The Potential of Nanomedicines for Delivery of Gaseous Signaling Molecules

Gaseous signaling molecules are a group of small gas molecules that are produced endogenously by the actions of a series of enzymes in the human body. These molecules play essential roles in maintaining homeostasis and regulating cellular processes including angiogenesis, vasodilation, apoptosis, inflammation and neurotransmission.[1-6] In addition, it has been revealed that unregulated production of these molecules is closely linked with the initiation and progression of many chronic diseases such as cancer, cardiovascular disorders and neurodegenerative diseases. Nitric oxide (NO) was the first molecule to be identified as an important signal mediator. Following this discovery, carbon monoxide (CO) and hydrogen sulfide (H_2S) have also been found to have regulatory roles in biological systems. In the past two decades, the physiological functions of these gaseous signaling molecules have been extensively studied to understand the mechanism of their actions and explore their potential therapeutic applications.

There are several unique chemical and physical properties shared by gaseous signaling molecules, which distinguish these molecules from conventional signaling molecules. First of all, the signal transductions of these molecules are not mediated by receptor-ligand interactions but instead rely on the covalent modification of target biomolecules. For example, NO and H_2S react with thiol groups of protein cysteine residues via S-nitrosation and S-sulfhydration, respectively.[7,8] Another example is that all three gaseous signaling molecules can coordinate with metal centers of metalloproteins.[3] These modifications induce conformational changes of proteins, eliciting biological responses. In addition to their chemical reactivity, gaseous signaling molecules, due to their small size, can diffuse rapidly in biological fluids and enter cells without the need for specific receptors. Also, since they exist as gas under ambient conditions, these molecules can be quickly eliminated from biological fluids by evaporation, which makes their accurate dosing difficult. Furthermore, these molecules have extremely short half-lives in biological systems (generally, milliseconds for NO and seconds for CO and H_2S). Based on these properties, it is unlikely for gaseous signaling molecules to migrate a great distance in the body. Therefore, the biological actions of these molecules occur only in close vicinity of where they are generated.

With the understanding of the biological significance of gaseous signaling molecules, their therapeutic use has attracted increasing interest. However, one of the challenges in translational research of gaseous signaling molecules is the lack of administration methods that enable delivery of these gases to the target site at a controlled rate and duration. A common approach is to use low molecular weight compounds that decompose to generate gaseous signaling molecules under physiological conditions (gas donors). Thus far, researchers have designed gas donors that decompose through different mechanisms, including hydrolysis and reactions with endogenous molecules such as enzymes and thiol-containing compounds. However, these gas donors generally show fast gas release and cannot maintain the therapeutic levels for prolonged periods of time. Another drawback is that the donor compounds and/or their decomposition byproducts often exert strong side effects. It should be noted that studies using different gas donors often result in contradicting outcomes. These results have generally been explained by the difference in their release profiles. However, the contribution of side effects caused by the

donor molecules cannot be ignored, and therefore overlooking these effects can lead to misinterpretation of results. In addition to these limitations, low aqueous solubility, rapid renal clearance and uncontrolled tissue distribution after systemic administration are potential problems, which lowers bioavailability and, thereby, therapeutic efficacy.

Nanomedicine-based gas donors have emerged to address the issues associated with small gas donors.[9] In this approach, the small gas donors are embedded in nanoparticles. While the nanomedicine approach has been well established for conventional therapeutic agents such as chemotherapeutics, there are additional design criteria to consider for gas delivery systems: 1) small gas donors must be bound to nanomedicines via a non-cleavable bond and 2) nanomedicines should not release decomposition products of the gas donors after gaseous signaling molecule release. Among nanoparticles that have been used for drug delivery, polymeric micelles, self-assembled core-shell type nanoparticles of amphiphilic block copolymers, have been used in the majority of studies reported thus far. Generally, these micelles are prepared from amphiphilic block copolymers with a core-forming segment conjugated with the gas donor. In this way, gas donors are stably immobilized in the micellar core. Importantly, selecting hydrophobic gas donors is beneficial since these structures can serve as hydrophobes to drive micellization and stabilize the micellar core. This approach was first exploited by Hubbell and coworkers.[10] They developed NO-releasing polymeric micelles with a core containing *N*-diazeniumdiolate (NONOate) groups, which showed sustained release of NO. The same group also developed CO-releasing micelles containing ruthenium carbonyl groups and showed that these micelles attenuated the proinflammatory response in lipopolysaccharide-treated macrophages.[11] Following these reports, polymeric micelles containing anethole dithiolethione groups have been developed for delivery of H_2S.[12] Thus far, a growing body of studies have used this design concept to develop polymeric micelles containing different gas donor groups and evaluated their therapeutic potential in *in vitro* and *in vivo* models.

The advantages of the micelle approach have been demonstrated in different contexts. First, sustained release of gaseous signaling molecules can be achieved by embedding gas donor groups within the micelle core. [10,12,13] Since the generation of gaseous signaling molecules is triggered by molecules in biological fluids (e.g., water, cysteine/glutathione, enzymes, hydrogen peroxide), a hydrophobic polymeric network of the core can serve as a physical barrier to limit the access of these trigger molecules to gas donors. It is worth mentioning that fine-tuning of gas release kinetics is possible by modulating thermodynamic stability and mobility of the micelle core.[14-16] In addition, further control of release profiles can be achieved by incorporating stimuli-sensitive motifs to the core-forming segment that induce micellar dissociation in response to specific stimuli.[17,18] In order to achieve quantitative gas release, polymeric micelles that release gases in response to UV, visible or NIR light have also been developed by conjugating photosensitive gas donors to the micellar core.[19,20] Apart from the release control, polymeric micelle-based gas donors show remarkably low toxicity compared to the small gas donors. This appears to be due to the altered intracellular distribution of polymeric micelles, which are not membrane permeable and thus taken up by cells via endocytosis.[9] Throughout the endocytosis process, polymeric micelles are confined within intracellular vesicles (i.e., endosomes, lysosomes), and do not contact with other intracellular organelles directly. This

inhibits unfavorable interactions between gas donors and intracellular components, preventing toxic side effects. Polymeric micelle-based gas donors can also offer an opportunity for “combination therapy”. Due to the crosstalk between different gaseous signaling molecules, a combination of different gases such as NO/H_2S and NO/CO is known to enhance therapeutic effects compared to the treatment with a single gas. Co-delivery of different gases can be achieved by immobilizing different gas donor molecules in the micelle core. In addition, polymeric micelle-based gas donors can be used as nano-carriers for other therapeutic agents, such as chemotherapeutics and anti-inflammatory drugs. While the mechanism is not fully understood, gaseous signaling molecules have synergistic effects with these conventional drugs to improve therapeutic outcomes. Lastly, as is well-documented in the field of nanomedicine, the pharmacokinetic issues of small gas donors can be solved using the micelle approach by avoiding the body’s clearance system (i.e., kidneys and reticuloendothelial system).

The past decade has witnessed a rapid evolution of delivery technologies for gaseous signaling molecules. Accumulating evidence clearly shows the potential of micelle-based gas delivery systems to improve therapeutic efficacy and safety of gaseous signaling molecules. However, there are still some questions that remain unanswered such as: what are the therapeutic levels of gaseous signaling molecules needed for specific applications? How long should the release last to ensure desired therapeutic outcomes? To answer these questions, further investigations are necessary to correlate release profiles of gaseous signaling molecules with their biological actions. In this regard, polymeric micelle-based gas donors can also be used as a tool to control delivery of gases to target cells to enhance understanding of the complex biology of gaseous signaling molecules. Furthermore, recent studies suggest that in addition to gaseous signaling molecules, their downstream products produced by cellular metabolism can also act as signaling mediators. For example, H_2S is known to be oxidized to per/polysulfide species through sulfur oxidation pathways, which are more reactive towards cysteine residues. Therefore, there is a growing interest in developing delivery systems for the species derived from gaseous signaling molecules.[21,22] In addition, reports have appeared on other endogenously produced gases such as sulfur dioxide (SO_2), methane (CH_4) and ammonia (NH_3) which can viewed as new members of gaseous signaling molecules. Although research on these gases is still in its infancy, development of delivery systems will be the next step to advance knowledge of the critical roles of gaseous signaling molecules in the human body. For further advancement of the field of gaseous signaling molecules, future research needs to focus on translational studies to introduce gas delivery technologies into clinical applications. This will pave the way for novel therapeutic strategies with the potential to treat various diseases.

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