vinyl alcohol) star polymers prepared via MADIX/RAFT polymerisation, Chem. Commun. (2004) 1546–1547.
[104] S. Duan, S. Cai, Q. Yang, M.L. Forrest, Multi-arm polymeric nanocarrier as a nitric oxide delivery platform for chemotherapy of head and neck squamous cell

carcinoma, Biomaterials 33 (2012) 3243–3253.
[105] S. Mura, J. Nicolas, P. Couvreur, Stimuli-responsive nanocarriers for drug delivery, Nat. Mater. 12 (2013) 991–1003.
[106] D. Schmaljohann, Thermo- and pH-responsive polymers in drug delivery, Adv. Drug Deliv. Rev. 58 (2006) 1655–1670.
[107] M. Bikram, J.L. West, Thermo-responsive systems for controlled drug delivery, Expert. Opin. Drug Del. 5 (2008) 1077–1091.
[108] C. Alvarez-Lorenzo, L. Bromberg, A. Concheiro, Light-sensitive intelligent drug delivery systems, Photochem. Photobiol. 85 (2009) 848–860.
[109] Y.Z. Zhao, L.N. Du, C.T. Lu, Y.G. Jin, S.P. Ge, Potential and problems in ultrasound-responsive drug delivery systems, Int. J. Nanomedicine 8 (2013) 1621–1633.
[110] R.X. Zhang, T. Ahmed, L.Y. Li, J. Li, A.Z. Abbasi, X.Y. Wu, Design of nanocarriers for nanoscale drug delivery to enhance cancer treatment using hybrid polymer and lipid building blocks, Nano 9 (2017) 1334–1355.
[111] M. Wei, Y. Gao, X. Li, M.J. Serpe, Stimuli-responsive polymers and their applications, Polym. Chem. 8 (2017) 127–143.
[112] Y. Kang, J. Kim, Y.M. Lee, S. Im, H. Park, W.J. Kim, Nitric oxide-releasing polymer incorporated ointment for cutaneous wound healing, J. Control. Release 220 ( (2015) 624–630.
[113] T. Ullrich, S. Oberle, A. Abate, H. Schroder, Photoactivation of the nitric oxide donor SIN-1, FEBS Lett. 406 (1997) 66–68.
[114] S. Sortino, S. Petralia, G. Compagnini, S. Conoci, G. Condorelli, Light-controlled nitric oxide generation from a novel self-assembled monolayer on a gold surface, Angew. Chem. Int. Ed. 41 (2002) 1914–1917.
[115] F. Karaki, Y. Kabasawa, T. Yanagimoto, N. Umeda, Y. Urano Firman, T. Nagano, Y. Otani, T. Ohwada, Visible-light-triggered release of nitric oxide from n-pyramidal nitrosamines, Chem.-Eur. J. 18 (2012) 1127–1141.
[116] N. Kanayama, K. Yamaguchi, Y. Nagasaki, PEGylated polymer micelle-based nitric oxide (NO) photodonor with NO-mediated antitumor activity, Chem. Lett. 39 (2010) 1008–1009.
[117] Z.S. Al-Ahmady, W.T. Al-Jamal, J.V. Bossche, T.T. Bui, A.F. Drake, A.J. Mason, K. Kostarelos, Lipid-peptide vesicle nanoscale hybrids for triggered drug release by mild hyperthermia in vitro and in vivo, ACS Nano 6 (2012) 9335–9346.
[118] K.J. Chen, H.F. Liang, H.L. Chen, Y.C. Wang, P.Y. Cheng, H.L. Liu, Y.N. Xia, H.W. Sung, A thermoresponsive bubble-generating liposomal system for triggering localized extracellular drug delivery, ACS Nano 7 (2013) 438–446.
[119] J. van der Zee, Heating the patient: a promising approach? Ann. Oncol. 13 (2002) 1173–1184.
[120] D.M. Hall, G.R. Buettner, R.D. Matthes, C.V. Gisolfi, Hyperthermia stimulates nitric-oxide formation - electron-paramagnetic-resonance detection of center-dot-no-heme in blood, J. Appl. Physiol. 77 (1994) 548–553.
[121] K. Janjetovic, M. Misirkic, L. Vucicevic, L. Harhaji, V. Trajkovic, Synergistic antiglioma action of hyperthermia and nitric oxide, Eur. J. Pharmacol. 583 (2008) 1–10.
[122] H.J. Al-Tamimi, P.A. Eichen, G.E. Rottinghaus, D.E. Spiers, Nitric oxide supplementation alleviates hyperthermia induced by intake of ergopeptine alkaloids during chronic heat stress, J. Therm. Biol. 32 (2007) 179–187.
[123] M.G.V. Heiden, L.C. Cantley, C.B. Thompson, Understanding the Warburg effect: the metabolic requirements of cell proliferation, Science 324 (2009) 1029–1033.
[124] M.V. Liberti, J.W. Locasale, The Warburg effect: how does it benefit cancer cells? Trends Biochem. Sci. 41 (2016) 211–218.
[125] G. Kocak, C. Tuncer, V. Butun, pH-responsive polymers, Polym. Chem. 8 (2017) 144–176.
[126] M. Hruby, C. Konak, K. Ulbrich, Polymeric micellar pH-sensitive drug delivery system for doxorubicin, J. Control. Release 103 (2005) 137–148.
[127] J.M. Swails, Y. Meng, F.A. Walker, M.A. Marti, D.A. Estrin, A.E. Roitberg, pH dependent mechanism of nitric oxide release in nitrophorins 2 and 4, J. Phys. Chem. B 113 (2009) 1192–1201.
[128] D. Pravdic, N. Vladic, I. Cavar, Z.J. Bosnjak, Effect of nitric oxide donors *S*-nitroso-*N*-acetyl-DL-penicillamine, spermine NONOate and propylamine propylamine NONOate on intracellular pH in cardiomyocytes, Clin. Exp. Pharmacol. P 39 (2012) 772–778.
[129] H.W. Choi, J. Kim, J. Kim, Y. Kim, H.B. Song, J.H. Kim, K. Kim, W.J. Kim, Light-induced acid generation on a gatekeeper for smart nitric oxide delivery, ACS Nano 10 (2016) 4199–4208.
[130] L.J. Tan, A. Wan, X.M. Zhu, H.L. Li, Visible light-triggered nitric oxide release from near-infrared fluorescent nanospheric vehicles, Analyst 139 (2014) 3398–3406.
[131] V.R. Zhelyaskov, K.R. Gee, D.W. Godwin, Control of no concentration in solutions of nitrosothiol compounds by light, Photochem. Photobiol. 67 (1998) 282–288.
[132] S.l.M. Shishido, A.B. Seabra, W. Loh, M. Ganzarolli de Oliveira, Thermal and photochemical nitric oxide release from *S*-nitrosothiols incorporated in Pluronic F127 gel: potential uses for local and controlled nitric oxide release, Biomaterials 24 (2003) 3543–3553.
[133] T. Liu, W. Zhang, T. Song, X. Yang, C. Li, Hollow double-layered polymer microspheres with pH and thermo-responsive properties as nitric oxide-releasing reservoirs, Polym. Chem. 6 (2015) 3305–3314.
[134] T.W. Liu, W. Zhang, X.L. Yang, C.X. Li, Hollow polymer nanoparticles with *S*-nitrosothiols as scaffolds for nitric oxide release, J. Colloid Interface Sci. 459 (2015) 115–122.
[135] H. Kimura, Physiological and pathophysiological functions of hydrogen sulfide, in: A. Hermann, G.F. Sitdikova, T.M. Weiger (Eds.), Gasotransmitters: Physiology and Pathophysiology, Springer Berlin Heidelberg, Berlin, Heidelberg, 2012, pp. 71–98.
[136] J.B. Haldane, Carbon monoxide as a tissue poison, Biochem. J. 21 (1927) 1068–1075.
[137] N.G. Abraham, A. Kappas, Pharmacological and clinical aspects of heme oxygenase, Pharmacol. Rev. 60 (2008) 79–127.
[138] D. Morse, A.M. Choi, Heme oxygenase-1: the "emerging molecule" has arrived, Am. J. Respir. Cell Mol. Biol. 27 (2002) 8–16.
[139] C.A. Piantadosi, Carbon monoxide, reactive oxygen signaling, and oxidative stress, Free Radic. Biol. Med. 45 (2008) 562–569.
[140] L. Rochette, Y. Cottin, M. Zeller, C. Vergely, Carbon monoxide: mechanisms of action and potential clinical implications, Pharmacol. Ther. 137 (2013) 133–152.
[141] S.W. Ryter, J. Alam, A.M. Choi, Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications, Physiol. Rev. 86 (2006) 583–650.
[142] S.W. Ryter, L.E. Otterbein, D. Morse, A.M. Choi, Heme oxygenase/carbon monoxide signaling pathways: regulation and functional significance, Mol. Cell. Biochem. 234-235 (2002) 249–263.
[143] J.R. Stone, M.A. Marletta, Soluble guanylate cyclase from bovine lung: activation with nitric oxide and carbon monoxide and spectral characterization of the ferrous and ferric states, Biochemistry 33 (1994) 5636–5640.
[144] R. Brenner, G.J. Perez, A.D. Bonev, D.M. Eckman, J.C. Kosek, S.W. Wiler, A.J. Patterson, M.T. Nelson, R.W. Aldrich, Vasoregulation by the beta1 subunit of the calcium-activated potassium channel, Nature 407 (2000) 870–876.
[145] J.L. Scragg, M.L. Dallas, J.A. Wilkinson, G. Varadi, C. Peers, Carbon monoxide inhibits L-type Ca2+ channels via redox modulation of key cysteine residues by mitochondrial reactive oxygen species, J. Biol. Chem. 283 (2008) 24412–24419.
[146] R. Motterlini, J.E. Clark, R. Foresti, P. Sarathchandra, B.E. Mann, C.J. Green, Carbon monoxide-releasing molecules: characterization of biochemical and vascular activities, Circ. Res. 90 (2002) E17–24.
[147] B.S. Zuckerbraun, B.Y. Chin, B. Wegiel, T.R. Billiar, E. Czsimadia, J. Rao, L. Shimoda, E. Ifedigbo, S. Kanno, L.E. Otterbein, Carbon monoxide reverses established pulmonary hypertension, J. Exp. Med. 203 (2006) 2109–2119.
[148] W. Durante, Carbon monoxide and bile pigments: surprising mediators of vascular function, Vasc. Med. 7 (2002) 195–202.
[149] L.E. Otterbein, F.H. Bach, J. Alam, M. Soares, H. Tao Lu, M. Wysk, R.J. Davis, R.A. Flavell, A.M. Choi, Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway, Nat. Med. 6 (2000) 422–428.
[150] D. Morse, S.E. Pischke, Z. Zhou, R.J. Davis, R.A. Flavell, T. Loop, S.L. Otterbein, L.E. Otterbein, A.M. Choi, Suppression of inflammatory cytokine production by carbon monoxide involves the JNK pathway and AP-1, J. Biol. Chem. 278 (2003) 36993–36998.
[151] J. Yoshida, K.S. Ozaki, M.A. Nalesnik, S. Ueki, M. Castillo-Rama, G. Faleo, M. Ezzelarab, A. Nakao, B. Ekser, G.J. Echeverri, M.A. Ross, D.B. Stolz, N. Murase, Ex vivo application of carbon monoxide in UW solution prevents transplant-induced renal ischemia/reperfusion injury in pigs, Am. J. Transplant. 10 (2010) 763–772.
[152] S.J. Dixon, B.R. Stockwell, The role of iron and reactive oxygen species in cell death, Nat. Chem. Biol. 10 (2014) 9–17.
[153] A. Nakao, G. Faleo, H. Shimizu, K. Nakahira, J. Kohmoto, R. Sugimoto, A.M. Choi, K.R. McCurry, T. Takahashi, N. Murase, Ex vivo carbon monoxide prevents cytochrome P450 degradation and ischemia/reperfusion injury of kidney grafts, Kidney Int. 74 (2008) 1009–1016.
[154] Y.K. Choi, C.K. Kim, H. Lee, D. Jeoung, K.S. Ha, Y.G. Kwon, K.W. Kim, Y.M. Kim, Carbon monoxide promotes VEGF expression by increasing HIF-1alpha protein level via two distinct mechanisms, translational activation and stabilization of HIF-1alpha protein, J. Biol. Chem. 285 (2010) 32116–32125.
[155] R. Motterlini, L.E. Otterbein, The therapeutic potential of carbon monoxide, Nat. Rev. Drug Discov. 9 (2010) 728–743.
[156] R. Motterlini, J.E. Clark, R. Foresti, P. Sarathchandra, B.E. Mann, C.J. Green, Carbon monoxide-releasing molecules, Charact. Biochem. Vasc. Activities 90 (2002) e17–e24.
[157] R. Foresti, J. Hammad, J.E. Clark, T.R. Johnson, B.E. Mann, A. Friebe, C.J. Green, R. Motterlini, Vasoactive properties of CORM-3, a novel water-soluble carbon monoxide-releasing molecule, Br. J. Pharmacol. 142 (2004) 453–460.
[158] M.G. Bani-Hani, D. Greenstein, B.E. Mann, C.J. Green, R. Motterlini, Modulation of thrombin-induced neuroinflammation in BV-2 microglia by carbon monoxide-releasing molecule 3, J. Pharmacol. Exp. Ther. 318 (2006) 1315–1322.
[159] S. Chlopicki, R. Olszanecki, E. Marcinkiewicz, M. Lomnicka, R. Motterlini, Carbon monoxide released by CORM-3 inhibits human platelets by a mechanism independent of soluble guanylate cyclase, Cardiovasc. Res. 71 (2006) 393–401.
[160] M.I. Guillén, J. Megías, V. Clérigues, F. Gomar, M.J. Alcaraz, The CO-releasing molecule CORM-2 is a novel regulator of the inflammatory process in osteoarthritic chondrocytes, Rheumatology 47 (2008) 1323–1328.
[161] H. Pfeiffer, A. Rojas, J. Niesel, U. Schatzschneider, Sonogashira and "Click" reactions for the N-terminal and side-chain functionalization of peptides with [Mn(CO)3(tpm)]+-based CO releasing molecules (tpm = tris(pyrazolyl)methane), Dalton Trans. (2009) 4292–4298.
[162] R.D. Rimmer, H. Richter, P.C. Ford, A photochemical precursor for carbon monoxide release in aerated aqueous media, Inorg. Chem. 49 (2010) 1180–1185.
[163] N.J. Farrer, L. Salassa, P.J. Sadler, Photoactivated chemotherapy (PACT): the potential of excited-state d-block metals in medicine, Dalton Trans. (2009) 10690–10701.
[164] K. Szacilowski, W. Macyk, A. Drzewiecka-Matuszek, M. Brindell, G. Stochel, Bioinorganic photochemistry: frontiers and mechanisms, Chem. Rev. 105 (2005) 2647–2694.
[165] A.E. Pierri, A. Pallaoro, G. Wu, P.C. Ford, A luminescent and biocompatible PhotoCORM, J. Am. Chem. Soc. 134 (2012) 18197–18200.
[166] A.R. Marques, L. Kromer, D.J. Gallo, N. Penacho, S.S. Rodrigues, J.D. Seixas, G.J.L. Bernardes, P.M. Reis, S.L. Otterbein, R.A. Ruggieri, A.S.G. Gonçalves, A.M.L. Gonçalves, M.N.D. Matos, I. Bento, L.E. Otterbein, W.A. Blättler, C.C. Romão, Generation of carbon monoxide releasing molecules (CO-RMs) as

drug candidates for the treatment of acute liver injury: targeting of CO-RMs to the liver, Organometallics 31 (2012) 5810–5822.
[167] F. Zobi, A. Degonda, M.C. Schaub, A.Y. Bogdanova, CO releasing properties and cytoprotective effect of cis-trans- [ReII(CO)2Br2L2]n complexes, Inorg. Chem. 49 (2010) 7313–7322.
[168] D. Nguyen, C. Boyer, Macromolecular and inorganic nanomaterials scaffolds for carbon monoxide delivery: recent developments and future trends, ACS Biomater. Sci. Eng. 1 (2015) 895–913.
[169] P.C. Kunz, N.E. Brückmann, B. Spingler, Towards polymer diagnostic agents — copolymers of N-(2-hydroxypropyl)methacrylamide and bis(2-pyridylmethyl)-4-vinylbenzylamine: synthesis, characterisation and Re(CO)3-labelling, Eur. J. Inorg. Chem. 2007 (2007) 394–399.
[170] N.E. Brückmann, M. Wahl, G.J. Reiß, M. Kohns, W. Wätjen, P.C. Kunz, Polymer conjugates of photoinducible CO-releasing molecules, Eur. J. Inorg. Chem. 2011 (2011) 4571–4577.
[171] U. Hasegawa, A.J. van der Vlies, E. Simeoni, C. Wandrey, J.A. Hubbell, Carbon monoxide-releasing micelles for immunotherapy, J. Am. Chem. Soc. 132 (2010) 18273–18280.
[172] H. Yin, J. Fang, L. Liao, H. Nakamura, H. Maeda, Styrene-maleic acid copolymer-encapsulated CORM2, a water-soluble carbon monoxide (CO) donor with a constant CO-releasing property, exhibits therapeutic potential for inflammatory bowel disease, J. Control. Release 187 (2014) 14–21.
[173] A.E. Pierri, P.-J. Huang, J.V. Garcia, J.G. Stanfill, M. Chui, G. Wu, N. Zheng, P.C. Ford, A photoCORM nanocarrier for CO release using NIR light, Chem. Commun. 51 (2015) 2072–2075.
[174] Y. Qian, J.B. Matson, Gasotransmitter delivery via self-assembling peptides: treating diseases with natural signaling gases, Adv. Drug Deliv. Rev. 110-111 (2017) 137–156.
[175] D. Zhang, I. Macinkovic, N.O. Devarie-Baez, J. Pan, C.-M. Park, K.S. Carroll, M.R. Filipovic, M. Xian, Detection of protein *S*-sulfhydration by a tag-switch technique, Angew. Chem. Int. Ed. 53 (2014) 575–581.
[176] W.M. Zhao, J. Zhang, Y.J. Lu, R. Wang, The vasorelaxant effect of $H_2S$ as a novel endogenous gaseous K-ATP channel opener, EMBO J. 20 (2001) 6008–6016.
[177] J. Pan, K.S. Carroll, Persulfide reactivity in the detection of protein *S*-sulfhydration, ACS Chem. Biol. 8 (2013) 1110–1116.
[178] C.E. Paulsen, K.S. Carroll, Cysteine-mediated redox signaling: chemistry, biology, and tools for discovery, Chem. Rev. 113 (2013) 4633–4679.
[179] L. Li, P. Rose, P.K. Moore, Hydrogen sulfide and cell signaling, Annu. Rev. Pharmacol. Toxicol. 51 (2011) 169–187.
[180] H. Kimura, Production and physiological effects of hydrogen sulfide, Antioxid. Redox Signal. 20 (2014) 783–793.
[181] Y. Kimura, Y. Goto, H. Kimura, Hydrogen sulfide increases glutathione production and suppresses oxidative stress in mitochondria, Antioxid. Redox Signal. 12 (2010) 1–13.
[182] R.C. Zanardo, V. Brancaleone, E. Distrutti, S. Fiorucci, G. Cirino, J.L. Wallace, Hydrogen sulfide is an endogenous modulator of leukocyte-mediated inflammation, FASEB J. 20 (2006) 2118–2120.
[183] Q. Zhang, H. Fu, H. Zhang, F. Xu, Z. Zou, M. Liu, Q. Wang, M. Miao, X. Shi, Hydrogen sulfide preconditioning protects rat liver against ischemia/reperfusion injury by activating Akt-GSK-3beta signaling and inhibiting mitochondrial permeability transition, PLoS One 8 (2013) e74422.
[184] W. Zhao, J. Zhang, Y. Lu, R. Wang, The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener, EMBO J. 20 (2001) 6008–6016.
[185] L. Liu, H. Liu, D. Sun, W. Qiao, Y. Qi, H. Sun, C. Yan, Effects of H(2)S on myogenic responses in rat cerebral arterioles, Circulat. J. 76 (2012) 1012–1019.
[186] Y. Zhao, T.D. Biggs, M. Xian, Hydrogen sulfide (H(2)S) releasing agents: chemistry and biological applications, Chem. Commun. (Camb.) 50 (2014) 11788–11805.
[187] Z.W. Lee, J. Zhou, C.S. Chen, Y. Zhao, C.H. Tan, L. Li, P.K. Moore, L.W. Deng, The slow-releasing hydrogen sulfide donor, GYY4137, exhibits novel anti-cancer effects in vitro and in vivo, PLoS One 6 (2011) e21077.
[188] Y. Zhao, S. Bhushan, C. Yang, H. Otsuka, J.D. Stein, A. Pacheco, B. Peng, N.O. Devarie-Baez, H.C. Aguilar, D.J. Lefer, M. Xian, Controllable hydrogen sulfide donors and their activity against myocardial ischemia-reperfusion injury, ACS Chem. Biol. 8 (2013) 1283–1290.
[189] T. Roger, F. Raynaud, F. Bouillaud, C. Ransy, S. Simonet, C. Crespo, M.P. Bourguignon, N. Villeneuve, J.P. Vilaine, I. Artaud, E. Galardon, New biologically active hydrogen sulfide donors, Chembiochem 14 (2013) 2268–2271.
[190] A. Martelli, L. Testai, V. Citi, A. Marino, I. Pugliesi, E. Barresi, G. Nesi, S. Rapposelli, S. Taliani, F. Da Settimo, M.C. Breschi, V. Calderone, Arylthioamides as $H_2S$ donors: l-cysteine-activated releasing properties and vascular effects in vitro and in vivo, ACS Med. Chem. Lett. 4 (2013) 904–908.
[191] Y. Zhao, H. Wang, M. Xian, Cysteine-activated hydrogen sulfide (H2S) donors, J. Am. Chem. Soc. 133 (2011) 15–17.
[192] N. Fukushima, N. Ieda, K. Sasakura, T. Nagano, K. Hanaoka, T. Suzuki, N. Miyata, H. Nakagawa, Synthesis of a photocontrollable hydrogen sulfide donor using ketoprofenate photocages, Chem. Commun. (Camb.) 50 (2014) 587–589.
[193] S.P.L. Cary, J.A. Winger, E.R. Derbyshire, M.A. Marletta, Nitric oxide signaling: no longer simply on or off, Trends Biochem. Sci., 31 231-239.
[194] W.P. Arnold, C.K. Mittal, S. Katsuki, F. Murad, Nitric oxide activates guanylate cyclase and increases guanosine 3′:5′-cyclic monophosphate levels in various tissue preparations, Proc. Natl Acad. Sci. USA 74 (1977) 3203–3207.
[195] B.D. Paul, S.H. Snyder, H2S signalling through protein sulfhydration and beyond, Nat. Rev. Mol. Cell Biol. 13 (2012) 499–507.
[196] O. Kabil, R. Banerjee, Redox biochemistry of hydrogen sulfide, J. Biol. Chem. 285 (2010) 21903–21907.
[197] V. Vitvitsky, O. Kabil, R. Banerjee, High turnover rates for hydrogen sulfide allow for rapid regulation of its tissue concentrations, Antioxid. Redox Signal. 17 (2012) 22–31.
[198] B.L. Predmore, D. Julian, A.J. Cardounel, Hydrogen sulfide increases nitric oxide production from endothelial cells by an Akt-dependent mechanism, Front. Physiol. 2 (2011) 104.
[199] Z. Altaany, G. Yang, R. Wang, Crosstalk between hydrogen sulfide and nitric oxide in endothelial cells, J. Cell. Mol. Med. 17 (2013) 879–888.
[200] C. Coletta, A. Papapetropoulos, K. Erdelyi, G. Olah, K. Modis, P. Panopoulos, A. Asimakopoulou, D. Gero, I. Sharina, E. Martin, C. Szabo, Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 9161–9166.
[201] R. Kodela, M. Chattopadhyay, K. Kashfi, NOSH-aspirin: a novel nitric oxide–hydrogen sulfide-releasing hybrid: a new class of anti-inflammatory pharmaceuticals, ACS Med. Chem. Lett. 3 (2012) 257–262.
[202] Q.X. Hu, D. Wu, F.F. Ma, S. Yang, B. Tan, H. Xin, X.F. Gu, X. Chen, S.Y. Chen, Y.C. Mao, Y.Z. Zhu, Novel angiogenic activity and molecular mechanisms of ZYZ-803, a slow-releasing hydrogen sulfide-nitric oxide hybrid molecule, Antioxid. Redox Signal. 25 (2016) 498–514.