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Review

Antioxidative nanomaterials and biomedical applications

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ABSTRACT

Oxidative stress, as results of deregulated production and accumulation of reactive oxygen and nitrogen species, is a common hallmark in a multitude of human diseases. Enormous studies have demonstrated that such imbalance of redox homeostasis is implicated in both disease initiation and progress. The development of antioxidants to target overexpressed reactive oxygen and/or nitrogen species, which is referred to as antioxidant therapy, has thus represented an important therapeutic option for the oxidative stress relevant diseases. Over the past decade, antioxidative nanotechnologies have been emerging as an alternative strategy and have shown many unique advantages over conventional antioxidants (such as enzymes and small molecules), owing to their advantageous pharmacokinetics and biodistribution, stable antioxidative activity, and more importantly, intrinsic multiple radicals scavenging properties. This review provides a comprehensive and up-to-date overview on antioxidative nanomaterials in terms of the category, their antioxidative activities and underlying mechanisms, and the potential biomedical applications. In addition, the challenges in this exciting field and future perspectives are also discussed.

Contents

Abbreviations: HIF-1, hypoxia-inducible factor; FOXO3, forkhead box O3; Nrf2, nuclear factor (erythroid-derived 2)-like 2; PKC, protein kinase C; SOD2, superoxide dismutase 2; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; MEK1/2, mitogen-activated protein kinase kinase; ERK1/2, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor alpha; NALP3, NACHT, LRR and PYD domains-containing protein 3; $p66^{Shc}$, a member of the Src homologous-collagen homologue adaptor.

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Introduction

The presence of free radicals in a variety of lyophilized biological materials was first confirmed by Commoner et al. using the electron paramagnetic resonance (EPR) spectroscopy in 1954 [1]. In the same year, Gerschman and colleagues put forward that the generation of oxidizing free radicals through partial reduction of oxygen might be a common mechanism for oxygen poisoning and injuries [2]. These oxidizing free radicals were soon thereafter defined as reactive oxygen species (ROS) by Harman [3], which at that time were proposed to play crucial roles in the aging process. Subsequent isolation of superoxide dismutase (SOD) from bovine erythrocytes provided further evidence for the existence of ROS in organisms [4]. Another milestone step in understanding the biological functions of oxidative species is the Nobel Prize-winning work by Murad et al., in which nitric oxide, a primary reactive nitrogen species (RNS), was first identified as a crucial endothelium-derived relaxing factor in the cardiovascular system [5]. Following the discovery, a body of evidence has supported that nitric oxide could act as a diffusible radical and a key messenger in cell signaling to regulate a series of cellular processes, and get involved in nitrosylation of various biomolecules [6]. Over the past few decades, these pioneered studies have inspired rapid and ever-growing research into the field of free radicals in living systems.

It now becomes clear that both ROS and RNS are important free radicals *in vivo*, which are generated as metabolites and signal molecules in living organisms during normal cellular metabolism and in response to environmental stimulation [7,8]. These species generally contain an uneven of electrons, enabling them to easily react with other molecules, a phenomenon referred to as oxidation [9]. Oxidation is associated with the regulation of various physiological and pathological events that take place in human body [10,11], and can also help fight off pathogens and pathogen-induced infections [12]. Normally, this oxidation process is tightly controlled by the cellular antioxidant defense, given the cytotoxicity of excessive ROS/RNS [13]. The term "oxidative stress" has thus been coined to represent an imbalance among ROS, RNS and the antioxidant defense, leading to a deregulated redox homeostasis and subsequent damages to various biomolecules (*e.g.*, DNAs, lipids, and proteins) and recruitment of many inflammatory mediators capable of triggering a series of secondary physiological events in the body over time [13–17]. Accumulated evidence has demonstrated the correlation of oxidative stress with the initial and progress of a myriad of diseases, most notably aging, autoimmune disorders, cardiovascular and neurodegenerative diseases [18–23]. Consequently, antioxidant therapy has been well recognized as a potentially powerful means for preventing and treating oxidative stress-associated diseases [24,25].

Antioxidants, sometimes called free-radical scavengers, are substances that can prevent or attenuate oxidative damages to cells caused by ROS/RNS [26]. They can be endogenous antioxidants (*i.e.*, formed within human body) or exogenous antioxidants (*i.e.*, from outside the body). Indeed, the use of antioxidants can be traced back to several decades when nutrition research findings have demonstrated that high-dose dietary supplement of antioxidant-rich foods could help protect the human body against diseases [27,28]. Accordingly, numerous efforts have been dedicated to explore naturally occurring materials (*e.g.*, antioxidative enzymes and small molecules) and their derivatives to prevent oxidative damages. Several small-molecule antioxidants have thus far been approved for the treatment of different diseases, such as stroke, Alzheimer's disease, cardiovascular diseases and inflammatory diseases (Fig. 1) [29]. However, these conventional antioxidants have intrinsic limitations, such as nonspecific distribution, rapid renal clearance, low delivery efficiency, vulnerable/specified antioxidative activities, among others [30].

The application of nanotechnology to biomedicine has considerably changed the pharmaceutical and biotechnology industries [31,32], and concurrently lead to a significant advance in the field of antioxidant therapy, showing promise to address the aforementioned challenges associated with conventional antioxidants and enable the development of entirely novel class of antioxidants [33–35]. Foremost, nanotechnology can significantly improve the pharmacokinetics of natural antioxidative molecules and protect them from rapid decay of anti-ROS/RNS activities under harsh stress or pH conditions. More impressively, many types of nanomaterials with inherent enzymatic activities have been emerging as powerful radical scavengers over the past decade, since the discovery of two antioxidative nanomaterials, fullerenes and cerium oxide (Fig. 2). These antioxidative nanomaterials, sometimes called as nanoantioxidants, have demonstrated higher antioxidative stability and stronger tolerance to harsh microenvironments, as compared to the natural antioxidants. In addition, multiple free radicals targeting antioxidative nanomaterials have recently moved into the spotlight [36,37], yielding promising results to minimize oxidative injuries derived from various ROS/RNS in different disease models. Meanwhile, it is worth noting that, while these antioxidative nanomaterials have shown astonishing potential in animal studies, their translation to human clinical trials remains elusive.

In this review, we aim to deliver a comprehensive overview of the important advances in the field of antioxidative nanotechnology. We briefly summarize the pathology and molecular mechanisms of oxidative stress as well as its major mechanisms that contribute to cellular and/or tissue injuries. To promote better understanding of the significance of antioxidative nanotechnol-

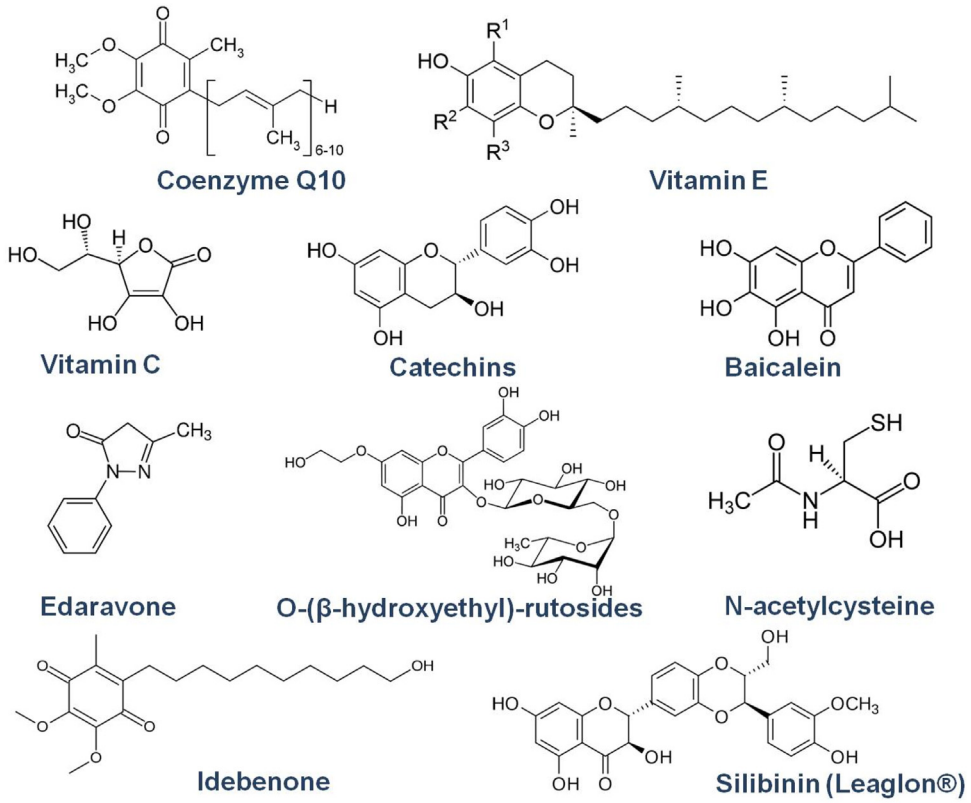

Fig. 1. Chemical structure of currently approved small molecular antioxidants by FDA for the treatment of different oxidative stress-related diseases, such as stroke, Alzheimer's disease, cancer, cardiovascular diseases and inflammatory diseases.

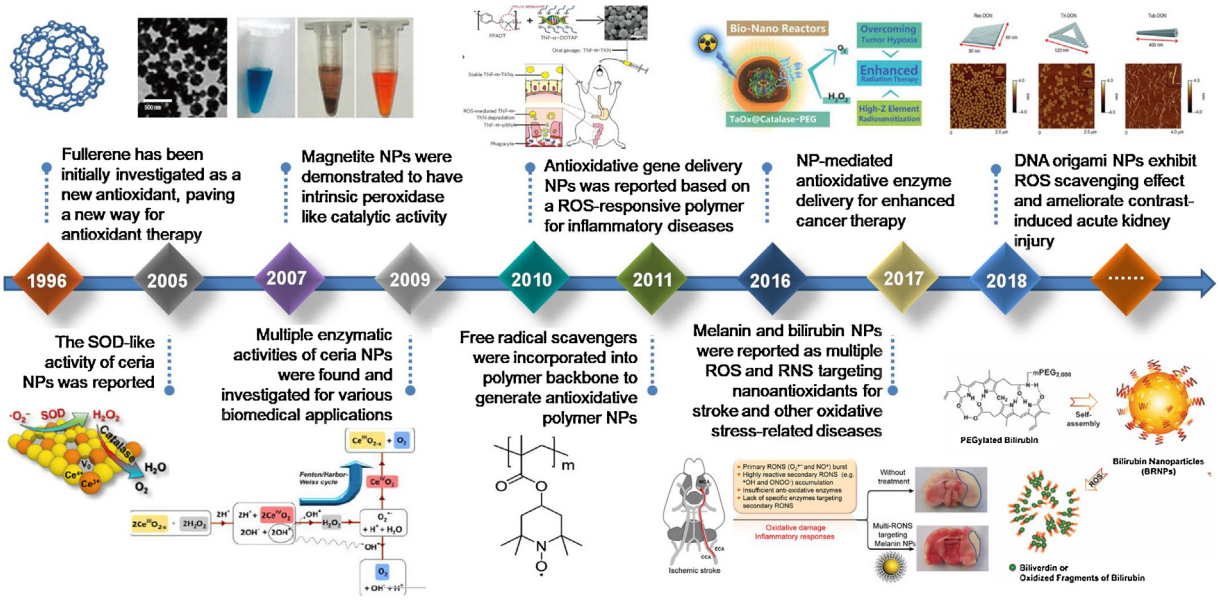

Fig. 2. Timeline of the development of some representative antioxidative nanotechnologies and nanomaterials.

ogy, we present thorough discussion regarding the antioxidative mechanisms and the factors that have impact on the anti-ROS/RNS activities of antioxidative nanomaterials. We also highlight their biomedical applications with a focus on recent findings for prevention and treatment of many important oxidative stress-related diseases. Lastly, we provide our perspectives on the greatest challenges and opportunities in antioxidative nanomedicine for clinical translation.

The pathology and molecular mechanisms of oxidative stress

Understanding the categories, sources, and reactivity of oxidizing species that contribute to oxidative stress is prerequisite and critical for the successful development of effective antioxidative therapeutics. In general, oxidative stress is dominated by two major categories of reactive species, ROS and RNS. Nevertheless, different

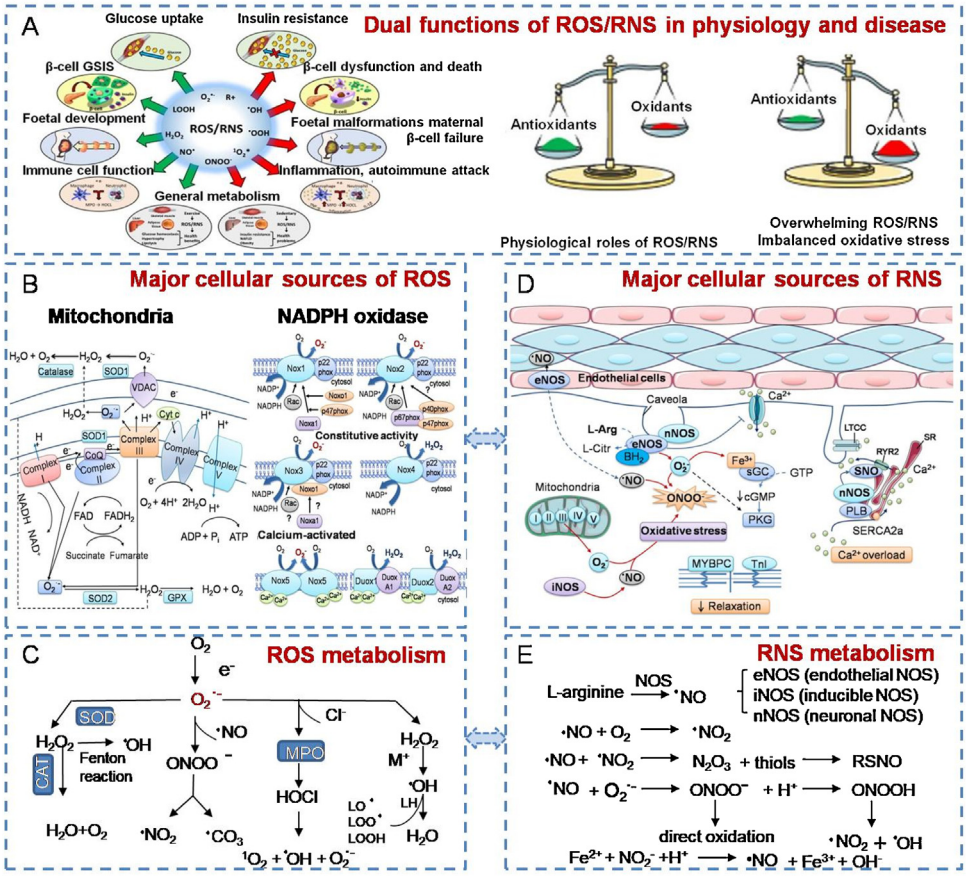

Fig. 3. ROS/RNS production, metabolism, and functions in human health. (A) The correlation and physiological roles between ROS/RNS and diseases. Reprinted with permission from Ref. [39]. Copyright 2016, Portland Press. (B) Major cellular sources of ROS production. Reprinted with permission from Ref [41]. (Copyright 2011, Nature) and Ref [42]. (Copyright 2014, Elsevier). (C) The metabolisms of ROS. (D) Major cellular sources of RNS production. Reprinted with permission from Ref. [54]. Copyright 2018, Nature. (E) The metabolisms of RNS.

ROS and RNS are regulated by different signals, and thus excessive production of these species will lead to diverse cellular dysfunction, such as dysfunctional energy metabolism, altered cell signaling and cell cycle control, impaired cell transport mechanisms, immune activation or suppression, and inflammation [38]. These physiological events have been implicated in many diseases (Fig. 3A) [39]. In this section, we overview the molecular and cellular origins and metabolism of ROS/RNS, and describe how ROS/RNS-induced oxidative stress affects cell functions.

The generation and metabolism of ROS

Superoxide anion radical ($O_2^{\bullet-}$) represents the main initial form of ROS, which is produced through monovalent reduction of molecular O_2 [40]. It functions as an essential signaling molecule in aerobic organisms and regulates different physiological processes such as host defense, blood pressure, aging, senescence, and apoptosis [39,40]. Mitochondria is known to be the biggest contributor to the generation of $O_2^{\bullet-}$ (Fig. 3B) [41,42]. An estimate of 1–2% of the 6.4 L/kg/day of total daily oxygen consumption would be converted into mitochondrial $O_2^{\bullet-}$ [43], and one adult with a weight of 80 kg would produce $O_2^{\bullet-}$ at 215–430 mmol per day. Among different components, complexes I, II and III in the mitochondrial respiratory chain are predominantly responsible for the generation of $O_2^{\bullet-}$ [41,44]. The $O_2^{\bullet-}$ formed from complexe II is commonly released into the mitochondrial matrix, whereas $O_2^{\bullet-}$ from complexes I and III enters both the intermembrane space and matrix (~80% *vs.* ~20%) of the mitochondria. In additon, the $O_2^{\bullet-}$ formed from complexe III is capable of transporting outside of the outer mitochondrial membrane by voltage-dependent anion selective channels. Nontheless, the detailed contribution of each complex to the overall $O_2^{\bullet-}$ production remains unclear.

Another major source of $O_2^{\bullet-}$ is the phagocytic prooxidant nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a membrane-binding enzyme also denoted as NOXs [45,46]. The NOX family is composed of five isoforms (NOX 1–5) and two oxidases (DUOX1-2), which catalyze the production of $O_2^{\bullet-}$ from univalent reduction between molecular O_2 and NADPH (Equation 1):

$$NADPH + 2O_2 \rightarrow NADP^+ + H^+ + 2O_2^{\bullet-} \quad (1)$$

While $O_2^{\bullet-}$ is known to be less active than other forms of ROS, it will be very detrimental to cells when it is produced uncontrollably or the antioxidant defense is deficient [47]. $O_2^{\bullet-}$ has a relatively short biological life span, and can be converted into H_2O_2 either by self-dismutation (particularly under acidic pH) or by SOD-mediated catalysis. Of note, H_2O_2 is the most stable form of ROS and has the capability of diffusing freely within and between cells. In additon to direct oxidative damage, $O_2^{\bullet-}$ and the byproduct H_2O_2 participate in the formation of other reactive species (Fig. 3C). For example, $O_2^{\bullet-}$ can further trigger the release of redox-sensitive iron from iron storage proteins (*e.g.*, ferritin and hemoglobin) under stress conditions, leading to the formation of highly reactive hydroxyl radicals ($^{\bullet}OH$) *via* Haber-Weiss cycle or Fenton-type reactions [48]. Likewise, other metal ions (*e.g.*, Cu^{2+}) can also catalyze the formation of $^{\bullet}OH$ through similar reactions, in which $O_2^{\bullet-}$ serves as the reducing agent. $^{\bullet}OH$ is a short-lived, yet extremely reactive, free radical that can react with virtually all surrounding biomolecules, thus acting as the major contributor of oxidative stress.

Hypochlorous acid (HOCl) represents another type of ROS that results from the reaction between H_2O_2 and Cl^- in body fluids in the presence of enzymes including myeloperoxidase and eosinophil peroxidase [49]. It is noteworthy that its reactivity/toxicity is 100–1000 times higher than those of $O_2^{\bullet-}$ and H_2O_2. Added to this, its reactive products with other bioreactive moleucles are even more toxic than HOCl itself, such as the liposoluble chloramines generated from the reaction of HOCl with amines [50]. On the other hand, the formed HOCl can in turn promote the generation of singlet oxygen ($^1O_2$), a reactive, diffusing, and long-lived radical that is highly reactive towards many biomolecules to initiate peroxidation [51]. Besides, HOCl can further produce more •OH upon reaction with other ROS. Taken together, •OH, HOCl, and $^1O_2$ have been considered as the secondary ROS and potentially induce oxidative damages to cells and tissues more severely than primary ROS ($O_2^{\bullet-}$ and H_2O_2). The prevention of oxidative injuries from these secondary ROS is therefore highly important in clinical settings under stress conditions. Unfortunately, there is no specific enzyme targeting these secondary ROS in organisms.

The generation and metabolism of RNS

RNS are a family of bioactive molecules arising from nitric oxide radical (•NO) and $O_2^{\bullet-}$ [39]. •NO is the primary [52], initial RNS endogenously synthesized as a bioproduct in virtually all types of organisms by converting L-arginine to L-citrulline under catalysis of various nitric oxide synthase (NOS) enzymes [53]. Endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS), and inducible nitric oxide synthase (iNOS) are three key NOS isoforms responsible for the generation of •NO (Fig. 3**D**) [54]. Among these three isoforms, eNOS is a low-activity enzyme and produces a small amount of •NO primarily in the vascular endothelium. nNOS produces •NO radicals in nervous systems [55]. The •NO radicals derived from eNOS and nNOS have many physiological functions as signaling molecules, such as regulation of cardiac function, cellular proliferation, platelet aggregation, and protection against oxidative injury induced by myocardial or cerebral ischemia. In contrast, iNOS has much higher activity than eNOS, and thus produces large amounts of •NO radicals in immune cells as a defense mechanism. Once it is activated, a near-micromolar •NO will be constantly formed for long periods of time.

Despite the diffusing capability through the plasma membranes, •NO has a very short lifetime, and thus react rapidly with surrounding molecules (Fig. 3E). In particular, •NO can interact with $O_2^{\bullet-}$ to form peroxynitrite ($ONOO^-$), the most cytotoxic and mutagenic RNS with the reactivity comparable to that of •OH. $ONOO^-$ is also an instable radical with the life time of about 1 s. It will convert into the acid form, peroxynitrous acid (ONOOH), in the equilibrium of trans/cis reverse isomerization. The acid form of $ONOO^-$ is a powerful oxidant, and can further undergo hemolytic decomposition to yield other free radicals such as •OH, $•NO_2$, and $CO_3^{\bullet-}$. Nevertheless, the knowledge on RNS biology is currently far behind of the ROS biology study, and their metabolic mechanisms remain largely unexplored, probably due to the difficulty in identifying various RNS and the complexity of RNS-activated signaling.

ROS/RNS in cellular function and signal transduction

While the detailed chemical mechanisms underlying the production and toxicity of ROS and RNS are still not well understood, it is evident that disturbance in any step involved in the ROS/RNS network can result in dysregulated redox homeostasis and orchestrate a cascade of intracellular signal alterations, leading to significant cellular dysfunction, cell death, and other chain reactions that may damage to organisms, which have been closely involved in the development and progress of many diseases. Moreover, oxidative injuries caused by the deregulated ROS/RNS homeostasis are seen in a variety of clinical settings either by acute overproduction of ROS/RNS or chronic accumulation of ROS/RNS. Since the influences of oxidative stress on cellular function and signal transduction have been systemically reviewed elsewhere [56–58], here we only demonstrate our emphasis on several critical factors that are closely associated with development and progress in oxidative stress-related diseases.

Initiating calcium influx

One representative effect as a result of deregulated levels of ROS/RNS is the change in calcium (Ca^{2+}) ion channels (Fig. 4**A)** [59,60]. Ca^{2+} is an important second messenger that regulates diverse cellular functions, such as secretion, contraction, gene expression, metabolism, cell survival and death. It enters the cells mainly through the transmembrane proteins, also known as calcium channels, whereas some special Ca^{2+} transport systems are responsible for intracellular Ca^{2+} metabolisms [59]. Specifically, mitochondrial Ca^{2+} uptake and transport play crucial roles in cellular physiology and pathophysiology including the control over energy production and spatiotemporal regulation of intracellular Ca^{2+} signals [61]. Since mitochondria is the major source of ROS, various Ca^{2+}-related signaling pathways in different organs might be activated and modulated upon oxidative stress challenge, thus reshaping the profile of local and global Ca^{2+} signals [62]. Studies have demonstrated that the Ca^{2+} entry mediated by voltage-dependent Ca^{2+} channels was redox sensitive. For example, switching of P/Q-type Ca^{2+} channels in neurons has been reported to be accelerated by the elevated H_2O_2 [63]. Likewise, excess ROS has been shown to stimulate the transport of Ca^{2+} into the vascular smooth muscle cells *via* L-type and T-type voltage-gated Ca^{2+} channels. Nevertheless, the redox-triggered alternation of Ca^{2+} channels is highly dependent on the types of target cells, the dose, time, and the categories of ROS. Previous studies have indicated that ROS had no obvious effect on the L-type Ca^{2+} channels in pancreatic β-cells [64]. In sharp contrast, the L-type Ca^{2+} channels was reported to be significantly influenced by ROS in skeletal muscles, which was presumably due to the extensive phosphorylation of these channels by various kinases under stress conditions [65].

Meanwhile, the increased level of Ca^{2+} might in turn modulate the ROS generating pathways to produce more ROS by enhancing the cellular metabolism. One representative example is the positive effect of Ca^{2+} on ROS generation in mitochondria. Ca^{2+} is able to promote mitochondrial citric acid cycle and oxidative phosphorylation, and the increased metabolic rate would result in more oxygen consuming for ATP synthesis, coupled with sustained release of ROS [66]. In addition, the family of NOXs has been found to be activated to produce ROS in a Ca^{2+}-dependent manner. Indeed, some enzymes such as NOX5, DUOX1 and DUOX2 have calcium-binding proteins, which require Ca^{2+} for the generation of ROS [67]. The cross-talk or interplay between Ca^{2+} and ROS has been demonstrated to contribute to the incidence and progress of many diseases such as cardiovascular diseases and neurodegenerative diseases, but more studies are demanded to fully reveal the underlying mechanisms behind the cross-talk between calcium ions and oxidative stress.

Autophagy

Autophagy is a multi-step intracellular degradative process responsible for maintaining cellular homeostasis through recycling cellular components and cleaning damaged organelles upon the response to different stress conditions [68]. It has been well demonstrated that ROS and RNS represent the major and early mediators in many pathways of intracellular autophagy (Fig. 4B) [69]. Some studies have supported that ROS/RNS may regulate autophagy through mTOR-dependent pathways in the cytoplasm

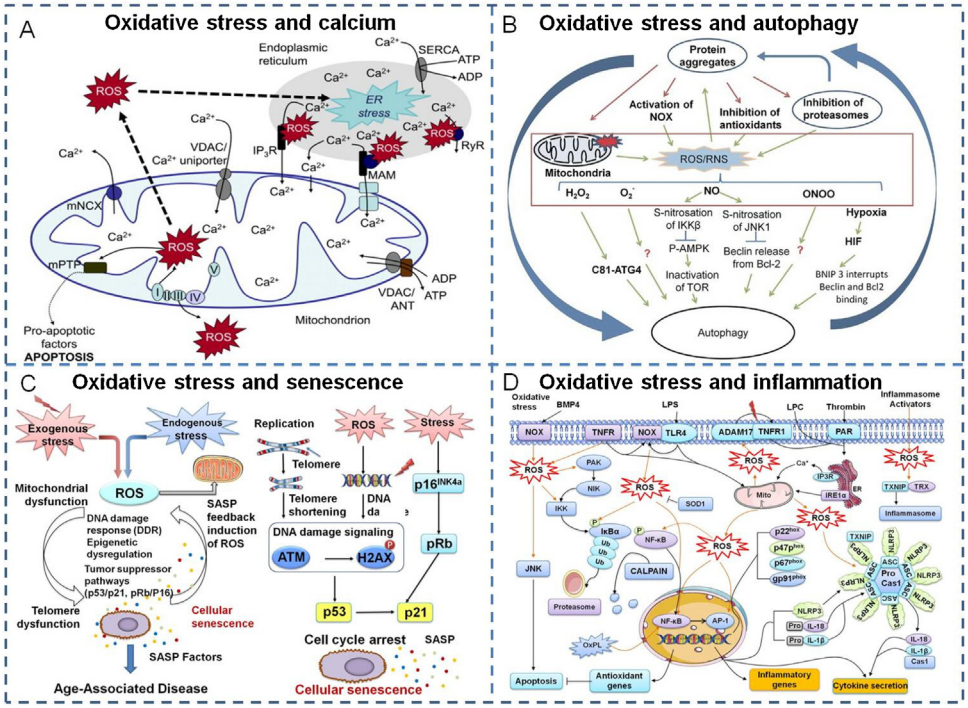

Fig. 4. ROS/RNS in cellular function and signal transduction, including (A) the calcium ion channel (Reprinted with permission from Ref. [59]. Copyright 2015, Elsevier), (B) autophagy (Reprinted with permission from Ref. [69]. Copyright 2012, Portland Press), (C) senescence (Reprinted with permission from Ref. [84]. Copyright 2015, European Respiratory Society), and (D) inflammatory signaling (Reprinted with permission from Ref. [94]. Copyright 2018, American Heart Association).

[70]. However, most literatures tend to support that ROS/RNS promote autophagy predominantly by ROS/RNS in mitochondria [71]. Notably, it is still not well established as to which oxidizing species exactly drive these pathways. A previous work from Chen et al. suggested that $O_2^{\bullet-}$ is the predominant ROS involved in the autophagy process upon stimulation by nutrient starvation [72]. Instead, H_2O_2 was required to induce this process by oxidizing autophagy proteases after starvation [73]. In an indirect manner, on the other hand, oxidative stress has proven to regulate the autophagy process by the AMP-activated protein kinase (AMPK), an enzyme sensitive to oxidative stress. In addition, modulation of autophagy would also occur after the expression of some autophagy-related genes (*e.g.*, HIF-1, tumor suppressor p53 and p16, cyclin-dependent kinase inhibitor p21, and Nrf2) was activated by transcription factors in the cytoplasm upon exposure to ROS [71,73].

At present, oxidative stress-induced autophagy has been widely suggested to be engaged in both onset and progression of some critical diseases, in particular cancer and many neurological disorders (*e.g.*, Alzheimer's disease, Parkinson's disease, and stroke) [74,75]. Of note, this process has seemingly contradictory roles as both cell survival and suppressor pathways. Autophagy has been proposed as an endogenously antioxidative strategy to clear the accumulated ROS through the aforementioned pathways, generally in the initiation of stress exposure. For example, autophagy was activated as a neuroprotective mechanism to decrease the ROS production and detoxify the oxidative toxicity [76]. Unfortunately, it might cause unexpected damages or other biological events under the stimulation of constant ROS burst. It has been observed that the accumulated ROS in cancer cells treated with chemotherapeutic drugs significantly activated cytoprotective autophagy, leading to persistent drug resistance and cancer cell survival. The following inhibition of autophagy restored the sensitivity of cancer cells to drugs [77]. Coincidentally, some studies also showed that pharmacological inhibition autophagy could delay the ROS burst in mitochondria and subsequently decrease brain injury [78].

Taken together, these studies highlight the complexity of the interaction between oxidative stress and autophagy and the essential roles of autophagy as a double-edged sword depending on the type and the stage of diseases associated with oxidative stress. An extensive knowledge will be necessary to balance these two cellular signaling mechanisms for more effective treatment of diseases.

Senescence

Cellular senescence is an essential biological phenomenon by which normal cells cease to divide, initially found by Leonard Hayflick and Paul Moorhead in the early 1960's [79]. It is the primary driver of aging-related dysfunction and pathologies that are implicated in many diseases such as cancer, diabetes, cardiovascular diseases, and neurodegenerative disorders [80,81]. Like cancer cells, senescent cells are able to diversify constantly and tend to make surrounding normal cells infected and senescent as a stress response, leading to dysfunction of tissues or organs [82]. It is becoming clearer that increased oxidative stress is a critical molecular mechanism for onset and stabilization of cellular senescence processes, thus promoting human aging and triggering the development of related diseases (Fig. 4C) [80,83,84]. Differing from the intrinsic "replicative senescence", which is provoked by endogenous stimuli (*e.g.*, extensive proliferation), diverse pathways are involved in the premature cellular senescence caused by oxidative stress as following:

(1) *Oxidative stress-dependent epigenetic modifications*. Oxidizing species (*e.g.*, $O_2^{\bullet-}$ and H_2O_2) have proven to be capable of regulating major epigenetic processes essential for all cell functions [85,86]. DNA methylation and histone acetylation are among two important epigenetic modification events, which have been widely observed during aging process. Numerous studies have illustrated the positive effects of ROS signaling on the induction of DNA methylation. It is well-known to be catalyzed by methyltransferases *via* positively charged

intermediate S-adenosyl-L-methionine. ROS could shape this process as a powerful nucleophile. In parallel, ROS could further increase the DNA methylation level *via* introducing methylation of the promoter in some critical age-related genes, such as PKC, SOD2, P16, and others.

(2) *Oxidative stress-dependent DNA (mtDNA) damage*. The theory of free radical in aging has been adapted to the study of cellular senescence [87]. As mitochondria is the most abundant cellular source of ROS, mitochondrial genome is thus regarded as the primary susceptible target for oxidative stress-induced mutations and deletions, resulting in progressive deterioration of mitochondrial function over time. Previous experiments in various cell culture models have illustrated that ROS, in particular •OH, has strong mutagenicity by damaging mtDNA *via* the formation of thymine glycol and 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxodG) from pyrimidine and guanine, respectively [88]. Furthermore, •OH could stimulate the occurrence of single-strand breaks, or even double-strand breaks, resulting in loss of the mtDNA integrity.

(3) *Regulation of age-related gene expression*. ROS can increase the expression of age-related genes [89], such as Ras, P53, P16, and P21. At the same time, ROS could potentially alter the functions of normal microRNA and activate miR-210 and miR-494. All these events modulate the cellular functions when cells are subjected to senescence.

(4) *Autophagy*. In addition to direct introduction of senescence, overexpression of P53 by excessive ROS could also inhibit intracellular autophagy process, contributing to mitochondrial dysfunction and subsequent cellular senescence [80,90].

It is worth noting that, similar to the case of cellular autophagy, senescence by itself can further aggravate oxidative stress in tissues and organs that harbor senescent cells. On the one hand, senescent cells secrete a series of senescence-associated secretory phenotype (SASP), which comprises of pro-inflammatory cystokines (*e.g.*, IL-6 and IL-8) and other bioactive peptides, through the stress-activated NF-κB signaling [91], thus enhancing the production of ROS and/or RNS. On the other hand, the activated DNA damage response in senescent cells can additionally cause senescence-associated mitochondrial dysfunction (SAMD) and increase ROS production. Importantly, SAMD would promote autocrine positive feedback loop and ultimately amplify the ROS-associated DNA damages for stabilizing senescence [92]. Moreover, the ROS/RNS released from senescent cells can accelerate the senescent process in normal cells under a senescent bystander effect [91]. These observations point to a clear relationship between oxidative stress and cellular senescence, yet more profound molecular explanations are highly demanded to better understand the roles and consequences of oxidative stress in cellular senescence and the related diseases.

Crosstalk with inflammatory signaling

The development of the systemic inflammatory response syndrome is a well-studied direct consequence in the body in response to oxidative stress caused by ROS/RNS and their metabolites [93]. Normally, acute inflammatory responses by the host are essential against foreign pathogens and beneficial for tissue repair, regeneration, and wound healing, but will become detrimental if they are unchecked and become self-perpetuating [10]. The evidence gathered from preclinical animal studies and clinical settings points to central roles of oxidative stress in inflammatory signaling [17], including rapid activation of immune cells, secretion of large amounts of pro-inflammatory cytokines, and infiltration of various inflammatory cells. In these events, ROS/RNS act as both signaling molecules and inflammatory mediators, which are closely correlated with a wide range of acute and chronic inflammatory diseases.

ROS derived from the NOX family and mitochondria were initially identified as the most important inflammatory mediators for triggering and regulating the amplitude of inflammatory responses (Fig. 4D) [94], which have been introduced in many reviews [95–97]. Since high expression of NOX family including NOX1, NOX4, DUOX1, and DUOX2 was observed in epithelial cells, liver, kidney, thyroid and exocrine glands, vascular tissues, and along mucosal surfaces (*e.g.*, lung and gastrointestinal tract), ROS produced by NOX overactivation significantly contribute to the vascular inflammation in chronic inflammatory diseases, cancer progression, fibrosis, atherosclerosis, and numerous other neurodegeneration disorders [42]. On the other hand, ROS derived from mitochondria can stimulate lipid peroxidation, resulting in further activation of phospholipase C and inositol trisphosphate-triggered calcium channels [98]. Meanwhile, mitochondrial ROS are able to regulate inflammasome to secrete inflammatory caspases and mediators (*e.g.*, TNF-α, IL-6, IL-1β, and IL-18) in macrophages. For example, the well-characterized form of inflammasome NALP3 has been reported to be highly sensitive to redox and can be rapidly activated by mitochondrial ROS, resulting in high-level TNF-α, IL-1β and IL-18 upon LPS stimulation [99]. Mitochondrial ROS can also disrupt endothelial functions through oxidation of crucial cellular signaling proteins such as tyrosine phosphatases. Collectively, mitochondria becomes one promising therapeutic target for the management of various oxidative stress-related diseases in the areas of interest to industry [100].

Recent advances in the understanding of chemical biology of oxidizing species have gradually recognized the key roles of RNS in mammalian immunity and inflammation. So far, •NO is the most widely studied and well understood RNS responding to cytokines and other signaling processes at inflammatory sites [101]. Originally, •NO was identified to be generated in the activated macrophages and neutrophils that represent the typical prototype of •NO-producing cells. Notwithstanding, some other cell subsets that belong to innate and adaptive immunity have further been documented to maintain the propagation of •NO through upregulation of different nitric oxide synthase isoforms (mainly from iNOS) [102]. Additionally, •NO rapidly reacts with ROS and produces the far more reactive $ONOO^-$ and other RNS as byproducts, which have been intriguingly associated with pathogenesis of many disease states. Of note, the immune effects of •NO and its metabolites are kind of self-contradictory. Under infectious conditions, these RNS predominantly display antimicrobial and antiviral activities through either immunostimulatory (pro-inflammatory) or immunosuppressive (anti-inflammatory) effect. Moreover, •NO has also shown the capability of reversing drug resistance by means of suppressing ATPase activity of p-glycoprotein in human cancer cells with multi-drug resistance. Conversely, in some other diseases such as chronic and/or acute inflammation, atherosclerosis, stroke, and diabetes, the upregulation of iNOS and excessive •NO and $ONOO^-$ in activated macrophages and neutrophils contribute to barrier breakdown and increased vascular permeability [103–106]. In cerebral ischemia, for instance, the continuously accumulated •NO in the ischemic area can orchestrate a series of pathological events including inflammatory responses, which lead to more ROS/RNS production, creating a 'vicious circle' with amplified injuries to the penumbral tissues (*e.g.*, blood brain barrier breakdown, the growth of cerebral edema, and the rise of intracranial pressure) [107,108].

To date, the crucial roles of ROS/RNS-derived oxidative stress in shaping immune activities have been well recognized. A full understanding of the ROS/RNS pathology in the immune system will provide clinically relevant markers and potential therapeutic targets for real-time monitoring of the therapeutic efficacy dur-

ing the treatment or post-treatment care of many inflammatory diseases.

Natural antioxidant defense and the limitations

The antioxidant has been defined as 'any natural or man-made substance that can prevent, decrease, or eliminate oxidative damage to a target molecule' by Halliwell and Gutteridge [109]. On the whole, antioxidants are roughly divided into two categories: endogenous biomolecules and exogenous compounds [110]. Indeed, there are varieties of endogenous antioxidative molecules (*e.g.*, antioxidative enzymes and low-molecular-weight molecules) in human body that play pivotal roles in maintaining intracellular redox homeostasis [26]. At the same time, the levels of these natural antioxidants represent important biomarkers that reflect the extent of oxidative stress in targeting cells and tissues [111].

SOD, catalase (CAT), and glutathione peroxidase (GPx) are among the most important enzymes in the natural antioxidant defense. SOD is well known to catalyze the dismutation of $O_2^{\bullet-}$ into O_2 and H_2O_2, and the byproduct H_2O_2 undergoes decomposition into water and molecular O_2 in the presence of CAT. In parallel, GPx is capable of converting peroxides (*e.g.*, H_2O_2 and fatty acid hydroperoxides) into alcohols, oxidized glutathione dimers, and water, in the presence of reduced glutathione as the substrate. Combination working of CAT, SOD, and GPx is generally required for the prevention of oxidative damage. Therefore, measurement of these three enzymes could be used to evaluate the redox status in the target of interest, and any activity imbalance of these catalytic enzymes has thus been implicated in various disorders, particularly in neurodegenerative diseases [112]. However, the levels of these enzymes are normally not high enough to match excessive radical formation, and their adequate replenishment is generally impossible under oxidative stress burst [113]. Moreover, their antioxidative activities are susceptible to dysregulated redox microenvironments and pH changes, and some of them are only capable of scavenging specific ROS/RNS (*e.g.*, $O_2^{\bullet-}$ and H_2O_2) [7]. In addition, there is no specific enzyme that targets the secondary ROS/RNS (such as $^{\bullet}OH$ and $ONOO^-$).

Another important class of natural antioxidants in human body is endogenous macromolecules (*e.g.*, bilirubin, selenoprotein, albumin, and ferritin) and small biomolecules (*e.g.*, glutathione, cysteine, and other biothiols, α-lipoic acid, vitamins, ascorbic acid as well as its oxidized product dehydroascorbic acid) [114]. Indeed, these endogenous biomolecules have been considered as biomarkers of oxidative stress and antioxidative therapeutics for many years [111]. For example, antioxidants vitamins including vitamins A, C and E have thus far received most extensive studies, and can be administrated in a large dose and for extended periods of time. Recently, vitamin D has emerged as an antihypertensive effector with the capability of decreasing renin-angiotensin aldosterone system activity, modulating endothelial function, and regulating vascular oxidative stress. Besides, α-lipoic acid, a naturally synthesized dithiol that acts as an essential cofactor for enzymes involved in the mitochondrial bioenergetics, has been clinically approved and currently used in Germany for the treatment of diabetic neuropathy [115]. Although these bioactive molecules serve to prevent or reduce oxidative damage, they are also vulnerable to redox and pH status, which might explain their limit clinical benefits. Even worse, high-dose supplements of these biomolecules might be harmful in some cases. For example, high-dose supplement of vitamin E carries the risk of bleeding for those patients who are simultaneously taking anticoagulant [116]. Some other safety concerns, such as increased hemorrhagic stroke and cancer incidence, have also been indicated.

In addition to these natural antioxidants, plenty of exogenous antioxidants such as synthetic compounds and molecules arising from food supplements have also demonstrated the potential in antioxidant therapy, by targeting various cellular signaling pathways and upregulating endogenous antioxidant defense. Notably, such antioxidants have not been clinically validated or have failed to prevent oxidative stress-caused damages, which is presumably due to their activity or safety concerns. These barriers highlight the importance of developing innovative antioxidative strategies to broadly and efficiently target various ROS/RNS species for effective antioxidant therapy.

Antioxidative nanomaterials and ROS/RNS-targeting mechanisms

The use of antioxidative nanomaterials has recently attracted considerable interest as a novel class of antioxidants, owing to many advantages of nanomaterials over conventional antioxidants including (Fig. 5), but not limited to the following: (i) nanomaterials can significantly improve the antioxidative stability of natural antioxidants and their pharmacokinetics, by protecting these agents from environmental oxidation/reduction and acid attacks; (ii) many nanomaterials by themselves have enzymatic activities with much higher catalytic efficiency and tolerance to environmental changes than enzymes and small molecules; (iii) emerging nanomaterials have demonstrated robust scavenging capabilities of multiple radicals, especially the secondary ROS/RNS, providing a broad antioxidant defense against diverse ROS/RNS for more effective treatment of oxidative stress-related diseases; (iv) by integrating diagnostic function into a single antioxidant nanoparticle (NP), the multifunctional antioxidative nanomedicine is capable of monitoring the pharmacokinetics and accumulation, yielding important insights into the pathological process longitudinally for predictive and personalized antioxidant therapy; and (v) facile preparation and storage of antioxidative nanomaterials allow for potential pharmaceutical industry. Thus far, numerous antioxidative nanomaterials have been reported (Table 1 and Table 2) and shown promising preclinical therapeutic effects in a wide range of oxidative stress-associated disease models. In this section, we will provide an overview of different antioxidative nanomedicines regarding radical scavenging activity, mechanisms, and influence factors to better understand this emerging field.

Antioxidant delivery nanomaterials

Natural antioxidant delivery nanomaterials

Given the vulnerable activities of natural antioxidants, attempts have initially been made to develop natural antioxidant delivery nanomaterials, aiming for effective delivery of antioxidants to targeted cells or tissues. Nanotechnology-mediated delivery of SOD has received great attention, as $O_2^{\bullet-}$ is the primary ROS overproduced in various diseases. Actually, native and modified SOD proteins have been investigated as antioxidative therapeutics in clinical trials. However, the efficacies of SOD-based antioxidant therapy are currently conflicting and many clinical trials in large animals have failed to demonstrate a significant therapeutic benefit [117,118]. One of the possible reasons is the unfavorable pharmacokinetics and the short half-life of SOD (the circulating half-life of wild type bovine SOD1 in the blood is only about six minutes) [119]. To this end, various SOD-loaded NPs have been reported [120–126]. For instance, SOD encapsulated in the biodegradable polyketal (PKSOD) microparticles has shown significantly prolonged circulation time and retention in the ischemic heart after reperfusion, under protection by the microparticles against proteolytic degradation [120]. The as-prepared PKSOD microparticles

Table 1
Representative antioxidative nanomaterials and their antioxidative mechanisms and biomedical applications.

Antioxidative nanomaterials	Categories		Antioxidative mechanisms	Indications	Refs.
Antioxidant delivery nanomaterials	Natural antioxidant delivery nanomaterials		Depending on the activity of the delivered enzyme or small molecule	Cancer, diabetes, inflammatory bowel disease, cardiovascular diseases, lung transplantation injury	[120,126,138,169,284,294]
	Antioxidative unite-containing polymer nanomaterials		Mainly focusing on H_2O_2	Atherosclerosis, cancer, ischemia-reperfusion injury	[146,150,151,154,160,170]
	Antioxidative gas delivery nanomaterials (e.g., H_2)		Selective scavenging •OH and $ONOO^-$, but inert to $O_2^{\bullet-}$ and H_2O_2.	Cancer, tissue inflammation, ischemia-reperfusion injury	[175–178]
	Antioxidative gene delivery nanomaterials		Silencing Nox family, apoptosis, and inflammation-related genes	Traumatic brain injuries, brain ischemia-reperfusion injury, myocardial infarction, DSS-induced colitis	[182–184,303]
Nanomaterials with instrinsic antioxidative activity	Metal-based NPs	Nobel metal NPs (e.g., Au, Pt, Pd, and their alloy)	Peroxidase, catalase, or oxidase mimic activity	Cancer, Antibacterial, tissue inflammation	[190–194,207–209, 215,220,321]
		Metal oxide NPs (CeO_x, Fe_3O_4, ZrO_2, MnO_x, V_2O_5, and mixed metal oxide NPs, etc.)	CeO_x: multiple ROS and RNS targeting Fe_3O_4: peroxidase and catalase like activities MnO_x: peroxidase and catalase like activities ZrO_2: GPx-like activity	Acute kidney injury, ischemic stroke, cancer; sepsis, Parkinson's disease, Alzheimer's disease, type-2 diabetes, obesity, wound healing, other inflammation	[199–206,227–234, 188,189,210,211,218]
		Transition metal sulfides (MoS_2, $MoSe_2$, WS_2, WSe_2)	$O_2^{\bullet-}$, •OH, and ABTS radicals	Acute kidney injury, Alzheimer's disease,	[244–248]
		MOF (e.g., Zn^{2+}, Co^{2+}, Cu^{2+}-modified nMOFs)	Peroxidase, GPx or laccase like activity	Wound healing in diabetes	[196–198]
	Carbon-based NPs	Fullerene, graphene, carbon nanotube, carbon nanoclusters, heteroatom-doped carbon	Typical scavenging capability of $O_2^{\bullet-}$ and •OH	Ischemic stroke, tissue inflammation, obesity, traumatic brain injuries, radial injuries, bacterial infections	[249–262]
	Bio-inspired polymer NPs	PEG-modified bilirubin NPs	Multiple ROS and RNS targeting	Inflammatory diseases, diabetes	[269–273]
		PEG-modified melanin NPs	Multiple ROS and RNS targeting including $O_2^{\bullet-}$, •OH, H_2O_2, •NO, and $ONOO^-$	Ischemic stroke	[274]
		DNA and analogue nanostructures	Unclear antioxidative mechanism	Acute kidney injury	[287]

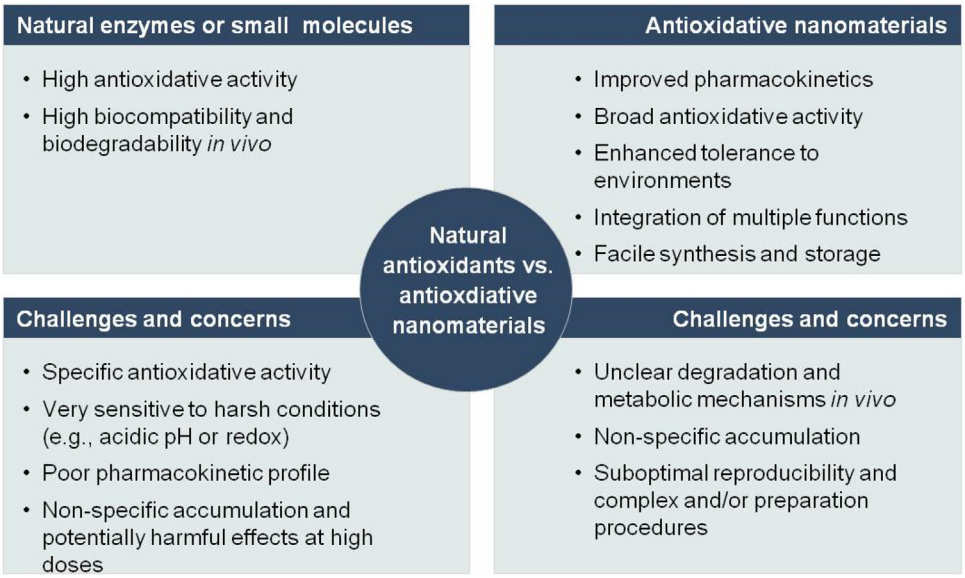

Fig. 5. Comparison between the natural antioxidant defense and nanomaterial-based antioxidants.

Table 2
Overview of the features and limitations of various antioxidative nanomaterials.

Categories		Features	Limitations
Antioxidant delivery nanomaterials	Natural antioxidant delivery nanomaterials	High antioxidative activities inherent from natural antioxidants	Specific or unstable antioxidative activities; Limited loading efficiency
	Antioxidative unite-containing polymer nanomaterials	Stimuli responsive release of antioxidative unite for controlled antioxidation; Improved biocompatibility and biodegradability	Unclear RNS targeting abilities
	Antioxidative gas delivery nanomaterials	Rapidly diffusing into tissues and crossing membranes; Selective scavenging of $^{\bullet}OH$ and $ONOO^{-}$; Capable of regulating antioxidative signaling pathways	Relatively narrow antioxidative defense
	Antioxidative gene delivery nanomaterials	Enhanced antioxidative selectivity; High efficiency	Potential adverse effects from gene nanocarriers
Nanomaterials with intrinsic antioxidative activity	Metal-based antioxidative nanomaterials	Multiple radicals scavenging; Controlled and pH-switched enzymatic activities; Reversible antioxidative effects of some metal-based nanomaterials (e.g., Ce NPs)	Non-degradability and long-term retention *in vivo* upon administration; Potential metal-induced toxicity
	Carbon-based antioxidative nanomaterials	Multiple radicals scavenging; Low cost and easy operation; Outstanding antioxidative stability against stringent conditions	Non- (or quite slow) biodegradability; Susceptible activities to many factors (e.g., pH, temperature, and physicochemical properties of materials); Inert to $^{\bullet}NO$ and $ONOO^{-}$; Safety concerns
	Bioinspired polymer-based antioxidative nanomaterials	Good biocompatibility and biodegradability; Broad and stably scavenging activities towards various ROS/RNS under different conditions	The development of such material is currently very limited; Unclear metabolism routes and long-term safety risk *in vivo*

reduced both extracellular and intracellular $O_2^{\bullet-}$ levels and sustainably release SOD within the myocardium in a mouse model of ischemia/reperfusion. Notwithstanding, SOD-loaded NPs can only detoxify the oxidative damages from $O_2^{\bullet-}$, concurrently promoting the release of H_2O_2 as byproducts and therefore triggering additional oxidative injury. In order to further eliminate the accompanied H_2O_2, CAT and SOD were subsequently co-delivered using different nanocarriers such as lipid NPs, inorganic NPs, and polymer NPs, which have proven to be more potent than those loaded with single enzyme [127–131]. In order to avoid disturbance of ROS signaling in normal cells, targeted delivery of these enzymes has further been studied using NPs functionalized with monoclonal antibodies, small molecules, peptides, among others [132–134].

Meanwhile, low-molecule-weight antioxidative molecules, such as vitamins and ascorbic acid, have also received extensive studies [135,136]. FDA-approved poly(lactic-co-glycolic acid) (PLGA) has received great attention for constructing nanoantioxidants, given its biocompatibility, biodegradability, and tunable mechanical properties [137,138]. Nevertheless, the use of these nanoantioxidants still faces incredible challenges, largely due to their hydrophobicity and subsequently poor loading capacity of these hydrophilic antioxidative molecules. In contrast to hydrophobic polymers, liposomes offer an advantage in the encapsulation

and delivery of hydrophilic antioxidative molecules, given the unique lipid bilayer structure of liposomes favorable for delivering both hydrophilic and hydrophobic drugs. Notably, liposomes also pose limitations including low solubility, short half-life, and high production costs. Moreover, the phospholipids in liposomes are subjected to oxidation, resulting in potential leakage of encapsulated molecules, especially for low-molecular-weight molecules. Another obstacle for these antioxidative nanomedicines arises from cytotoxicity of these small molecules, as we discussed above.

As a vitamin analogue, Coenzyme Q10 (CoQ10) is another well-known radical scavenger that is endogenously synthesized in virtually all types of cells, whose primary role is to act as an essential intermediate within the mitochondrial oxidative respiratory chain [139]. It is generally considered safe, and has intrinsic ability to scavenge free radicals and inhibit lipid peroxidation. CoQ10 was also recognized to have significant impact on the expression of many genes mainly involved in cell signaling, metabolism, and inflammation. Oral administration of CoQ10 has been approved by FDA and is now a frequent antioxidative strategy in many diseases with the potential to provide a significant symptomatic benefit. Nevertheless, the bioavailability of CoQ10 is generally low, due to its large molecular weight (863 Da), strong hydrophobicity, and thermolability, thus demanding administration at high doses. To address this problem, various nanoformulation strategies like micelles, CoQ10-cyclodextrin complex, liposomes, solid lipid NPs, and polymer NPs have been developed to improve the pharmacokinetics, biodistribution, and ultimately the therapeutic efficacy of CoQ10 [140–144]. Of note, the molecular mechanisms underlying the pleiotropic effects of CoQ_{10} nanoformulations have yet to be completely understood.

To further improve the loading capacity of antioxidants and minimize the toxicity of nanocarriers, researchers have attempted to directly utilize antioxidants as the unit for antioxidative nanomaterials. One representative example is a self-assembled micellar nanocomplexe containing green tea catechin developed by Chung and co-workers [145]. In their work, (−)-Epigallocatechin-3-O-gallate (EGCG), an ingredient of green tea with multifunctions including antioxidant, anti-inflammation, and neuroprotective effects, was used to produce micellar nanocomplexe along with an anticancer protein and poly(ethylene glycol)-EGCG through a self-assembly method for combined therapy. Such strategy hold a great promise to drastically enhance the therapeutic efficacy and lower the toxicity concern associated with the carriers.

Antioxidative unite-containing polymer nanomaterials

Apart from the aforementioned natural antioxidant-loaded polymer NPs, there is another category of antioxidative polymer nanomedicine with synthetic antioxidative moieties integrated into the polymer chains (Fig. 6). These antioxidative polymer nanomaterials may be roughly divided into the following two categories:

(i) Phenol group-containing polymer nanomaterials. Phenolic compounds in plants have long been suggested as effective antioxidants due to the low bond dissociation energies of O—H, and can protect plants against UV radiation-induced oxidative injury by scavenging H_2O_2. Mechanisms underlying the H_2O_2 scavenging of phenol groups could be attributable to their capability of accelerating the decomposition of H_2O_2 into water [146–150]. The primary mechanism is directly linked to two steps [151]. The first involves the donation of a hydrogen atom or electron to free radicals as below:

$$Radical^{\bullet} + ArOH \rightarrow Radical(H) + ArO^{\bullet}$$

$$Radical^{\bullet} + ArOH \rightarrow Radical(H)^{-} + ArOH^{\bullet -}$$

The resulting phenoxyl radicals will then react with radicals for additional scavenging. In general, the H_2O_2-scavenging activities of these compounds are largely dependent on the hydrogen and electron-donating ability of phenolic compounds and the stability of the formed phenoxyl radicals after dehydrogenation. When the hydrogen atoms in aromatic rings are substituted by electron-donating hydroxyl groups, the nucleophilicity of phenoxyl radicals will be increased. As a consequence, the H_2O_2-scavenging ability of these phenolic compounds would be enhanced. Nevertheless, the stability of the phenoxyl radicals is also affected by the substitution position. Among different types of phenolic compounds, catechols with two hydroxyl groups in ortho position were demonstrated to be the most effective antioxidants [152]. Beyond direct ROS scavenging, monophenols and polyphenols have also been reported to be able to bind transition metal ions as inert complexes, leading to the regeneration of the potent chain-breaking α-tocopherol for further antioxidation [153].

Notwithstanding effectiveness, drawbacks have emerged over time. Phenolic compounds tend to be easily oxidized, especially in the oxygen atmosphere. To solve these limitations, these phenolic compounds have been encapsulated within nanocarriers. For instance, phenolic compounds have been formulated into lipid-based nanocarriers including nanoemulsions, liposomes, and solid nanostructures (Fig. 6A). These formulations could dramatically improve the pharmacokinetics of phenolic compounds and increase their bioavailability, solubility, and stability [154]. To further achieve controlled antioxidation, many studies have been carried out to construct stimuli-responsive polymer NPs by integrating phenolic compounds into the polymer backbone. For instance, tannic acid, a promising antioxidative polyphenol, was covalently conjugated to the poly(methacrylic acid) using ethylene glycol dimethacrylate as a biodegradable cross-linker, leading to antioxidative polymer NPs with controllable tannic acid release and activity for more effective antioxidative protection compared to free tannic acid [155]. Alternatively, phenols have been directly polymerized into the polymer chain with ROS-responsive linkers (Fig. 6B) [156–158]. Under physiological conditions, these polymer NPs were stable, yet released free phenolic compounds under redox microenvironments.

(ii) Free radical trapper-containing polymer nanomaterials. During examination of the free radical scavenging abilities of antioxidants, different radical trappers are typically used in EPR experiments, depending on the types of tested free radicals. Indeed, these ESR trappers can be used as a novel class of free radical scavengers. One sample is 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO), a well-known nitroxide radical-containing ROS trapper (*e.g.*, $O_2^{\bullet -}$ and $^{\bullet}OH$). It is a stable radical, and can capture the unpaired electrons in other free radicals [159]. Efforts have thus been devoted to developing antioxidative polymer nanomaterials by integrating TEMPO or its derivatives into the polymer backbones [160–167]. Commonly, TEMPO has been covalently conjugated to an amphiphilic polymer chain by polymerization or postmodification strategies. After conjugation, the polymer NPs inherited the scavenging capabilities of the free TEMPO molecule towards $^{\bullet}OH$, $O_2^{\bullet -}$, and H_2O_2, yet showed improved pharmacokinetics *in vivo*. In another work, Nagasaki et al. have developed a pH-responsive polymer micelle (RNP^{pH}) using amphiphilic block copolymers that containing TEMPO moiety in the backbone *via* amine linkage in a side chain of the hydrophobic segment [160,168]. Under physiological condition, the antioxidative effects of TEMPO moieties in RNP^{pH} were blocked. In contrast, the assembled RNP^{pH} undergo disintegration at pH below 7.0 as the result of amino group protonation in the hydrophobic core, leading to the release of TEMPO for enhanced ROS scavenging (Fig. 6C). Such "off-on" strategy holds promise to improve the therapeutic efficiency and minimize side effects.

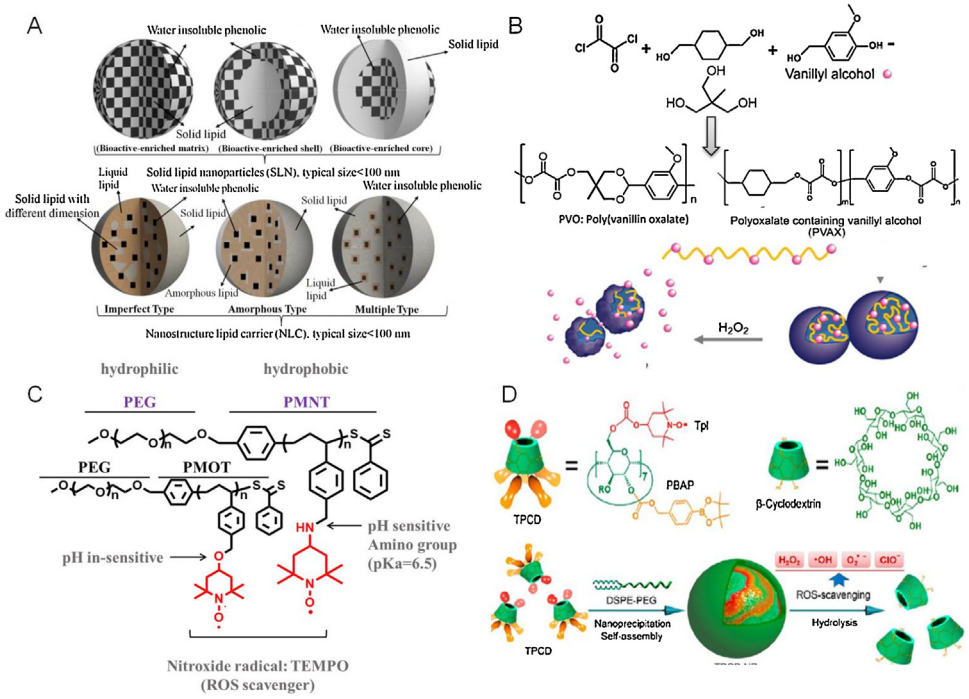

Fig. 6. (A) Schematic illustration of various lipid-based nanocarriers for loading phenolic compounds. Reprinted with permission from Ref. [154]. Copyright 2018, Elsevier. Examples of polymer nanomaterials composed of the polymer chain containing (B) antioxidative phenol groups (Reprinted with permission from Refs. [156–158]. Copyright 2016, 2014, and 2013, Elsevier), (C) free radical trapper (TEMPO or its derivative) (Reprinted with permission from Ref. [160]. Copyright 2018, Elsevier), and (D) both phenol and free radical trappers (Reprinted with permission from Ref. [170]. Copyright 2018, American Chemical Society).

To further extend the antioxidative efficacy, Hu and co-workers have recently designed an innovative strategy by combining a TEMPO analogue with antioxidative boronic groups to concurrently target multiple reactive species (Fig. 6D). This novel antioxidant was synthesized by covalently conjugating phenylboronic acid pinacol ester and Tempol onto the β-cyclodextrin scaffold (abbreviated as TPCD) [169,170]. Tempol is a SOD-mimetic agent, while phenylboronic acid pinacol ester is a H_2O_2-eliminating compound. Benefiting from the synergistic antioxidation of these molecules, the assembled TPCD NPs showed broad-spectrum ROS-scavenging and effectively scavenged multiple ROS including H_2O_2, $^{\bullet}OH$, $O_2^{\bullet-}$, and HOCl, leading to reduced oxidative stress, decreased inflammation and inflammatory cell infiltration in atherosclerotic plaques. Notably, the antioxidative efficiency of radical trapper-containing polymer NPs is largely limited to ROS, and their RNS scavenging abilities remain largely unexplored.

Antioxidative gas delivery nanomaterials

The concept of using gas for therapeutic purposes has been suggested as a "green" therapeutic approach and showed promising results in treating many diseases, because gaseous molecules, such as oxygen (O_2), hydrogen (H_2), nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S), can modulate many important physiological processes [171]. H_2 is among one important physiological regulator with antioxidative, anti-inflammatory and anti-apoptotic effects, and has been applied for the prevention of oxidative stress-induced damages in almost all organs [172]. The unique features of H_2-based therapy include (i) rapidly diffusing into tissues and crossing membranes to reach subcellular compartments (*e.g.*, mitochondria and nuclei), which are closely associated with ROS generation and DNA damage; (ii) selectively scavenging the most active ROS, $^{\bullet}OH$ and $ONOO^-$, yet having no obvious activity toward $O_2^{\bullet-}$ and H_2O_2; (iii) increasing antioxidative enzymes such as CAT and SOD; (iv) inhibiting expression or activation of pro-apoptotic factors (*e.g.*, caspase proteins 3 and 8, and B-cell lymphoma-2); (v) downregulating pro-inflammatory and inflammatory cytokines (*e.g.*, TNF-α, IL-1β and IL-6); and (vi) acting as a gaseous signal modulator for multiple pathways (*e.g.*, MEK1/2-ERK1/2, NF-κB, JNK, and Nrf2) [172,173]. Inhalation of H_2 with air and administration (oral or intravenous drip infusion) of H_2-riched water are two common strategies for H_2-based therapy. Nevertheless, the absorbed H_2 by the body through these strategies is very limit and might be insufficient for eliminating the overproduced ROS, because inhalation may not be suitable for continuous H_2 administration for safety concern (explosive risk upon mixing with air), and H_2 has relatively low water solubility [174]. To address these limitations, attempts have recently been made to develop H_2 delivery nanoplatforms, aiming to improve local therapeutic concentration of H_2 in the diseased sites. One frequently used method is using nanocarriers to deliver molecular H_2. For example, He et al. fabricated an ultrasound-visible H_2 delivery platform by loading H_2 within octafluoropropane-based microbubbles (H_2-MBs) [175]. This strategy allows for not only three-fold higher H_2 content per unit volume of solution compared to H_2-saturated saline for more efficient scavenging of $^{\bullet}OH$ in living tissues, but also real-time monitoring of delivery process using ultrasound imaging (Fig. 7A). Nonetheless, concerns of this strategy still remain including low H_2 loading capacity and suboptimal stability (about 10 min). An alternative approach is in-situ generation and spatiotemporally controlled release of H_2 by stimulus. He and coworkers reported local generation of H_2 by using $PdH_{0.2}$ nanocrystals with self-catalysis, bio-reductivity, and high near-infrared (NIR) photothermal conversion effect [176]. Superior to previous study, this nanoplatform released bio-reductive H_2 under NIR irradiation *via* a self-catalysis process in a power-dependent manner, whereas very low level H_2 was released at physiological conditions (Fig. 7B). In another typical example, Au NPs, ascorbic acid, and chlorophyll co-delivered liposomes were developed for photocatalytic production of H_2, inspired by natural photosynthesis [177]. Upon irradiation, the photosensitizer chlorophyll would be excited to generate an

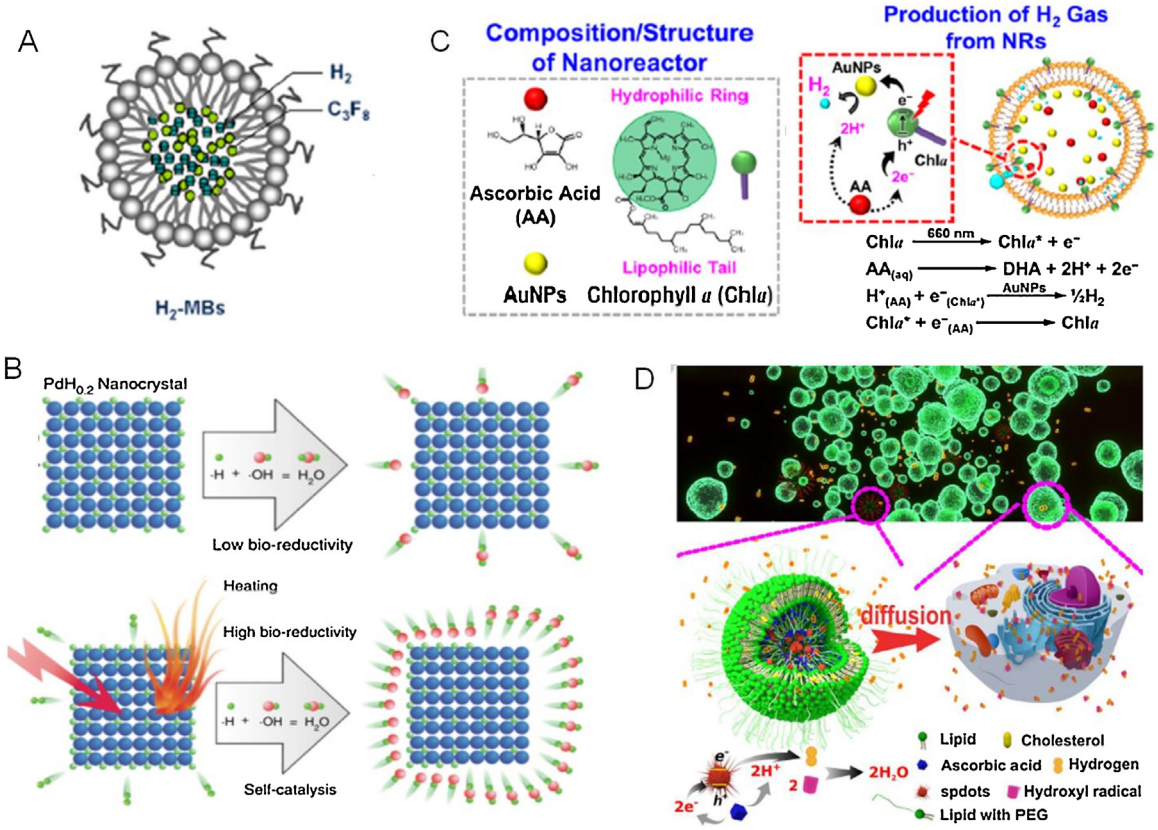

Fig. 7. (A) Schematic illustration of the structure of H_2-MBs. Reprinted with permission from Ref. [175]. Copyright 2017, American Chemical Society. (B) NIR-triggered H_2 generation from $PdH_{0.2}$ nanocrystals. Reprinted with permission from Ref. [176]. Copyright 2018, Nature. (C) The mechanisms behind the H_2 generation by the photosynthesis mediated by liposome-based nanoreactors. Reprinted with permission from Ref. [177]. Copyright 2017, American Chemical Society. (D) Schematic illustration of Pdots compartmentalized liposomes as photocatalyst for *in situ* generation and release of H_2. Reprinted with permission from Ref. [178]. Copyright 2019, Wiley.

electron-hole pair. The generated hole would subsequently accept an electron from ascorbic acid to return back its ground state. The Au NPs were used as the catalyst to collect electrons and protons from the excited chlorophyll and the oxidized ascorbic acid to produce H_2 (Fig. 7C). Likewise, Zhang et al. recently described a light-driven hydrogen evolution strategy using semiconducting polymer dots (Pdots)-loaded liposomes [178]. Meanwhile, ascorbic acid was also loaded in the liposomes as a sacrificial electron donor. Upon light irritation, the charge transfer between the Pdots and donor was initiated for *in situ* generation of H_2. The formed H_2 could diffuse across the lipid bilayer to counteract $^{\bullet}OH$ overexpressed in diseased tissues to produce non-toxic H_2O (Fig. 7D).

In short, the gas-based antioxidative nanotechnology, to the best of our knowledge, is currently limited to the delivery of H_2, and the potential antioxidative effects of other gases remain largely unexplored. As aforementioned, H_2 can only target $^{\bullet}OH$ and $ONOO^-$, and thus H_2-based antioxidative strategy might not be powerful enough in those diseases with burst of other radicals or other reactive species. Furthermore, how to control the H_2 delivery more specifically and whether H_2 would interrupt normal functions and metabolism of endogenous gaseous signaling need to be carefully investigated in future studies.

Antioxidative gene delivery nanomaterials

The development of antioxidative gene delivery nanomaterials is a new area in antioxidant therapy. Strikingly, gene-mediated antioxidative strategy has the potential to essentially and specifically inhibit the oxidative stress-induced damages without significant unexpected interference in normal redox metabolism and signaling. Thus far, this field is still in its infancy, and few efforts have been made to deliver therapeutic nucleic acids such as plasmid DNA [179,180], siRNA [181–183], or pDNA [184] mediated by nanotechnology to regulate oxidative stress-related genes for the treatment of different human diseases.

One direct antioxidative gene therapeutic strategy is to selectively upregulate the expression of antioxidative enzyme genes, given the critical roles of natural antioxidant enzymes. For instance, co-delivery of CAT and SOD plasmid DNAs using liposomes has been investigated [180]. The as-prepared antioxidative liposomes could efficiently suppress the overproduction of $O_2^{\bullet-}$ and H_2O_2 and significantly attenuate oxidative stress in a mouse model of retinal ischemia-reperfusion. An alternative approach is to knockdown specific genes responsible for ROS/RNS production or their upstream signaling pathways. As aforementioned discussion, one promising gene target is the NOX family, the major source for mitochondrial ROS production. Very recently, Davis et al. have constructed acid-degradable polyketal nanoplatform for co-delivery of NOX2-specific siRNA and an endosome-disruptive molecule to the cardiac macrophages in the infarct heart. NOX2 acts as the active center for NADPH oxidase, which contributes to the burst of $O_2^{\bullet-}$ in the infarct heart [182]. The silencing of NOX2 in cardiac macrophages mediated by the acid-degradable polyketal nanoplatform significantly inhibited the activity of NADPH oxidase, thus suppressing the production of $O_2^{\bullet-}$ in the infarct heart. Similarly, another study reported by Camici et al. utilized injectable PEI as the vector for complexing siRNA against $p66^{Shc}$ [183], an isoform of the mammalian adaptor protein Shc that mediates the production of ROS in several disease states. Knockdown of $p66^{Shc}$ was found to efficiently blunt NADPH activity and ROS generation. To further improve the targeting delivery and therapeutic effect, Bhatia et al.

have engineered a peptide-based RNAi nanosystem composed of a membrane interactive peptide and a targeting peptide towards caspase 3, whose activation has been suggested as a critical mechanism for oxidative stress-induced apoptosis in many different diseases. In that work, these peptide-based targeted NPs could efficiently deliver caspase 3 specific siRNA into the tissue adjacent to the injured site and knockdown the expression of caspase 3, thus decreasing the oxidative stress-induced apoptosis. To amplify the antioxidative efficacy, a recent work reported the design of using cationic amphiphile incorporating vitamin C or E as hydrophilic heads for gene delivery [184]. The transfection efficiency of these antioxidative liposomes was found to be comparable or even higher than that of the commercial transfection agent. With the strong radical scavenging capabilities of vitamin C and vitamin E, these liposomes could be a promising non-viral gene delivery platform for antioxidant therapy.

Despite high efficiency and specificity, currently studied gene therapeutic nanomedicines typically require the use of cationic lipids or compounds for efficient endosomal escape of the therapeutic genes. However, the potential toxicity associated with those cationic materials is one of the most important barriers limiting the clinical translation of gene therapy, which may be ascribed to, as least in part, the promotion of ROS generation and subsequent deleterious effects on antioxidant therapy. The development of novel powerful cationic compound-free nanocarriers is highly desirable for translational purposes. Additionally, antioxidative strategies through specific regulation of oxidative stress-related genes are rare, and continuous efforts also need to contribute to the exploration of other potential cell signaling pathways linking oxidative stress to ensure adequately gene-mediated antioxidative intervention.

Nanomaterials with intrinsic antioxidative activity

Metal-based antioxidative nanomaterials

The inherent enzymatic properties of some specific metal and metal oxides have been known for decades. These metal-based antioxidative nanomaterials were also defined as nanozymes by Yan and coworkers in 2007, when iron oxide nanomaterials by themselves could act as a peroxidase mimic. Thereafter, various metal and metal oxides have been rapidly developed as artificial enzymes (Table 1). In this part, these metal-based antioxidative nanomaterials will be overviewed accordingly to their enzymatic activities, with a highlight on recent advances in this field.

(i) Peroxidase-like metallic nanomaterials. Yan and coworkers are the first investigators that found intrinsic horseradish peroxidase (HRP)-like activity of ferromagnetic NPs that was similar to natural HRP at a pH of 3.5 [185]. This study has opened a new way for developing novel effective artificial antioxidants to replace natural enzymes. Till date, a large variety of metallic nanomaterials have showed peroxidase-like activities, for instance, metal oxides [186–189], noble metal NPs [190–194], metal sulfides [195], and metal organic frameworks (MOFs) [196–198].

V_2O_5 nanomaterials have recently received much attention as a novel category of metal oxide-based antioxidative nanomaterials. Mugesh and coworkers reported that V_2O_5 nanowires exhibited GPx-like activity in the presence of GSH and could protect cells from oxidative damage [188,189]. Following mechanistic investigation indicated that GSH could be bund to the surface of V_2O_5 nanowires for reduction of H_2O_2, yielding a stable sulfenate-binding intermediate that would subsequently undergo hydrolysis into a dihydroxo intermediate. The dihydroxo intermediate would further react with H_2O_2, and the byproduct would in turn participate in next-circle reduction reaction, similar to the case of natural GPx. In addition, fine tuning of enzymatic activity of V_2O_5 could be achieved by engineering the surface of V_2O_5 nanomaterials. The authors further prepared four V_2O_5 nanocrystals with different morphologies including nanowires, nanoflowers, nanosheets, and nanospheres. A careful comparison of the reactivity of the V=O bond in these four types of nanocrystals towards H_2O_2 suggested that the formation of V-peroxido species was most favored on the surface of nanospheres, which demonstrated the highest activity in the reduction of H_2O_2 among four samples. More impressively, the V_2O_5 nanospheres are capable of reducing H_2O_2 for multiple cycles without significant loss of catalytic activity, and their activity after storage in water for six months was still comparable to that of freshly prepared materials. Subsequent detailed atomic-level understanding of V_2O_5 surface properties has revealed that the variations in the GPx-like activity were largely ascribed to the different formation rates of V-peroxido on the nanostructure surface.

MOFs are another class of metal-based antioxidative nanomaterials that emerge in recent years. The highly porous structures of MOFs would facilitate the exposure of active sites inside the nanostructures to free radicals, thus allowing for effective antioxidation. Recent efforts have also been made to develop MOFs as enzyme mimic. Initially, metal ions such as Cu^{2+} and Fe^{3+} were incorporated into MOFs as part of the frameworks, which enabled peroxidase-mimicking functions of MOFs [198]. Differing from this strategy, Willner and co-workers utilized bipyridine, a metal-ion ligand, as the crosslinking unit, for coordination with metal ions to yield nanoscale MOFs with catalytic activities in analogy to HRP and NADH peroxidase [197]. Despite potent H_2O_2 scavenging, the particle size of currently studied antioxidative MOFs is relatively large for *in vivo* applications. In addition, the suboptimal solubility and stability under physiological conditions remain concerns for biomedical studies.

(ii) Catalase-like metallic nanomaterials. Cerium NPs, also referred to as nanoceria or CNPs, are well-known CAT-like metallic nanomaterials. Among various metallic antioxidative nanomaterials, the antioxidative mechanisms of CNPs have been widely studied [199–205]. Specifically, the coexistence of Ce^{3+} and Ce^{4+} contributes to the formation of oxygen vacancies, which could act as the catalytic centers. Accumulated evidence has indicated that their free radical scavenging activities largely depend on reversible binding oxygen and shifting between two valent states of Ce^{3+} (the reduced form) and Ce^{4+} (the oxidized form) on the surface of NPs [206]. The catalytic process follows an exhaustive catalytic mechanism, involving initial reduction of Ce^{4+} into Ce^{3+} by H_2O_2, accompanied by combination of oxygen vacancies with H_2O_2 for Ce^{3+} oxidation. Apart from CNPs, a number of other types of metallic nanomaterials have demonstrated striking CAT mimic behaviors, ranging from noble metal NPs (*e.g.*, Ag and Pd) [207–209] to metal oxide NPs (*e.g.*, Fe_3O_4 and MnO_2) [210,211]. Moreover, the rapid advance in nanotechnology has been broadening the way of exploring other novel metallic nanomaterials with intrinsic enzymatic features. For instance, a recent paper reported, for the first time, bovine serum albumin-stabilized IrO_2 NPs as a novel CAT-like antioxidative nanomaterials, based on the highly catalytic activity of IrO_2 NPs for oxygen evolution reaction over a broad pH range [212]. However, the catalytic mechanism was currently unclear.

(iii) Oxidase-like metallic nanomaterials. Relative to peroxidase- and CAT-like metallic nanomaterials, oxidase-mimicking metal-based antioxidative nanomaterials are limited [213–220]. In addition to CAT activity, noble metal NPs including Au, Ag, Pd, Pt and their alloys NPs are also well-known glucose oxidase-like antioxidants, producing gluconate and H_2O_2 [215]. Using the density functional theory (DFT) simulation, a plausible mechanism associated with simple reaction-dissociation of the absorbed molecule oxygen on the surface of these NPs was proposed [206]. Two steps are involved in this mechanism. Oxygen molecule is first dissociated into oxygen adatoms under the catalysis of metals, and the generated oxygen adatoms would absorb

hydrogen from substrates. In combination with experimental verification, the oxidase-like activity of these noble metal NPs was found to be significantly dependent on the metal compositions and exposed facets. Derived from redox state conversion of catalytic metal ions, some other types of metallic nanomaterials have also shown oxidase-mimic properties. For example, Mn_2O_3 and $CoFe_2O_4$ NPs demonstrated catalytic activities in TMB oxidation and luminol respectively [216,219], while copper-nucleotide coordination NPs possessed laccase-mimic behavior for phenol oxidation [218]. Notably, oxygen or other nutriments will be consumed and generally converted into secondary ROS for the function of oxidase and oxidase-like nanomaterials.

(iv) Superoxide dismutase-like metallic nanomaterials. Since $O_2^{\bullet-}$ is the primary radical under stress conditions, investigations have also been devoted to evaluating the SOD activity of metallic nanomaterials. Aside from peroxidase and CAT-like activities, the aforementioned noble metal NPs have additionally demonstrated SOD-like property. Given the fact that $O_2^{\bullet-}$ tends to capture proton from water to generate $HO_2^{\bullet}$, the potential catalytic mechanism was proposed to be the adsorption of $HO_2^{\bullet}$ on the surface of these NPs, accompanied by rearrangements of $HO_2^{\bullet}$ to produce O_2 and H_2O_2 [206]. Similarly, CNPs are also effective in catalysis of $O_2^{\bullet-}$ dismuation. Notably, the catalytic process for $O_2^{\bullet-}$ was different from that for H_2O_2. During catalysis, $O_2^{\bullet-}$ would first combine with the oxygen vacancies around Ce^{3+} in CNPs, followed by electron transformation from Ce^{3+} and capture of protons from the solution to release H_2O_2. After structural rearrangement in the presence of another $O_2^{\bullet-}$, the formed CNP intermediate would catalyze the conversion of H_2O_2 into O_2, similarly to the CAT mimic activity-mediated processes. In addition to these NPs, other types of metallic NPs exhibited SOD-like property. Samples include Mn_3O_4 NPs [221], $Mn_2P_2O_7$ NPs [222], copper hydroxide NPs [223], and $FePO_3$ NPs [224], which share similar catalytic mechanisms that are related to the disproportionation of $O_2^{\bullet-}$ mediated by redox state conversion of different metal ions (*e.g.*, Mn^{2+}/Mn^{3+}, Cu^{2+}/Cu^{+}, and Fe^{2+}/Fe^{3+}).

(v) Influence factors on the enzymatic activity of metallic nanomaterials. Evidently, the aforementioned NPs, and many other types of metallic nanomaterials, typically possess two or even more enzymes-like characters to target multiple ROS, which is advantageous for practical antioxidant therapy, given the aforementioned ROS pathology and metabolism. For example, the functions of oxidases and peroxidases are accompanied by the formation of H_2O_2 and $^{\bullet}OH$ radials as products, which may induce additional oxidative injury. Noteworthy, the multienzyme activity of metallic nanomaterials is strongly dependent on many environmental factors.

Interestingly, the enzymatic activity of several metallic nanomaterials has been found to be pH-switchable. A combination of experimental tests and theoretical verifications carried out by Li et al. has provided an in-depth insight into the enzymatic activities of noble metal nanomaterials [225]. At acidic conditions, these NPs generally showed peroxidase-like properties derived from base-like decompositions of H_2O_2 on the metal surfaces, whereas the CAT-like activity will be switched on and increased as function with pH increase owing to acid-like decompositions of H_2O_2 on the surfaces. Such pH-switchable CAT-like activity has also been observed in porous Co_3O_4 NPs [217], which demonstrated higher catalytic activity at pH ranging from 6 to 10, as compared with acidic pH conditions. Likewise, iron oxides were reported to show powerful CAT-like activity at neutral pH, yet peroxidase-like activity under acidic lysosome pH [226].

On the other hand, the size and surface chemistry of metallic nanomaterials have significant impacts on their catalytic performance. One typical example is CNPs. They have demonstrated potent and stable multienzymes activity including SOD, CAT, oxidase, peroxidase, ATPase, and phosphatases. Furthermore, they have the capacity to actively scavenge varieties of both ROS and RNS in cells and animal models, such as $O_2^{\bullet-}$, $^{\bullet}OH$, DPPH $^{\bullet}$, H_2O_2, $ONOO^-$, and $^{\bullet}NO$ [227–234]. In general, the ratio of Ce^{3+} and Ce^{4+} ions on the particle surface is a key factor for the activities of CNPs. At a bulk state, cerium oxides exist in either Ce^{3+} or Ce^{4+} form. When the particle size is decreased to nanoscale, nevertheless, cerium oxides are composed of a mixture of Ce^{3+} or Ce^{4+} on the surface and concurrently show a size-dependent loss of oxygen atoms form the particle surface, leading to the generation of oxygen vacancies (defect sites). Commonly, the smaller the size, the more the Ce redox couple (Ce^{3+}/Ce^{4+}) and oxygen vacancy. The significance of the Ce^{3+}/Ce^{4+} ratio on enzymatic activity of CNPs was further established by another study, in which ultrasmall CeO_2 NPs with an average diameter of 4 nm demonstrated enhanced catalytic properties, as compared to larger NPs. Notably, the correlation of the Ce^{3+}/Ce^{4+} ratio *versus* antioxidative activities of CNPs depends on the category of radicals. For example, Korsvik et al. found that CNPs with a high ratio of Ce^{3+}/Ce^{4+} were more powerful scavengers of $O_2^{\bullet-}$ than those with lower Ce^{3+}/Ce^{4+} ratios on the particle surface [205]. In contrast, a higher CAT activity was observed in CNPs with a lower Ce^{3+}/Ce^{4+} ratio, as Ce^{4+} is required to decompose H_2O_2 [202,235]. Likewise, CNPs with a low Ce^{3+}/Ce^{4+} ratio also exhibited more effective $^{\bullet}NO$ scavenging than high Ce^{3+}/Ce^{4+} ratios [204,235]. Interestingly, CNPs with both high and low Ce^{3+}/Ce^{4+} surface ratios were able to efficiently eliminate $ONOO^-$ by accelerating its decomposition into non-toxic or less toxic substances [203,236]. At present, the recycling Ce redox couple is widely accepted as the main source for the intrinsic antioxidation of CNPs, but the biological significance of the oxygen defect sites remains largely unknown.

In the case of the surface chemistry effect on the antioxidative activities, Perez et al. found that dextran coating significantly improved water solubility of CNPs and offered them a unique pH-dependent antioxidative activity, yet did not compromise the CNPs' autocatalytic behavior due to the diffusion capability of H_2O_2 and peroxyl radicals after hydrophilic dextran coating [237]. These dextran-coated CNPs were also able to response to radicals and come back to their initial amount of Ce^{3+} and Ce^{4+} after redox reaction, whereas a large amount of Ce^{4+} remained even 10 days after addition of H_2O_2 at pH 4.0. The possible reason could be the interference of the cyclical regenerative or autocatalytic nature caused by the high concentration of protons at low-pH environments, thus inhibiting the ability of CNPs to scavenge more free radicals. The thickness of surface coating, on the other hand, was found to strongly affected the amount of radicals that reach to CNP surface and subsequent scavenging capability of CNPs. It is evident that the antioxidative activity of CNPs would greatly benefit from thin coating of the surface stabilizer. In a very recent study, an elegant experiment performed by Hyeon and co-workers has illustrated that selective elimination of extracellular, intracellular, and mitochondrial ROS could be achieved through tailoring the surface coating and size of CNPs [238].

In parallel, the composition and morphology of metallic NPs are considered as another two important factors for their catalytic performance. For example, manganese oxides with different compositions have demonstrated varied multienzyme activities. MnO_2 has been reported to possess inherent SOD and CAT mimic activities [239]. In another study, Mn_3O_4 NPs with flower-like morphology (Mnf) were first illustrated by Singh and colleagues to exhibit higher triple-enzyme activity including SOD, CAT and GPx as compared to other types of manganese oxides and other metal oxides [240]. Interestingly, the multienzyme activity was size and morphology dependent, and Mn_3O_4 with other three morphologies (cube, polyhedron, and hexagonal plate) and commercially available Mn_3O_4 materials showed negligible or much lower enzymatic

activities. Moreover, these NPs were also effective scavengers for •OH. Since the Mn^{2+}/Mn^{3+} couple is known to have high affinity for H_2O_2 and $O_2^{\bullet-}$, the remarkable multi-enzyme activity of Mnf was ascribed to the mixed oxidation states of manganese (Mn^{2+}/Mn^{3+}), along with large surface area and large pore size. The antioxidation of Mn_3O_4 NPs was further confirmed by other studies [241–243].

Lately, few-layer MoS_2 nanosheets, one typical type of two-dimensional transition metal dichalcogenide materials (TMDs), have also demonstrated multienzyme-like properties including SOD, CAT, and peroxidase under physiological conditions (Fig. 8) [244–247]. In combination with several analytical methods, the molecular mechanism underlying the multienzyme activity was ascribed to the $Mo^{6+}\leftrightarrow Mo^{4+}$ recycle process on the surface of MoS_2 nanosheets. Notably, unlike other peroxidase-like metallic nanomaterials, the peroxidase activity of MoS_2 nanosheets was due to the electron transferring between TMB and H_2O_2 instead of generating •OH. Moreover, they were effective to eliminate $O_2^{\bullet-}$, •OH, DPPH•, and •NO (Fig. 8A), which was presumably due to the presence of defects and unpaired electrons on the surface of MoS_2 nanosheets. A further study compared the ROS scavenging activity among four different TMDs including MoS_2, WS_2, $MoSe_2$, and WSe_2 (Fig. 8**B**) [247]. No significant difference in scavenging •OH was observed for these four TMDs, whereas MoS_2 was more powerful for eliminating ABTS radicals. Interestingly, for $O_2^{\bullet-}$, WS_2 and WSe_2 displayed more powerful scavenging activities than the other two TMDs. A mechanistic study based on EPR, X-ray photoelectron spectroscopy (XPS), and DFT calculations suggested that the antioxidation of TMDs was proposed to involve the following two processes. TMDs would first undergo oxidization by ROS, donating hydrogen to ROS to produce hydroxylated TMDs ($(HO)_3$-TMD). Thereafter, further oxidation of $(HO)_3$-TMDs by ROS gave rise to formation of sulfur-vacant TMDs and sulfate anions, followed by the generation of HO-TMD for scavenging ROS *via* hydrogen transfer. In a very recent work performed by Ni and his colleagues, ultrasmall Mo-based polyoxometalate (POM) nanoclusters with a readily variable valence state of molybdenum ions have been reported as novel antioxidative nanomaterials (Fig. 8**C**) [248]. Similar to the aforementioned MoS_2 nanosheets, these POM nanoclusters were very sensitive and showed concentration-dependent scavenging of three representative ROS, H_2O_2, $O_2^{\bullet-}$, and •OH, and another well-studied ABTS free radicals. Nevertheless, the mechanism behind the robust ROS-scavenging performance of POM nanoclusters was attributed to shifting between the reduced (Mo^{5+}) and oxidized (Mo^{6+}) forms through the bridging oxygen bonds, instead of $Mo^{6+}\leftrightarrow Mo^{4+}$ recycle process in MoS_2 nanosheets.

While an explosion of fundamental and practical studies have been carried out for the applications of metallic nanomaterials as promising antioxidants in both cellular and *in vivo* animal models of different oxidative stress-related diseases, their clinical translation may face incredible challenges due to the potential metal-related adverse effects, high cost, or complex manufacture procedures. Moreover, antioxidative nanomaterials that only scavenge ROS may not be effective enough for practical antioxidant therapy, given the generation of both ROS and RNS in oxidative stress-related diseases. Nevertheless, very few of currently studied metallic nanomaterials, to the best of our knowledge, have demonstrated powerful intrinsic capabilities in eliminating both ROS and RNS.

Carbon-based antioxidative nanomaterials

Carbon-based nanomaterials represent another biggest class of antioxidative nanomaterials. Since the pioneering work regarding antioxidative activity of fullerene in 1996, various carbon nanomaterials with different structures and compositions, including carbon nanotubes (CNTs) [249,250], carbon particles [251], carbon nanoclusters [251,252], graphene and graphene quantum dots (GQDs) [253–255], carbon quantum dots (CQDs) [256], and others [257,258], have been explored as scavengers towards free radicals in the treatment of many diseases [37,259,260]. The advantages of carbon-based antioxidative nanomaterials over natural enzymes include low cost, easy operation, robust catalytic activity towards many free radicals, and outstanding stability against stringent conditions, among others. Currently, carbon-based antioxidative nanomaterials have received extensive experimental and theoretical studies in term of their antioxidative mechanisms, catalytic efficiencies, structure-dependent activities, and impact factors on activities.

In general, molecules or compounds containing conjugated C=C chains typically have free radical scavenging capabilities (Fig. 9A). Fullerene and its derivative are the best-characterized carbon-based antioxidative nanomaterials. They have shown remarkable scavenging activities for •OH and $O_2^{\bullet-}$, which was dominated by a combined electron transfer and adduct formation mechanisms (Fig. 9**B,C**). Using a well-defined H_2O_2-TMB reaction system, together with UV–vis analysis, Qu et al. have suggested that the peroxidase-like catalytic activity of GO containing carboxyl groups (GO−COOH) was largely due to the electron transfer from the top of the valence band of GO to the lowest unoccupied molecular orbital (LUMO) of H_2O_2 [254]. Aiming at a deeper understanding of the enzyme-like mechanisms of carbon-based nanomaterials, kinetic studies of catalytic activities of GQDs and their derivatives were performed by the same group. It was found that different oxygen-containing functional groups in these materials have significant effects on the peroxidase mimic activity. The -C = O groups behavior as active sites for converting H_2O_2 into •OH, and the O = C-O- groups act as the substrate binding sites for H_2O_2. However, the C—OH groups showed deleterious effects on catalytic activity of GQDs. Later on, carbon clusters, as a new type of carbon-based antioxidative nanomaterials, have attracted great attentions [252]. They have demonstrated extraordinarily high and stably antioxidative activity of carbon clusters toward $O_2^{\bullet-}$ mediated by a SOD-mimic self-dismutation mechanism. Notably, unlike the aforementioned antioxidative mechanisms for CNT, GO and their derivative, the SOD-mimic activity was attributable to the intrinsic stable radicals in the carbon network, which acts as the active centers for catalysis of $O_2^{\bullet-}$. However, similar to those types of carbon nanomaterials, they were also inert to •NO and $ONOO^-$.

The antioxidative activities of carbon nanomaterials are largely influenced by many factors. The surface chemistry of carbon nanomaterials is essential. For example, fullerenes with abundant carboxyl groups ($C_{60}(C(COOH)_2)_2$) on the surface were found to be much less active compared to those carboxyl-free fullerenes ($C_{60}(OH)_{22}$) and $Gd@C_{82}(OH)_{22}$ in scavenging free radicals (Fig. 9**D**) [261]. Based on the fact that carbonyl groups in carbon nanomaterials can act as active centers during the oxidative dehydrogenation process, studies carried out by Grande et al. have also indicated that decreasing the level of non-active carboxyl groups and hydroxyl groups in CNTs through modest oxidization was able to dramatically enhance their peroxidase activity and biocatalytic efficiency in a TMB-H_2O_2 reaction system [255]. Similarly, remarkable influence of the chemical composition of graphene QDs on their free radical scavenging activities was also reported, where graphene QDs with high-level sp^2 hybridized carbon domains and strong hydrogen donor behavior were generally very effective in antioxidation (UV absorption) and scavenging of ROS (*e.g.*, DPPH• and •OH radicals), as compared to highly crystalline graphene QDs and carbon black NPs that contain a large amount of sp^3 hybridized carbon. Moreover, the antioxidative activities could be further enhanced by doping electron-rich heteroatoms, such as nitrogen and sulfur, due to the increased electron density in the carbon nanostructure. Very recently, metallic heteroatom selenium (Se) was doped into the carbon QDs [256]. Interestingly, the as-prepared Se-doped carbon QDs exhibited not only efficient scavenging in •OH, but also

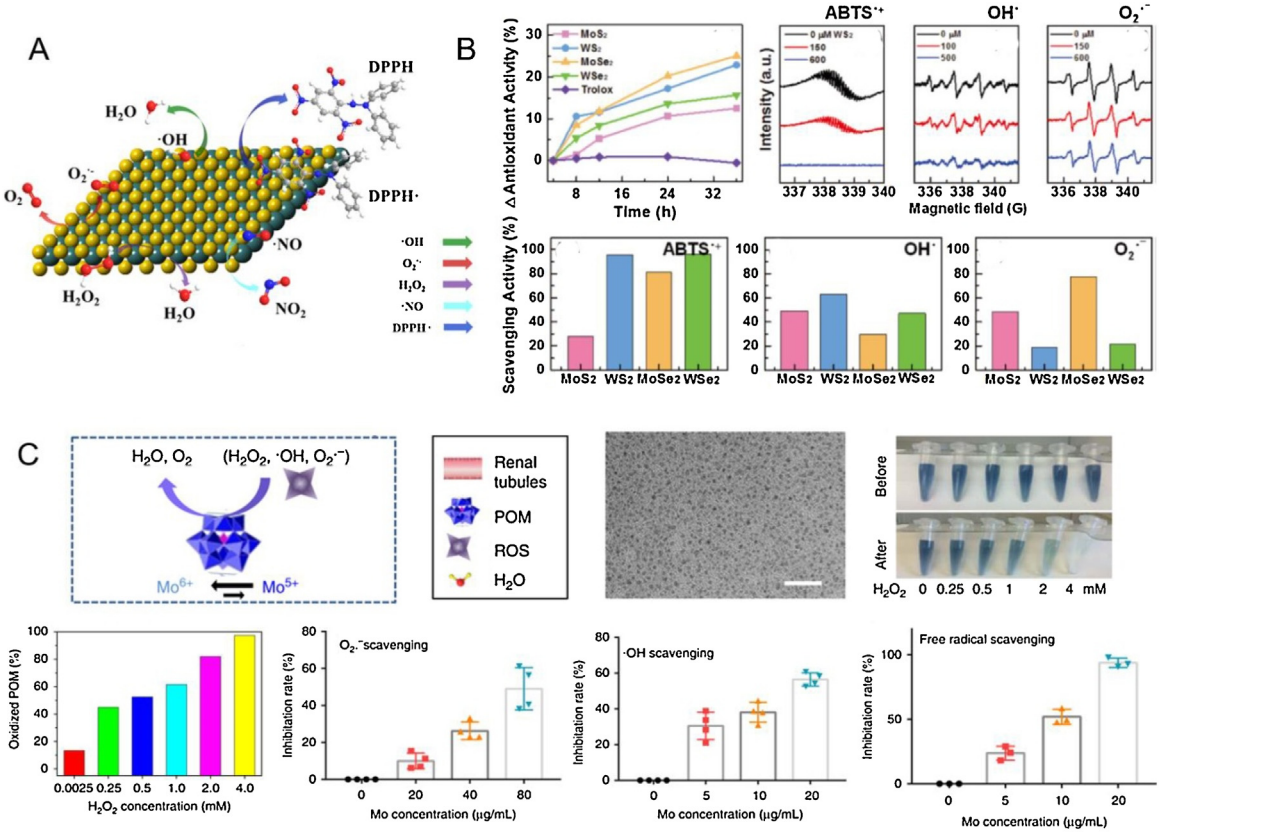

Fig. 8. (A) Schematic illustration of the intrinsic antioxidative activity of MoS_2 nanosheets. Reprinted with permission from Ref. [245]. Copyright 2018, American Chemical Society. (B) Comparison of antioxidative activities among four different TMDs. Reprinted with permission from Ref. [247]. Copyright 2018, Wiley. (C) The characterization and antioxidative activity of POM nanoclusters. Reprinted with permission from Ref. [248]. Copyright 2018, Nature.

significant excitation-dependent and reversible redox-dependent luminescence, which makes them very promising for simultaneously imaging and protecting organisms from oxidative stress. Additionally, Fe^{3+}-doped mesoporous carbon nanospheres (MCNs) have recently been developed through a biomimetic strategy, inspired by the molecular structures and mechanisms of natural enzymes [262]. Fe dopants could serve as catalytic centers, and the unique mesoporous structure further facilitated reaction of substrates with catalytic centers. As a result, Fe^{3+}-MCNs displayed enhanced catalytic activity than traditional Fe_3O_4 NPs and better thermal stability than natural horseradish peroxidase (HRP), allowing for circulating utilization.

Another important factor that significantly affects the antioxidative activities of carbon nanomaterials is the reaction condition. Unlike natural enzymes, carbon nanomaterials usually hold higher biocatalytic activity at relatively higher temperatures and acidic pH environments. For example, the optimal reaction for CNTs-mediated catalysis requires a temperature of ~ 40 °C and a pH value of 4.0 [263–265]. On the other hand, different types of carbon nanomaterials possess their own optimal substrate concentration. Excessive free radicals will in turn overwhelm the catalytic activity of carbon nanomaterials, which was ascribed to the ping-pong mechanism, similar to the case of catalysis mediated by HRP [266,267].

To date, great efforts have been made to developing various carbon-based nanomaterials as versatile antioxidants, and intensive investigations have been performed in animal models of oxidative-stressed diseases to evaluate their therapeutic potential. Nonetheless, the catalytic efficiency of carbon-based antioxidative nanomaterials is generally lower as compared to natural enzymes. Thus, searching for new nanomaterials with high enzyme activity is still on demand. It should also be mentioned that the antioxidative mechanisms of various carbon nanostructures have not been fully understood, and in-depth mechanistic studies will be beneficial for better understanding their working mechanisms and facilitating future design of carbon-based antioxidative nanomaterials for more effective antioxidant therapy.

Bioinspired polymer-based antioxidative nanomaterials

Biocompatibility and biodegradability are widely recognized as prerequisite and two critical factors in determining whether a newly developed antioxidative NP is applicable for *in vivo* applications. Despite the aforementioned advances in antioxidative nanomaterials based on inorganic or artificial polymer nanomaterials, emerging concerns regarding their safety, which is also defined as nanotoxicity, have attracted considerable attention. It has been proved that these exogenous nanomaterials may cause potential deleterious side effects, such as induction of oxidative stress, mitochondrial respiration, activation of immune cells, genotoxicity, among others [268], which also represent great challenges for their clinical translation. Development of biocompatible and biodegradable nanomaterials using naturally occurring biopolymers from organisms have recently inspired the generation of a new class of antioxidative nanomaterials, with the potential for minimizing adverse effects caused by administration of exogenous nanomaterials.

Bilirubin, a natural metabolite in human body, has been suggested as a potent endogenous antioxidant for scavenging ROS and protecting cells from damage caused by oxidative stress. However, the poor water solubility of natural bilirubin has significantly restricted its potential uses as a medicine. To address this limitation, Jon and co-workers have recently reported the synthesis

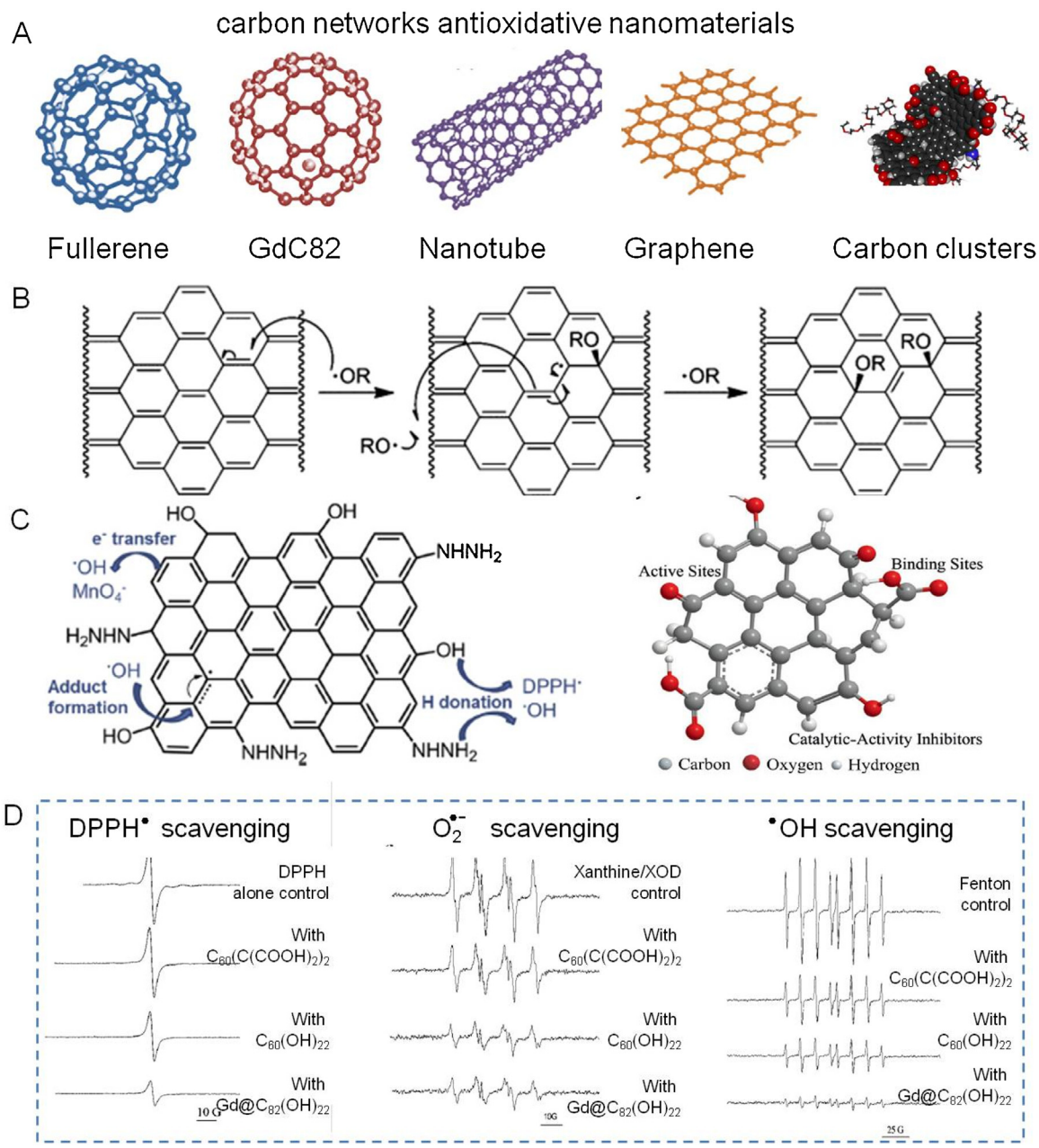

Fig. 9. (A) Schematic illustration of carbon-based antioxidative nanomaterials. (B) Antioxidative mechanisms of carbon nanotubes. Reprinted with permission from Ref. [251]. Copyright 2012, American Chemical Society. (C) Antioxidative mechanisms of graphene quantum dots (GQDs). Reprinted with permission from Ref [255]. (Copyright 2017, Elsevier) and Ref [259]. (Copyright 2018, Wiley). (D) Radical scavenging activities among different types of carbon nanomaterials. Reprinted with permission from Ref. [261]. Copyright 2009, Elsevier.

of PEGylated bilirubin (PEG-BR) NPs through a combined covalent conjugation and self-assembly method [269–273]. PEG-BR NPs inherited the effective detoxifying effect of natural bilirubin toward H_2O_2. The antioxidative mechanism was ascribed to the redox reaction triggered by ROS, where the water insoluble bilirubin would be oxidized into water soluble biliverdin [270]. Using different disease models, PEG-BR NPs have demonstrated effective protection of different cells from oxidative stress-associated injuries. In addition, they could also decrease inflammation from activated macrophages by suppressing cytokine release. Notably, total ROS levels were examined and the detailed ROS scavenging capabilities of PEG-BR NPs in targeting different ROS species need further characterization.

Very recently, inspired by the protective function of natural melanin in human skin against UV irradiation-induced oxidative damage, our group has developed a straightforward method to prepare highly water soluble and size-controlled melanin NPs and systemically investigated their antioxidative mechanisms in targeting different ROS and RNS (Fig. 10) [274]. It was found that melanin NPs could serve as a SOD mimic towards $O_2^{\bullet-}$ with a comparable catalytic activity to SOD, yet possess much stronger catalytic stability with no obvious decay in the catalytic activity even after one-year storage at 4 °C or under different pHs. The mechanism behind the robust and stable catalysis of $O_2^{\bullet-}$ might be ascribed to the stable unpaired electrons in the stacked units of melanin, which could function as active centers and facilitate the electron removal from $O_2^{\bullet-}$ to molecular oxygen. Aside from $O_2^{\bullet-}$, melanin NPs can also scavenge •OH, because it could strongly chelate with transition metal ions (*e.g.*, Cu^{2+} and Fe^{3+}), thereby blocking the Fenton-type reaction-mediated generation of •OH. In

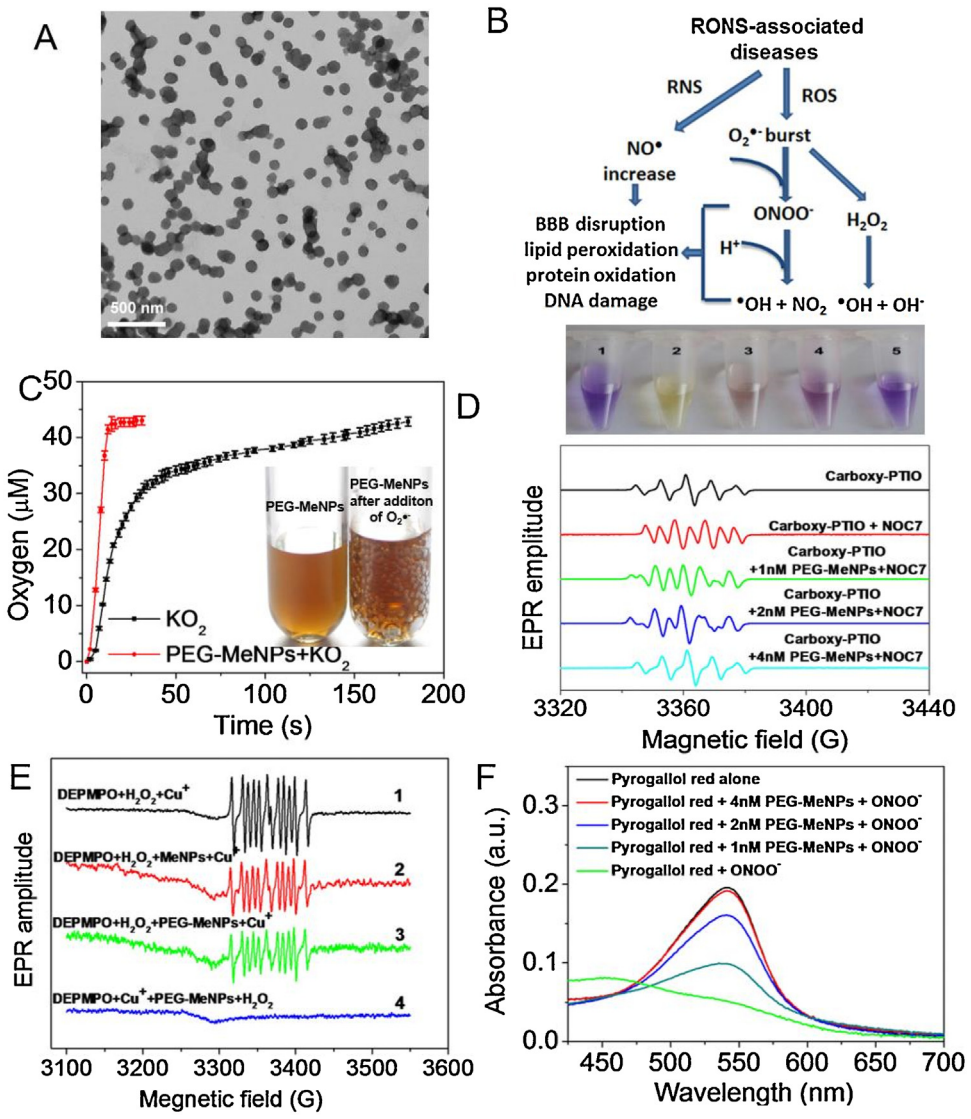

Fig. 10. Characterization and antioxidative activity of PEG-MeNPs towards different ROS/RNS. Reprinted with permission from Ref. [274].Copyright 2017, American Chemical Society.

addition to ROS, melanin NPs further demonstrated potent antioxidation towards RNS including •NO and $ONOO^-$. Such scavenging capability was presumably ascribed to the presence of the residual catechol groups in melanin NPs, which could react with these two types of RNS through nitration and nitrosation reaction-mediated mechanisms. Nevertheless, nitration and nitrosation reactions are strongly dependent on the categories of phenols, and the exact structure of melanin remains currently unclear, highlighting the need of further studies to reveal the detailed reaction processes and possible products of melanin NPs after reaction with RNS. Thereafter, researchers further found that after encapsulation of hemoglobin (Hb) within polydopamine (the major component of melanin), the resulting NPs could not only scavenge ROS and other free radicals, but also carry molecule oxygen and inhibit the generation of methemoglobin and ferryl Hb [275].

The demand for powerful and affordable antioxidative nanomedicines has encouraged researchers to draw inspiration from nature. However, the development of antioxidative nanomaterials composed of naturally occurring bioactive compounds is still in its infancy, and few types of such materials have been reported. Additionally, the long-term adverse effects as well as the possible influence on normal metabolisms by those bioinspired antioxidative nanomaterials are currently unclear.

Biomedical applications of antioxidative nanomaterials

The current body of evidence for oxidative damage in many important human diseases is vast. Furthermore, the potential of antioxidative nanotechnology has been well characterized in established experimental models. Highlights of some recent significant findings will be presented as below.

Ischemia and ischemia-reperfusion injuries

Ischemia (or hypoxia) is a restriction in blood supply to an organ, inducing rapid activation of a series of ischemic cascades. Over the past several decades, numerous studies have revealed that high-level free radicals including ROS and RNS generated following ischemia could trigger severe oxidative damage to or dysfunction of organs [276,277]. Moreover, ROS and RNS can orchestrate a series of pathological events, such as the aforementioned calcium influx, activation of inflammatory signaling, vascular cellular senescence, which in turn lead to more ROS/RNS production, cre-

ating a 'vicious circle' with amplified injuries to tissues [278]. Organ ischemia underlies in many clinical conditions including stroke [276], myocardial infarction [277], atherosclerosis [279], acute kidney injury [280], limb ischemia [281], among others. It is necessary to make blood supply return to tissues for prevention of further ischemia-caused damage. During the reestablishment of blood flow, however, a sequential chain of pathophysiological cascades will be triggered, such as impairment of mitochondrial electron transport chain, massive intracellular Ca^{2+} release, neutrophil recruitment, acute inflammation, and production of more ROS and RNS which, in turn, aggravate apoptotic or necrotic cell death. These pathophysiological events are generally called reperfusion injuries or secondary injuries.

Brain is an organ characterized by great demand of oxygen and other nutrition as well as high levels of redox-active metals (*e.g.*, Cu and Fe), and thus it is particularly vulnerable to oxidative stress [282]. It has been well documented that large amounts of ROS and RNS can continuously accumulate and are highly involved in brain ischemia and reperfusion cascades. Antioxidant therapy is therefore highly important in clinical settings to prevent brain from these injuries. Unfortunately, there is no clinically validated therapeutics that can effectively inhibit ROS/RNS-amplified brain injury. In recent years, the use of antioxidative nanomaterials has recently attracted considerable attention as a second category of antioxidants for prevention of organs from ischemia and ischemia-reperfusion injuries, given the many merits of antioxidative nanotechnology as we discussed above. Previously, Kim and co-workers have pioneered the novel antioxidative treatment option of ischemic stroke using amine-modified SWNTs [250]. In a rat model of stroke, pretreatment with amine-modified SWNTs could protect neurons from excessive oxidative stress and enhance behavioral recovery of rats following ischemia-reperfusion. Moreover, pretreatment with amine-modified SWNTs could reduce apoptosis, inflammatory responses and overactivation of glial cells, and thus dramatically decrease the infarct area. Notably, these CNTs were treated into rats before the occlusion was initiated, and further studies are therefore needed to prove therapeutic benefits of these CNTs. From the view point of practical applications, Hyeon and colleagues reported efficient protection of brain in a rat model of ischemic stroke by using ultrasmall CNPs with an average size of 3 nm [228]. CNPs were significantly accumulated in the penumbra area in the ischemic hemisphere following intravenous injection, benefiting from the small size and prolonged circulation half-life of CNPs, as well as the extensive breakage of the blood-brain barrier caused by ischemia. After a stroke, the animals treated with CNPs demonstrated reduced apoptotic cell death and smaller infarct area as compared to the sham group, owing to CNP-mediated scavenging of ROS and down-regulation of pro-apoptotic proteins. Aiming at improving the biocompatibility of antioxidative nanomaterials, our preliminary study also revealed, for the first time, that the PEGylated melanin NPs (PEG-MeNPs) exhibited an enzymatic activity in targeting $O_2^{\bullet-}$ and could further scavenge the downstream toxic metabolites of $O_2^{\bullet-}$ (*e.g.*, H_2O_2 and $^{\bullet}OH$) and RNS (*e.g.*, $ONOO^-$ and $^{\bullet}NO$), all of which can contribute to lipid peroxidation, protein oxidation, and DNA damage in ischemic brain [274]. Our progressive experiments clearly suggested that PEG-MeNPs efficiently detoxified ROS and RNS, and concurrently depressed the expression of inflammatory mediators/cytokines, prophylactically preventing brain damage in the animal model of ischemic stroke (Fig. 11A).

Kidney is another organ that is very susceptible to oxidative stress. For decades, renal ischemia-reperfusion injury has been confirmed to be a common cause of acute renal failure. Unfortunately, there is no effective therapeutics available in the clinic for such disease. Similar to brain ischemia, renal ischemia is also characterized by overproduction of ROS/RNS and inflammation activation [283]. Antioxidative nanomaterials have recently found extensive studies in several acute and chronic kidney diseases. On the basis of intrinsic antioxidative functions of natural polyphenolic compounds, Chen et al. developed a nanocarrier composed of distearoylphosphatidylethanolamine-polyethylene glycol for delivery of curcumin, a hydrophobic polyphenol that widely exists in the rhizome and exhibits a wide range of biological functions including antioxidative activity [284,285]. Curcumin has been reported to significantly prevent renal tubular epithelial cells against free radicals, but its poor aqueous solubility significantly restricts clinical applications. In comparison, the as-prepared curcumin-NPs dramatically enhanced the bioavailability of curcumin by prolonging the pharmacokinetics, leading to reduced oxidative stress, apoptosis, and down-regulation of apoptotic markers (*e.g.*, Caspase-3 and GRP-78) both *in vitro* and *in vivo*. Moreover, curcumin-NPs relieved oxidative stress-induced cell apoptosis in the AKI model, as verified by the decreased levels of serum creatine phosphokinase, creatinine and urea, and less histological damage in renal tubules. In contrast, no significant difference in lipid peroxidation was observed between the AKI group and curcumin-free group [284]. Alternatively, a type of traditional medicine herbs, berberine, was formulated into a nanoformulation to form BBR-NPs [286]. Similar to the above study, administration of BBR-NPs efficiently prevented renal injury both morphologically and functionally in a rat model as compared to the free berberine, by reversing oxidative stress and decreasing the expression of proteins that are involved in the mitochondrial stress pathways. Notably, most studied antioxidative nanomaterials for kidney diseases typically showed large particle size, and only a small fraction of NPs were able to accumulate in the kidney due to the size selection of kidney, bringing concerns on interference with the redox signaling in normal cells or tissues and potential side effects to other organs. To address this problem, ultrasmall molybdenum (Mo)-based polyoxometalate (POM) nanoclusters with an average size below 10 nm were developed by Cai and co-workers as antioxidants to ameliorate AKI in mice [248]. Owing to the ultrasmall size, these nanoclusters showed high rates of accumulation in kidney and broad antioxidative activities against various ROS including H_2O_2, $O_2^{\bullet-}$, and $^{\bullet}OH$, mediated by variable valence state between Mo^{5+} and Mo^{6+}. *In vitro* experiments confirmed the potential of POM nanoclusters against harmful oxidative stress and therapeutic efficacy of AKI induced by ROS. Alternatively, the same group reported a new paradigm for kidney diseases using DNA origami nanostructures (Fig. 11B). Despite large size, all DNA origami nanostructures (DONs) with different shapes preferentially accumulated in kidneys of both healthy mice and mice with rhabdomyolysis-induced AKI [287]. Such active accumulation of DONs in kidney may be attributed to three factors: (1) escape from unnecessary enzyme digestion due to the compact structures of the fully folded DONs; (2) the less chance of forming protein corona on the negatively charged DONs during circulating in the blood which facilitates NP's accumulation in the liver and spleen; and (3) the specific morphology and size of DONs allow for glomerular filtration and urinary excretion *in vivo*. Consistent with the previous study, rectangular DONs possess intrinsic ROS scavenging ability ($^{\bullet}OH$, $O_2^{\bullet-}$ and H_2O_2) *via* oxidation of DNA bases. Along with active kidney accumulation, these striking properties enabled rectangular DONs for effective renal protection with efficacy similar to N-acetylcysteine, an antioxidant clinically used for treating contrast-induced AKI and preventing kidney function injuries from nephrotoxic agents. Moreover, ^{64}Cu-labeling DONs were synthesized through hybridizing ^{64}Cu-labelled ssDNA with side-arms designed on DONs. With these radiolabelled DONs, the kidney-targeting and therapeutic efficiencies of DNA origami frameworks could be monitored by PET imaging. These promising results clearly suggest that DNA nanotechnology could become a potential therapeutic option for the

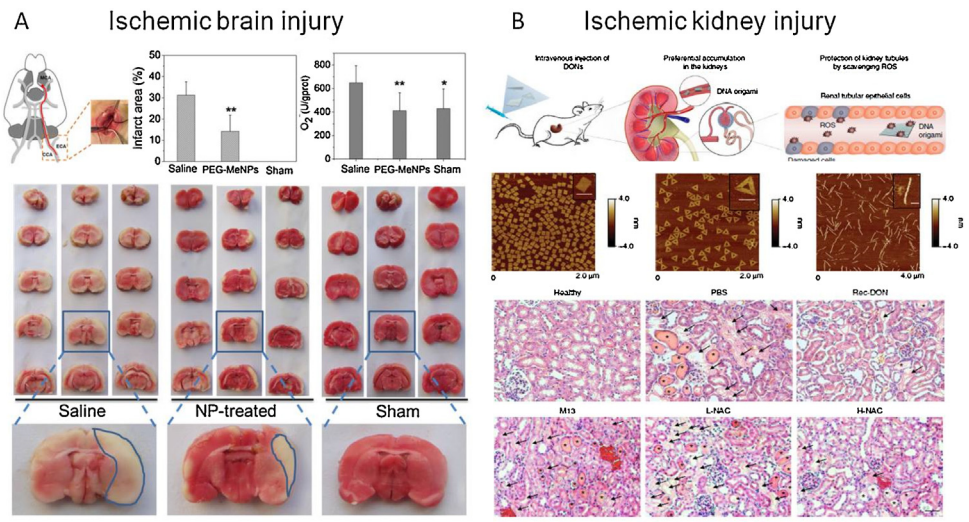

Fig. 11. Applications of antioxidative nanomaterials in ischemic injuries. (A) Administration of PEG-MeNPs could protect brain tissues from oxidative stress-induced damage by scavenging ROS. Reprinted with permission from Ref. [274]. Copyright 2017, American Chemical Society. (B) Antioxidative DNA DONs with different morphologies showed high accumulation in the kidney and demonstrated renal protection in an animal model of AKI. Reprinted with permission from Ref. [287].Copyright 2018, Nature.

treatment of AKI and other renal diseases. Nevertheless, the cost of this technology might become a major concern for clinical translation.

In recent years, antioxidative nanomaterials have also found broad applications in the treatment of cardiovascular diseases. Oxidative stress and inflammation have been key players for the pathogenesis of cardiac remodeling in cardiovascular diseases. Atherosclerosis is among the leading cause of various vascular diseases worldwide. The pathogenesis of atherosclerosis involves a number of proatherogenic events including low density lipoprotein oxidation, vascular dysfunction, formation of foam cells, generation of excessive ROS/RNS, the build-up of lipid-rich plaques in arterial walls, and inflammation cell recruiting in plaques [277]. Increasing evidence has demonstrated that nanotechnology-mediated targeted delivery of therapeutics to atherosclerotic plaque is one effective and promising strategy to attenuate vascular and systemic oxidative stress and suppress ROS accumulation in plaques of atherosclerosis. Recent progress further suggested that NPs with both antioxidative and anti-inflammatory activities could be a promising next generation of therapeutics against atherosclerosis [170,288–292]. For instance, targeting delivery of CAT to vascular endothelial cells using semi-permeable polymer nanocarriers was reported for detoxifying H_2O_2-induced vascular oxidative stress [289]. In another study, epigallocatechin gallate (EGCG), one characteristic antioxidtive compound in green tea, was formulated into chitosan-polyaspartic acid NPs *via* a self-assembling process. Oral administration of these NPs could efficiently decrease the level of lipid in the blood of rabbits with high-fat diet-induced atherosclerosis more efficiently than the free EGCG [290]. Hu and colleagues recently engineered innovative antioxidative and anti-inflammatory NPs using β-cyclodextrin with covalent conjugation to Tempol and phenylboronic acid pinacol ester, with a broad-spectrum ROS eliminating ability for targeted atherosclerosis therapy [170]. *In vivo* studies in atherosclerotic mice indicated that the newly developed NPs concurrently attenuated inflammation and cell apoptosis. Moreover, the formation of oxidized LDL-induced foam cells was suppressed after treatment with NPs, subsequently preventing the progression of atherosclerosis and stabilizing vulnerable atherosclerotic plaques. To enhance the therapeutic efficiency, one study conducted by Fredman and coworkers engineered collagen-IV-targeted polymer NPs by covalent conjugation of collagen-IV binding proresolving peptide Ac-26 to combat advanced atherosclerosis in hypercholesterolemic mice [292]. These NPs showed a sustained release of Ac-26 and homing to atherosclerotic lesions, enabling stabilization of vulnerable plaques by suppressing the production of ROS and collagenase activity. Notably, these therapeutic benefits were not seen in mice without FPR2/ALX in myeloid cells. To further realize spatiotemporal control over the antioxidation, stimuli-responsive antioxidative nanoplatforms have further been explored under various stimulations such as light, pH, thermal stimulus, or redox. For example, Lee et al. have synthesized H_2O_2-responsive copolyoxalate (HPOX) NPs for diagnosis and therapy of ischemia-reperfusion injury [148]. HPOX NPs would undergo decomposition to release hydroxybenzyl alcohol (HBA) for radical scavenging in a dose-dependent manner. By co-delivery of an anti-inflammatory agent, these NPs exerted efficient antioxidative and anti-inflammatory activities in an animal model of ischemia-reperfusion.

Autoimmune diseases

As aforementioned discussion, oxidative stress can trigger a cascade of immune reactions such as increasing secretion of pro-inflammatory cytokines, recruitment or amplified infiltration of inflammatory cells, and activation of neutrophils and leukocytes, further promoting the formation of reactive free radicals for aggravated oxidative stress. Cumulatively, this provokes many inflammatory autoimmune disorders. Nevertheless, dietary antioxidants (*e.g.* vitamins) have only demonstrated modest clinical benefits in autoimmunity, presumably due to their suboptimal activity for radical annihilation, poor antioxidative stability, and detoxifying molecule-dependent activity. These limitations may potentially be overcome by nanomaterials with both ROS/RNS scavenging and anti-inflammatory properties.

Rheumatoid arthritis (RA), one representative chronic inflammatory autoimmune disorder that can lead to long-term synovitis and joint disability, is characterized by elevated ROS/RNS levels in both disease onset and progression [293]. In addition, macrophages and activated T-cells attack healthy tissues, as least in part, through oxidative mechanisms involving activation of NF-κB, TNF-α and NRF2, resulting in an extended inflammatory milieu and progressive joint destruction. The curcumin-loaded NPs with dual responses to oxidative stress and acidic pH involved in the inflammatory microenvironment have been constructed [294]. The

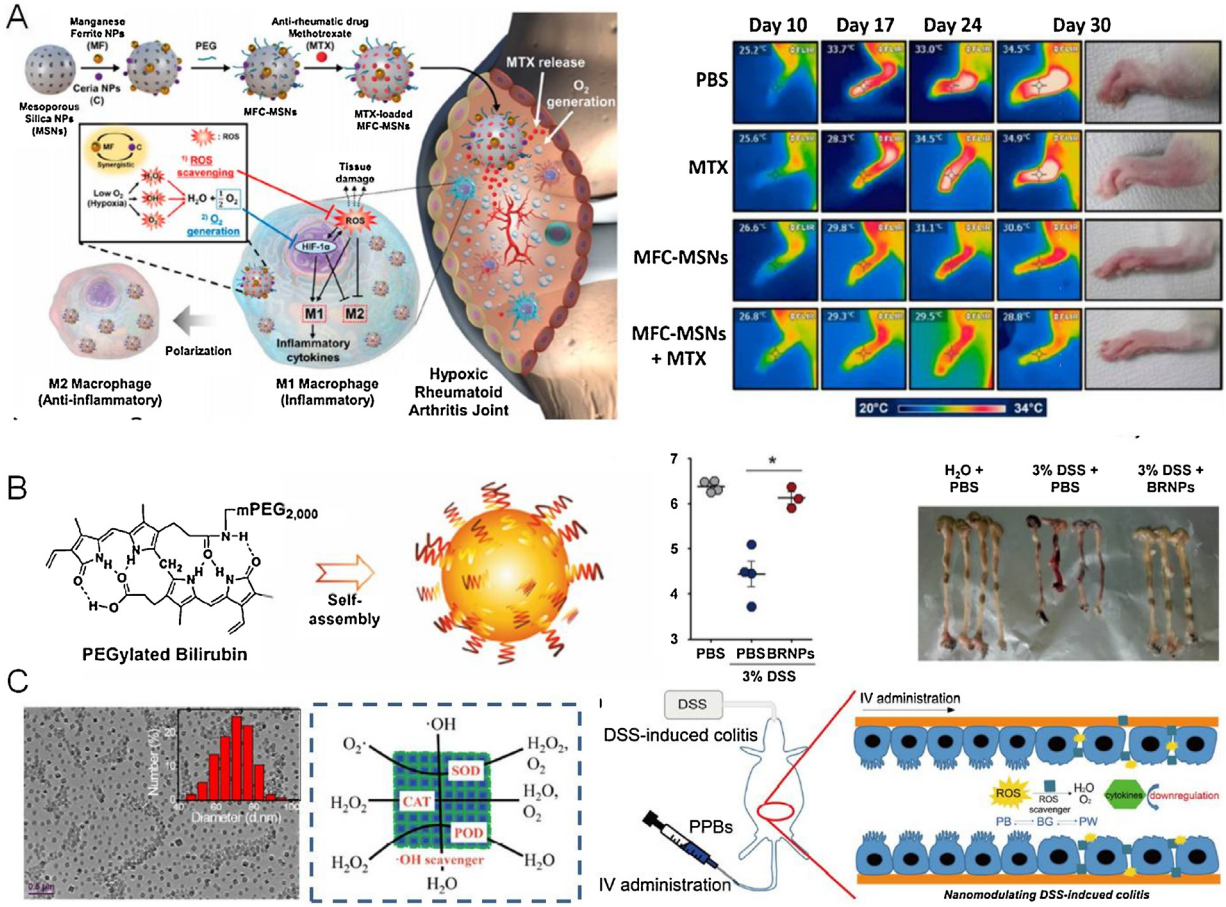

Fig. 12. Examples of using antioxidative nanomaterials for the treatment of autoimmune diseases. (A) Schematic illustration of MFC-MSNs for the treatment of RA as well as *in vivo* therapeutic effects of MFC-MSNs and antirheumatic drug MTX-loaded MFC-MSNs in an animal model of RA. Reprinted with permission from Ref. [295]. Copyright 2019, American Chemical Society. The use of (B) antioxidative bilirubin NPs (Reprinted with permission from Ref. [269]. Copyright 2016, Wiley) and (C) PPBs (Reprinted with permission from Ref. [301]. Copyright 2018, American Chemical Society) for ROS scavenging and anti-inflammation in DSS-induced colitis animal models.

feasibility of using these dual-responsive NPs for antioxidant and anti-inflammation has been examined in a mouse model with LPS-stimulated ankle inflammation. Upon local injection, these NPs could dramatically reduce ROS/RNS and rescue the ankle from LPS-induced inflammation, as compared to free curcumin and saline-treated groups. Considering that hypoxia and ROS would trigger the induction of pro-inflammatory M1 macrophages and reduction of anti-inflammatory M2 macrophages in RA synovium, a very recent work by Hyeon's group engineered manganese ferrite and ceria NPs-anchored mesoporous silica nanoparticles (MFC-MSNs) to simultaneously eliminate M1 macrophages and increase M2 macrophages [295]. The MFC-MSNs could not only scavenge ROS, but also produce O_2 for promotion of M2 polarization of macrophages under hypoxic and inflammatory conditions. Administration of MFC-MSNs into the rat model of RA successfully relieved the hypoxia and attenuated inflammation in the inflamed synovial joint. Moreover, the therapeutic effect could be further augmented following encapsulating another antirheumatic drug, leading to dramatically decreased hyperthermia and paw swelling (Fig. 12A).

Multiple sclerosis (MS) is another classic tissue-specific chronic autoimmune disease mediated by T cells [296,297]. $O_2^{\bullet-}$ produced by the mitochondria in response to T cell activation is an important second messenger during T lymphocyte activation and represents a promising target for regulating T cell activation [298]. Given this mechanism, poly(ethylene glycol)-modified hydrophilic carbon clusters (PEG-HCCs), prominent scavengers of $O_2^{\bullet-}$, were evaluated for immunomodulation [299]. Interestingly, a higher uptake of PEG-HCCs by T lymphocytes than by other splenic immune cells was observed, leading to efficient scavenging intracellular $O_2^{\bullet-}$ produced in T cells upon antigen stimulation. Moreover, the proliferation of the activated T lymphocytes and the production of proinflammatory cytokines were inhibited. In line with *ex vivo* studies, T lymphocyte-mediated inflammation was efficiently reduced after administration of PEG-HCCs in an *in vivo* model of MS. In another study performed by Mattson's group revealed that combined treatment with Lenalidomide (a thalidomide analogue with potent anti-inflammatory effects) and nanoceria could concurrently delay symptom onset and promote recovery, reducing white matter pathology and inflammatory cell responses more effective than either treatment alone [300].

Similar to the case of MS, oxidative stress also plays pivotal roles in the initiation and development of inflammatory bowel disease (IBD), a chronic inflammatory disorder in the gastrointestinal tract. Excessive ROS produced from infiltrated inflammatory cells in the intestinal mucosa could not only amplify inflammation, but also damage the mucosa and accelerate mucosal ulceration in the pathogenesis of IBD. Despite experimental and clinical benefits from antioxidants, only limited success has been achieved largely due to the following reasons: (i) harsh, acidic, and enzyme-abundant microenvironments in the gastrointestinal tract may significantly compromise the activities of many antioxidants; (ii) very limited accumulation of antioxidants at diseased sites; and (iii) narrow antioxidant defense of traditional antioxidative regimens given the severe stress condition in IBD. To this end, many

antioxidative NP-mediated strategies have been investigated for the treatment of IBD [169,269,301–303]. In view of the significance of SOD and CAT in scavenging ROS, Zhang et al. developed a SOD/CAT mimetic nanomedicine containing a H_2O_2-eliminating β-cyclodextrin-derived material and a free radical scavenger Tempol, aiming to effectively prevent and alleviate intestinal inflammation in IBD [169]. These enzymatic NPs could effectively scavenge multiple oxidizing species including $O_2^{\bullet-}$, $^{\bullet}OH$, and H_2O_2. Post oral delivery, these NPs remained stable in the gastrointestinal tract environments and efficiently accumulated at inflammatory sites due to concurrent enhancement of the epithelial permeability and physicochemical binding effects. Their broad antioxidant defense and stability enabled them to exhibit robust therapeutic benefits in mice with dextran sodium sulfate (DSS) or 2,4,6-trinitro benzene sulfonic acid induced acute or chronic colitis, with good safety profiles. Similarly, bilirubin NPs with powerful scavenging capability towards various ROS have been demonstrated to accumulate at the site of inflammation and reduce colitis in DSS-induced colitis animal models after intravenous administration (Fig. 12**B**) [269]. In a recent paper, polyvinylpyrrolidone (PVP)-modified Prussian blue NPs (PPBs) were reported to have a broader antioxidant defense and effectively scavenge $O_2^{\bullet-}$, $^{\bullet}OH$, H_2O_2, and $^{\bullet}OOH$, thus demonstrating significant therapeutic effects in mice with DSS-induced colitis without inducing obvious side effects (Fig. 12**C**) [301]. Considering the interaction and essential roles of ROS and TNF-α in the onset and duration of intestinal inflammation, Wilson et al. presented ROS-responsive thioketal NPs to orally deliver siRNA against TNF-α to the inflammatory area of intestinal. Using a mouse model of ulcerative colitis, the authors showed that these RNAi NPs could significantly protect siRNA from the harsh environment of the gastrointestinal tract and deliver it to the inflamed intestinal tissue, significantly down-regulating the expression of TNF-α in the colon and subsequently protecting mice from ulcerative colitis [303].

Additionally, antioxidative nanotechnology has also been extended to the treatment of necrotizing enterocolitis (NEC), a serious gastrointestinal disorder in the premature infant, in which the released pro-inflammatory high-mobility group box 1 (HMGB1) from the injured intestine activates Toll-like receptor 4 on microglial cells in brain, leading to accumulation of ROS and subsequent cognitive impairments. By targeting the activated microglia in a mouse model of NEC, Hackam and colleagues recently developed a microglia-targeting antioxidant based on N-acetyl-l-cysteine conjugated dendrimer NPs (D-NAC) [304]. The authors found that oral administration of D-NAC could efficiently reduce the microglial activation by means of scavenging the accumulated ROS and preventing NEC-associated neurological dysfunction in neonatal mice, suggesting the great potential of antioxidant therapy in protecting the developing brain in infants with NEC.

To date, while different attempts have emerged using nanocarriers to enrich the activity of antioxidant compounds for treating various types of autoimmune disorders, success in antioxidative nanotechnologies remains limited, largely due to the impacts of administration routes, the duration of treatment and dosage frequency on the treatment success.

Metabolic diseases

A metabolic disorder can occurs as a result of the changes in normal metabolic mechanisms by abnormal chemical reactions in the human body. Metabolic disorders have been suggested to be a critical mechanism in many diseases, where the metabolic profile often includes abnormal consumption of glucose, altered respiration and fermentation, and changes in the use of metabolic enzyme isoforms.

Cancer continues to be the primary global public health issue and will remain the top leading cause of death in the next few years worldwide. Although cancer has historically been viewed as a disorder of proliferation, accumulating studies have pointed out that cancer is primarily a metabolic disease [305]. The reprogramming of energy metabolism has become an emerging hallmark of cancer, whereby tumor cells have a preference for glucose consumption coupled with elevated lactic acid (lactate) production [306]. This process, referred to as aerobic glycolysis or the 'Warburg effect', is commonly observed in both primary and metastatic cancers [307]. One unique hallmark of many solid tumors is hypoxia, which in addition to promoting the shift of cell metabolism to aerobic glycolysis and lactate production, contributing to higher levels of ROS and RNS in tumor *vs.* normal cells. The elevated oxidative stress observed in cancer cells can also result from a decrease or inactivation of antioxidants such as antioxidative enzymes (*e.g.*, CAT, SOD, and GPx). It has been indicated that ROS and RNS play crucial roles in tumor growth and metastasis and contribute to immunosuppression and the development of drug resistance [308]. Therefore, antioxidants supplementation in tumor cells has been proposed as a promising therapeutic option for many years.

Given the important physiological regulation functions and selective radical scavenging property, H_2 has been suggested as a potential means to treat cancer for more than forty years [309]. The primary mechanism of H_2-based cancer therapy could be attributable to reduced intratumoural radical level by H_2 and down-regulated expression of anti-apoptotic genes. However, efficient delivery of H_2 and controllable release in tumor tissues remain challenging. To this end, He et al. utilized acid-responsive ammonia borane-loaded mesoporous silica (AB@MSN) to locally generate H_2 in tumor for efficient cancer therapy without inducing obvious side effects [310]. Meanwhile, the same group found that a synergistic anti-cancer effect in different tumor models when H_2-generated antioxidative NPs was combined with photothermal therapy (Fig. 13**A**) [176].

Tumor hypoxia, along with the elevated ROS, can directly promote therapeutic resistance, as some therapies require oxygen to be maximally cytotoxic, such as chemotherapy, radiotherapy, and photodynamic therapy (PDT). Additionally, they are capable of improving genetic instability in cancer cells, enabling rapid conversion of drug-sensitive cells to drug-resistant cells [311]. Therefore, many research groups have devised different strategies to improve tumor oxygenation using antioxidative NPs, by which anti-cancer efficacy of various therapies could be enhanced or tumor resistance to these therapies could be reversed [312–318]. Relieving tumor hypoxia can be achieved by decomposing endogenous H_2O_2 in tumor to generate oxygen *in situ* mediated by enzymatic NPs. On one hand, enzyme CAT has been formulated within NPs. For instance, Liu's group reported TaOx nanoshells that combine high-Z element and high CAT loading as bio-nanoreactors for enhanced radiotherapy [318]. Such bio-nanoreactors could dramatically improve the tumor oxygenation by CAT-catalyzed decomposition of endogenous H_2O_2 into oxygen within the hypoxic tumor microenvironment, concurrently enhancing the therapeutic efficacy of radiotherapy and overcoming hypoxia-induced radiotherapy resistance (Fig. 13B). In an attempt to overcome the O_2-dependent PDT that suffers from the low therapeutic effect in clinical application as a result of the hypoxic tumor microenvironment, self-assembly of CAT and chitosan-Ce6 NPs has recently been reported by Shen et al. [315]. Under acidic environments, these NPs would undergo disassembly upon irradiation, leading to rapid O_2 release catalyzed by CAT and subsequently continuous formation of cytotoxic singlet oxygen for more effective PDT. Similar PDT outcomes have been obtained for other oxygen self-supplement NP formulations, such as CAT-loaded mesoporous silica [317].

In addition to NP-mediated direct delivery of CAT, NPs with CAT-like activities have also found wide applications for combined therapy [212,319–321]. One recent study by Jiang and co-workers

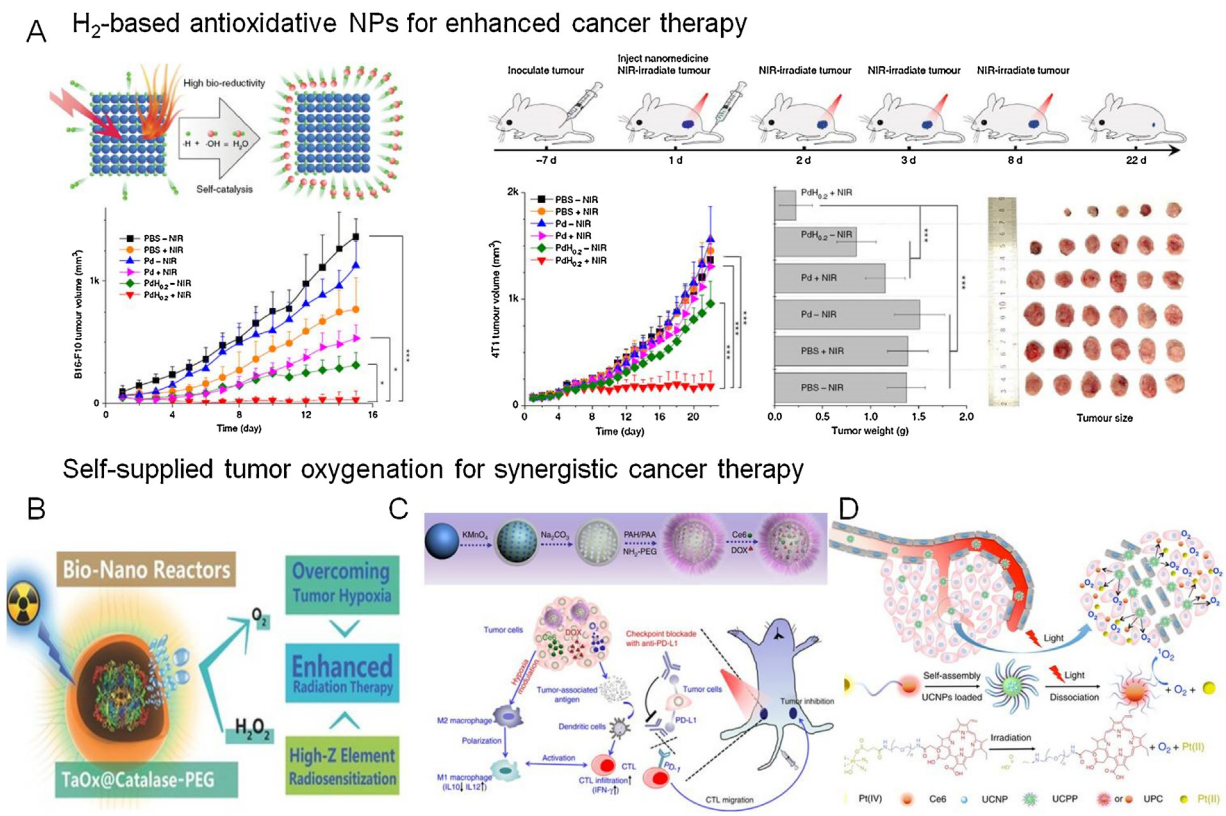

Fig. 13. Examples of using antioxidative nanomaterials for enhanced cancer therapy. (A) Schematic illustration of H_2-generated $PdH_{0.2}$ NPs for tumor oxygenation and their synergistic anti-cancer effect in different tumor models. Reprinted with permission from Ref. [176]. Copyright 2018, Nature. (B) Schematic illustration of TaOx nanoshell-based bio-nanoreactors for tumor oxygenation and enhanced cancer radiotherapy. Reprinted with permission from Ref. [318]. Copyright 2016, Wiley. (C) Schematic illustration of the CAT-mimic hollow MnO_2 NPs for synergistic chemo-PDT and anti-tumor immune therapy. Reprinted with permission from Ref. [320]. Copyright 2017, Nature. (D) Schematic illustration of the NIR light-controlled and O_2/Pt(II) self-generating nanoprodrug for synergistic photo-chemo cancer therapy. Reprinted with permission from Ref. [321]. Copyright 2018, Nature.

reported the use of CAT-mimic BSA-IrO_2 NPs that can overcome tumor hypoxia, enhance phototherapy, and protect normal cells against H_2O_2-induced oxidative stress and inflammation [212]. Liu's group reported an intelligent biodegradable hollow MnO_2 NPs for *in situ* catalysis of endogenous H_2O_2 in tumor into oxygen to overcome tumor hypoxia (Fig. 13C), leading to synergistic chemo-PDT therapy and a series of anti-tumor immune responses [320]. Alternatively, Yan and his colleagues developed a novel NIR light-controlled and O_2/Pt(II) self-generating nanoprodrug for synergistic photo-chemo therapy [321]. In that work, an amphiphilic oligomer Ce6-PEG-Pt(IV) was self-assembled on the surface of upconversion NPs (UCNPs) to yield the nanoprodrug. Upon NIR irradiation, UCNPs converted the NIR light into visible light, triggering the decomposition of Pt(IV) to release bioactive Pt(II) and excitation of Ce6 for synergistic cancer therapy. During this process, sustained generation of oxygen could compensate the oxygen consuming during PDT and alleviate the tumor hypoxia (Fig. 13D). Despite encouraging results, it has to be noticed that the tumor endogenous H_2O_2 is limit (10–50 μM) and varies among different solid tumors [322], which may restrict the wide applications of this strategy. To overcome the potential issue, liposome-mediated delivery of exogenous H_2O_2 and CAT has therefore been reported by Liu and colleagues. In their work, CAT-loaded liposomes and H_2O_2-loaded liposomes were separately administrated, and their enhanced radio-immunotherapy effects were systemically investigated using a patient-derived xenograft tumor model. Sustained release of H_2O_2 and subsequent long-term tumor oxygenation improvement have been achieved [316], offering remarkably enhanced therapeutic effects in cancer radiotherapy. Moreover, the relieved tumor hypoxia reversed the immunosuppressive tumor microenvironment, further enhancing the combined radiotherapy with checkpoint blockade immunotherapy.

Obesity is another representative metabolic disorder that occurs as results of persistent accumulation of body fat and excess release of free fatty acid into various organs [323]. The global incidence of obesity has doubled during the past few decades. Presently, some medications approved by FDA for long-term treatment of obesity can lead to significant loss of the mean weight and a clinically meaningful 1-year weight loss. However, obesity requires long-term treatment, and thus the efficacy and particularly the safety of anti-obesity medications must be evaluated during long-term use [324]. Notably, current small molecule-based medications generally yield significant off-target side effects with long-term use, highlighting the need of developing novel therapeutics.

Compelling evidences have indicated that interplay between ROS and adipogenesis in adipose tissues involving chronic low-grade systemic inflammation is the causal link between obesity and its comorbidities [325], such as cardiovascular diseases, stroke, type-2 diabetes, cancer, and respiratory diseases. In fact, studies have indicated that ROS is necessary for lipid accumulation, and cut down of carbohydrate intake could diminish both the body weight and oxidative stress markers [326]. Oxidative stress could also activate pathways to promote increased accumulation of white adipose tissues [327,328]. From this perspective, much hope was placed on the prevention and long-term treatment of obesity with antioxidative nanomaterials. Studies on the nanotechnology-mediated antioxidant therapy in obesity are limit, and very few antioxidative NPs have been reported thus

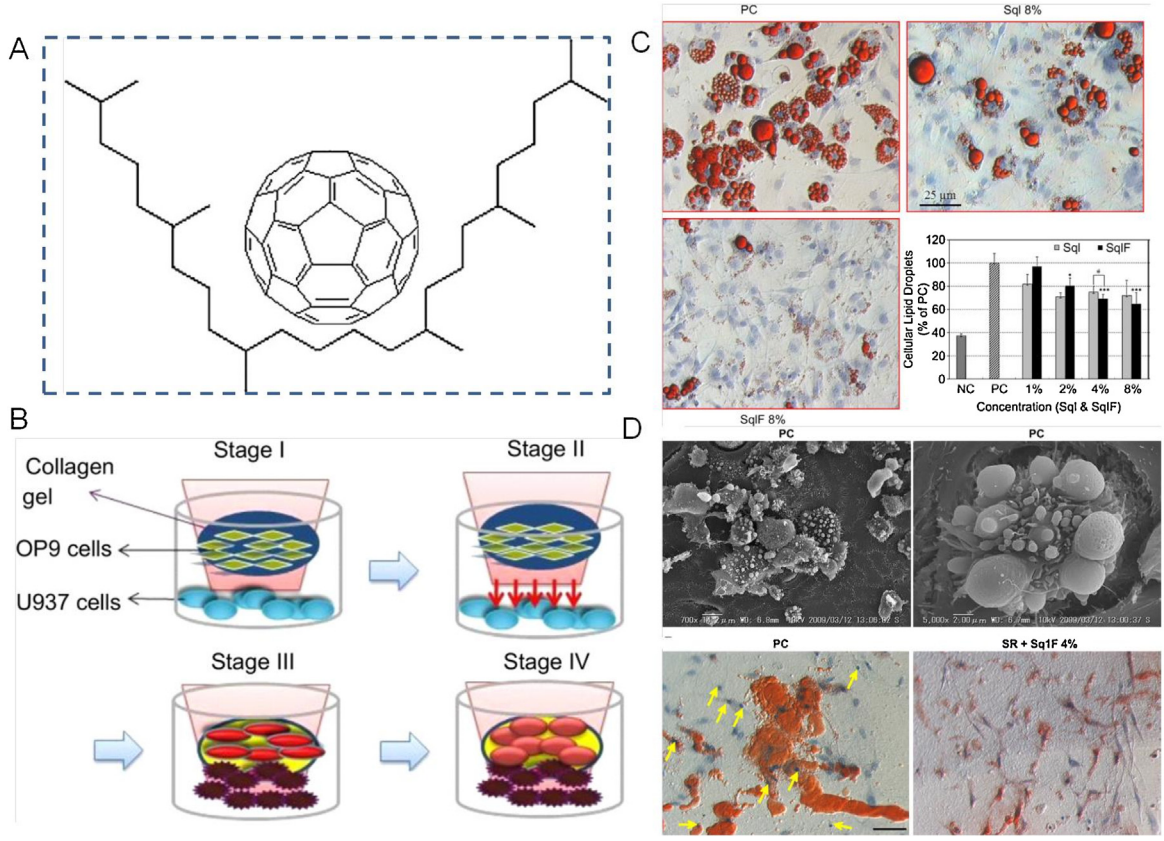

Fig. 14. (A) Schematic illustration of the structure of Sql-fullerene. (B) Schematic illustration of the three-dimensional inflammatory adipose-tissue equivalent for studying the anti-obesity effect of Sql-fullerene in OP9 cells. (C,D) Effects of Sql-fullerene on the prevention of lipid droplet accumulation and macrophage infiltration in a three-dimensional inflammatory adipose-tissue equivalent (ATE). Reprinted with permission from Ref. [331]. Copyright 2010, Elsevier.

far [329,330]. An early effort has been directly devoted to the utilization of nanoceria as a novel anti-obesity pharmaceutical formulation, given its broad and efficient antioxidative activity. In rat MSCs, administration of nanoceria suppressed adipogenic differentiation by inhibiting the generation and accumulation of ROS, which is essential for MSC differentiation into adipocytes [329]. Later on, it has proven that the mRNA transcription of some genes involved in adipogenesis and the accumulation of triglycerides in 3T3-L1 pre-adipocytes could be hindered by nanoceria [330]. Upon intraperitoneal administration into rats, nanoceria did not induce obvious toxic effects, but instead efficiently slowed down the weight gain and decreased the levels of insulin, triglycerides, leptin, and glucose in plasma. Similar anti-obesity effect has also been observed for fullerene and its derivatives [331]. Using a three-dimensional inflammatory adipose-tissue model containing OP9 cells, researchers found that Sql-fullerene significantly inhibited insulin-rich serum replacement-stimulated differentiation of OP9 cells and the following intracellular lipid droplet and ROS accumulation caused by monocyte activation and macrophage filtration in this tissue model (Fig. 14).

As a typical comorbidity of obesity, diabetes is generally accompanied by a high level of oxidative stress [323]. It was believed for a few decades that patients suffering from this disease would benefit from exogenous antioxidant supplementation as prevention or adjunct therapy [332]. Although dietary antioxidant consumption has demonstrated beneficial outcomes, most clinical antioxidant trials so far have failed to show significant health benefits of antioxidants [333]. One possible reason may arrive from the mutual interaction between oxidative stress and inflammation in pathophysiological conditions of diabetes, and antioxidants in clinical trials failed in specifically targeting both oxidative stress and inflammation. From this viewpoint, it can be assumed that antioxidants with simultaneous anti-inflammatory and ROS/RNS scavenging capabilities could be alternative effective medications for patients with diabetes. Recently, a variety of antioxidative nanomaterials have proven to protect against diabetes-related oxidative stress and unexpectedly exhibited metabolic outcomes in streptozotocin-induced diabetic rat models. Examples include nanoceria [334], Se NPs [335], and noble metal NPs [336], which have shown capability of suppressing the oxidative stress and enhancing the antioxidant defense system by increasing high density lipoprotein or decreasing the adenosine diphosphate/adenosine triphosphate ratio and inflammatory cytokines.

Pancreatic islet transplantation is a potential curative, noninvasive treatment option for type-1 diabetes. However, massive cell death during the first 72 h post-implantation caused by isolation stress and hypoxia have significantly impeded successful implantation [337]. Hypoxic stress of islets activates natural factor kB signaling pathways, leading to the secretion of large amounts of pro-inflammatory cytokines and downregulation of anti-inflammatory cytokines. Recently, Fullagar et al. reported that bilirubin NPs formulated with pluronic 127-chitosan could effectively decrease the cell death in murine islets treated with bilirubin NPs compared to untreated islets, which was presumably due to the bilirubin-mediated upregulation of protective genes (HO-1 and bcl-2) and downregulation of proinflammatory genes including MCP-1, caspase-8, caspase-3, TNF-α, and iNOS [338]. Instead of encapsulating poor soluble bilirubin within NPs, Lee and co-workers synthesized PEG-modified bilirubin NPs *via* covalent conjugation of PEG and bilirubin and self-assembly as an anti-oxidative and anti-inflammatory agent for pancreatic islet xenotransplantation [273]. Similar *in vitro* protection effects of islet cells against

oxidative stress and activated macrophages were obtained. In a pancreatic islets xenotransplantation animal model of diabetes, PEG-modified bilirubin NPs demonstrated significantly prolonged islet graft survival compared to free bilirubin treatment. Impressively, the rational design of antioxidative NPs could further lead to a synergistic or enhanced therapeutic efficacy. A recent work from Gu's lab described a core-shell microneedle (MN) array patch composed of H_2O_2-responsive cross-linked poly(vinyl alcohol) (PVA) gel simultaneously loaded with GOx, CAT and insulin for the treatment of diabetes [339]. The embedded GOx in the patch could stimulate the production of H_2O_2 for rapid release of insulin under the elevated blood glucose levels in diabetes, while the CAT scavenged excessive H_2O_2 to minimize the safety risk of H_2O_2-induced inflammation. These data suggest the potential of antioxidative nanomaterials as a potent remedy for diabetic complications.

Other diseases

Wound healing continues to be a great challenge for physicians and contributes to the increasing healthcare costs, as this process is complicated and could easily be interrupted, resulting in the formation of chronic, non-healing wounds. The elevated ROS generation in the wounded area has been implicated in triggering many deleterious events including cellular senescence, inflammation, and finally fibrotic scarring [340]. Wound care thus requires protection of the wound from oxidative stress and infection during the healing period. Given the powerful and stable free radical scavenging and anti-inflammatory activities, antioxidative nanomaterials have been further used for wound care [341–344]. For example, a copper ion-eluting thermoresponsive antioxidative hydrogel consisting of copper-based metal organic framework (HKUST-1) and poly(polyethylene glycol citrate-co-*N*-isopropylacrylamide) (PPCN) was reported [341]. The HKUST-1 could slow down the release of copper ions, and the antioxidative hydrogel would further decrease copper ion toxicity, thus demonstrating reduced cytotoxicity and apoptosis, increased *in vitro* migration of dermal cells, and accelerated wound closure rates in diabetic mice. RNAi nanotechnology has also been employed to enhance the wound healing. siRNA against Keap1, a key repressor of Nrf2 in antioxidant pathways, was formulated into a stable lipoproteoplex NPs to target diabetic wounds with severe oxidative stress [344]. Treatment with these NPs restored Nrf2-mediated endogenous antioxidant function, accelerated wound closure, and augmented redox homeostasis in the wound environment. In another study, ceria nanocrystals with a broad antioxidative activity have also been extended to regenerate wound healing [342]. The ultrasmall ceria nanocrystals were decorated on the surface of tissue adhesive mesoporous silica NPs (MSN-Ceria) to restore the tissue integrity and function in the wounded skin. The MSN-Ceria inherited both tissue adhesion of silica and antioxidative capabilities of ceria, accelerating the wound healing process of the wounded skin with much skin appendage morphogenesis and less scar formation (Fig. 15).

Antioxidative nanotechnology has further found potential applications in the cosmeceutical industry, as oxidative stress has long been believed as a great contributor to the accelerated senescence and aging. The major merits of using antioxidative nanotechnology in cosmeceuticals include improved stability of the cosmetic ingredients (*e.g.*, vitamins, unsaturated fatty acids, and other antioxidants) after encapsulation within NPs, targeted delivery of active ingredients to the desired site, and sustained release of active ingredients for long-term therapeutic purpose and decreased treatment frequency. Up to date, many antioxidative molecules-loaded NPs such as liposomes, fullerene, metal oxides, gold and silver NPs have been explored in cosmeceutical treatments such as radio damage, wrinkles, and hair damage [345,346].

Besides, melanin NPs have also been extended for the treatment of oxidative stress-induced periodontal disease, given the ROS-induced inflammatory reactions during the disease progress. Using a murine periodontitis model, it has been shown that melanin NPs was capable of removing ROS and decreasing periodontal inflammation without inducing any side effects [347]. With the advances in understanding the significance of nanotechnology in the field of antioxidant therapy, we believe that antioxidative nanomaterials will find more broad applications in diverse biological and medical fields.

Challenges in clinical translation

Antioxidative nanotechnology is fast becoming a novel class of therapeutic option and has shown great benefits for safe and effective treatment of numerous human diseases associated with oxidative stress and inflammation. However, it still faces considerable challenges for clinical transition from bench to bedside.

At present, the health and safety profiles of exposures to these bioactive NPs are not yet fully understood. The unique physicochemical and structural properties of nanoscale materials that are unusual or not seen with larger particles as a result of the quantum size effect and large surface area to volume ratio could account for a number of nano-bio interactions that can lead to toxic effects. It has been well recognized that the particle size, surface charge, hydrophobicity/hydrophilicity, coating, and steric effects would dictate the compatibility of NPs with immune systems [348]. Nonetheless, the physicochemical and biological rules of each factor remain poorly understood. In general, the smaller the particle is, the larger surface area to volume ratio and the stronger chemical and biological activities it will show. The greater chemical reactivity of nanomaterials can potentially increase the production of ROS, which reflects one of the primary toxicity mechanisms of NPs and has been observed in diverse nanomaterials such as carbon nanotubes, fullerenes, and some metal oxides [268]. The ROS generation property seems to be a paradox with the activity of those antioxidative nanomaterials, raising a question of what levels of exposure may be acceptable for effective radical scavenging in disease yet without inducing excess oxidative stress in normal tissues. Furthermore, NPs can also generate toxicity by several other mechanisms including protein misfolding, DNA injury, membrane perturbation, and direct physical damage. At present, bioinspired polymer nanomaterials or bioresponsive engineered polymer nanomaterials may hold promise for practical translation, benefiting from their appealing biocompatible and biodegradable features. Many preclinical data have also shown no obvious adverse effects associated with the administration of these antioxidative nanomaterials [349]. Nevertheless, the long-term toxicity (*e.g.*, genotoxicity), metabolism, degradation mechanisms, and clearance routes of these nanomaterials remain to be clarified in future studies for promoting clinical translation.

Aside from potential toxicity, the activity and stability of antioxidative nanomaterials, in particular *via* enzymatic catalysis, could be affected by the particle size, component, concentration, environmental conditions (*e.g.*, redox and pH), and others. Nevertheless, the detailed activity and stability of these antioxidative nanomaterials *in vivo* remain unclear. Despite ever-growing investigations and recent advance in the materials science and nanotechnology, a NP capable of scavenging various ROS and RNS with excellent stability is very limited. In particular, targeting delivery of antioxidative nanomaterials and spatiotemporally controlled antioxidant activity may provide a powerful means for simultaneously maximizing therapeutic effect and minimizing interference of redox balance in normal cells and tissues. Such active targeting is particularly important when tissue accumulation no longer depends on the

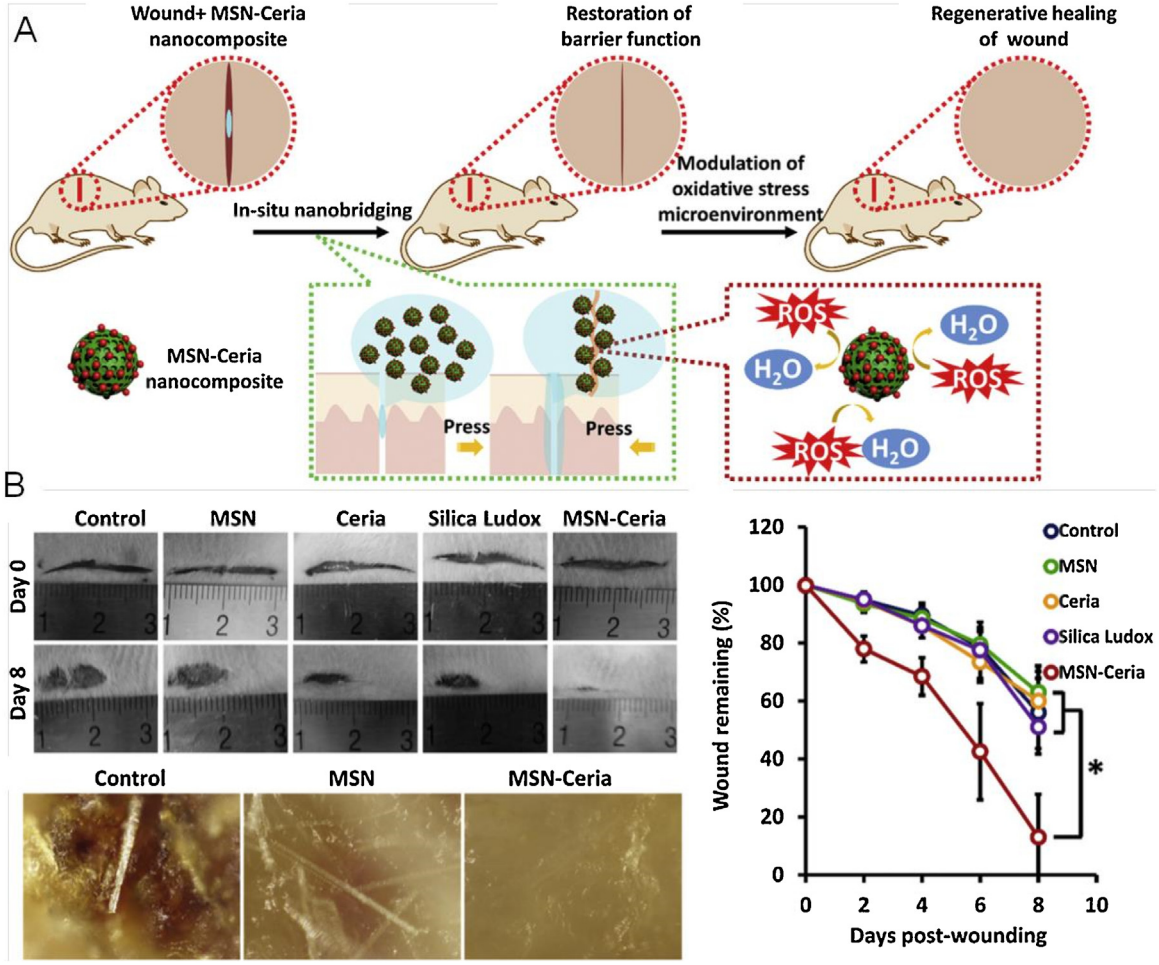

Fig. 15. (A) Schematic illustration of the synthesis of MSN-Ceria for wounding healing. (B) Wound repair effects of MSN-Ceria in the wounded skin. Reprinted with permission from Ref. [342]. Copyright 2018, Elsevier.

enhanced permeability and retention effect, such as vascular targeting. Moreover, antioxidative nanomaterials upon intravenous administration will face a complex multiple physiological and biological barriers that significantly limit site-specific bioavailability and thus prevent improved therapeutic outcomes [350]. These barriers include sequestration by the mononuclear phagocyte system (MPS), hemorheological/blood vessel flow limitations, nonspecific distribution, pressure gradients, cellular internalization, and endosomal/lysosomal escape. While substantial research efforts have attempted to increase half-life and propensity of antioxidants to accumulate at sites of injury, few antioxidative nanomaterials have to date adequately addressed these barriers and demonstrated the capability of selectively regulating redox status in the target tissues/cells.

An even greater challenge behind the clinical development of antioxidative nanomaterials arises from the redox pathology. Heterogeneity with regard to the type, concentration, and metabolism of different oxidative species is a distinct character in many oxidative stress-related diseases. Specifically, the redox status varies since the onset of disease, which largely determines the dose and frequencies of antioxidants. Specific and sensitive monitoring of ROS/RNS dynamics is therefore required to advance our understanding of the pathological basis of ROS/RNS in organisms. Currently, many approaches have been employed for sensitive and specific detection and monitoring of ROS/RNS *in vivo* [351,352]. EPR measurement of free radicals may offer great specificity and sensitivity towards ROS/RNS detection, and its ability to assay *in vivo* avoids artefactual perturbation of their levels. Nevertheless, the specificity of free radical identification by spin trapping could be ambiguous in some cases. Moreover, while spin trapping provides very useful measurements of the relative amounts of free radicals, the assay results are difficult to be translated into absolute quantities. It may also be possible to see some biological impact of the spin traps themselves. Proper controls are thus needed to ascertain whether perturbations arise from the spin trap. Although a large numbers of emerging techniques (*e.g.*, fluorescence and photoacoustic imaging) allow for sensitive and species-specific identification of ROS/RNS, optimization of imaging probes in terms of their biocompatibility, photostability, solubility, organ targeting, imaging depth, and tolerance to biological environments, is still required. Currently, the rigorous quantification and profiling of various ROS and RNS have remained an incredible challenge, highlighting the need of developing robust techniques that are able to sensitively distinguish and quantify different radicals for promoting clinical translation of antioxidant therapy.

Additionally, facile and scale-up manufacture procedure for high-performance antioxidative nanomaterials is another critical factor for clinical translation. Antioxidative nanomaterials that require very complicated or laborious synthetic procedures generally have limited potential for clinical translation, as they might be quite problematic to pharmaceutically manufacture on a large-scale. Current obstacles in pharmaceutical manufacturing for antioxidative nanomaterials include (i) suboptimal quality control, like size, polydispersity, morphology, encapsulation, surface

chemistry, purity and stability under physiological conditions; (ii) structural and physicochemical complexity of the antioxidative nanomaterial itself; (iii) high material and/or manufacturing costs, (iv) low production yield and batch-to-batch reproducibility, and (v) poor stability during long-term storage and upon clinical administration. Addressing these challenges is necessary to safeguard the applications of emerging antioxidative nanomaterials in the clinical setting.

Conclusions and perspectives

Over the past few decades, nanotechnology has made a revolutionary impact in the biomedical field, leading to great success in the development of a variety of functional nanomaterials for diverse therapeutic applications [29,30]. Given the fact that oxidative stress is a common hallmark in a multitude of human diseases, antioxidant therapy is moving rapidly as a new therapeutic modality. Antioxidative nanotechnology could be either nanoplatforms loaded with antioxidative compounds or enzymes or nanomaterials with intrinsic antioxidative activities. The versatility in designing nanostructures also provides the opportunity for creating novel formulations for targeted delivery that are potentially more powerful and safer. Extensive preclinical research has thus fur provided key information on design criteria for development of antioxidative nanomaterials. As a comprehensive overview of antioxidative nanotechnology, the underlying themes of this review are to demonstrate the current state with respect to the categories, activities, antioxidative mechanisms of various nanomaterials, as well as their potential applications in the treatment of oxidative stress relevant diseases.

While the past decade has witnessed an extensive body of work with numerous nanomaterials that demonstrate potent antioxidative and anti-inflammatory activities both *in vitro* and *in vivo*, we are still in the early stage of the clinical development of antioxidative nanomaterials. Several factors must be considered in future studies to promote clinical translation of antioxidative nanomaterials. The rapid growth of preclinical biomedical applications of the emerging antioxidative nanomaterials highlights the demands of specialized toxicology study in animal models to assess both acute and long-term chronic toxicity before translation to the clinical test. Indeed, study of nanomaterials with regard to toxicity has become a new branch of science, which is referred to as nanotoxicity. From this perspective, the vast array of existing and emerging antioxidative nanomaterials will require in-depth evaluation with respect to administration routes, *in vivo* biocompatibility, pharmacokinetics, biodistribution, metabolisms, toxicokinetics/toxicodynamics, and ultimately the expression of pathological sequelae. One promising area for antioxidative nanotechnology is the development of bio-inspired nanomaterials, which could be potentially advantageous in addressing the safety and metabolism concerns. Although several groups are working on the design of antioxidative nanomaterials using naturally occurring biomolecules as the building units, this area is still in its infancy and only a few bioinspired antioxidative nanomaterials have thus far been investigated.

Going forward, significant clinical benefits call for a thorough understanding of redox pathologies in different diseases and detailed antioxidative mechanisms of antioxidative nanomaterials, which will require deep cross-collaboration among experts from chemistry, materials science, engineering, physical, and biomedical fields. Since rationally designed antioxidative nanomaterials could lead to strong and stable antioxidative activity *in vivo* and subsequently better patient outcomes, we need to actively seek the best and cost-effective route for the manufacture of antioxidative nanomaterials that can selectively and efficiently modulate redox microenvironment in target sites with minimal systemic side effects. Another area of research that may revolutionize the field of antioxidant therapy is the pathology mechanistic study of oxidative stress *in vivo*. It is highly demanded to specifically identify which of oxidative species is primarily responsible for regulating a given biological event as well as its dynamics and contribution to both the initial and progress of oxidative stress-related diseases. The complexity also makes it highly necessary to develop novel and more clinically relevant *ex vivo* or *in vivo* animal models for robust evaluation of continuously emerging antioxidative nanotechnologies.

In summary, the field of antioxidative nanomedicine is rapidly advancing, and significant progress has also been made in the fundamental understanding of antioxidative nanomaterials. The publications showcased in this review are prime examples in the synthesis and exploration of novel antioxidative nanomaterials with *in vivo* antioxidative dynamics/activities superior to those traditional antioxidants. With the aforementioned challenges associated with antioxidative nanomaterials to be addressed in the foreseeable future, we expect that rapid and widespread applications of antioxidative nanotechnology for prevention and/or treatment of important human diseases will be achieved.

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