Chapter 8
Nanomaterials and Reactive Oxygen Species (ROS)

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Abstract One fundamental mechanism widely described for nanotoxicity from nanomaterials involves oxidative damage due to generation of free radicals and other reactive oxygen species (ROSs). Indeed, the ability of nanoscale materials to facilitate the transfer of electrons, and thereby promote oxidative damage or in some instances provide antioxidant protection, may be a fundamental property of nanomaterials. Effective methods are needed to assess oxidative damage elicited by nanoscale materials. The production of ROSs induced by nanomaterials is a double-edged sword, bringing not only the benefits of efficient nanomaterials for therapeutic treatment of diseases, but also possible health and environmental risks associated with them. Therefore, it is important to give a brief review on ROSs of nanomaterials and their relation in various biomedical applications.

Keywords Nanomaterial · Reactive oxygen species (ROSs) · Oxidative stress · Antioxidant · Biomedical application

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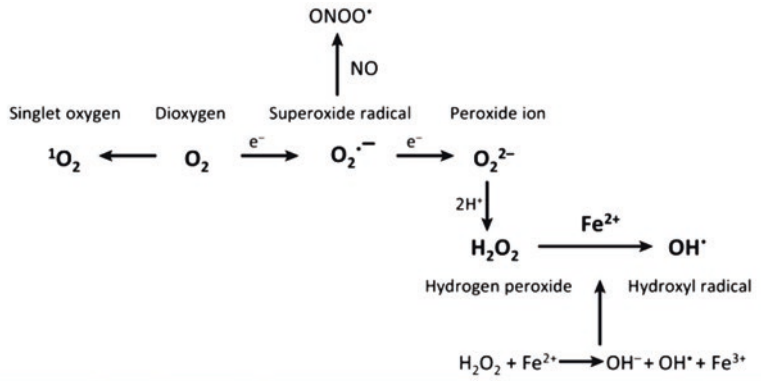
8.1 ROS in Biology System

Reactive oxygen species (ROSs) are a group of chemical species that are continuously generated, transformed, and consumed in all living organisms, which are regarded as unavoidable by-products of aerobic metabolism [1–4]. As a family of molecules that include at least one oxygen atom in each molecule but display higher reactivities relative to molecular O_2, ROSs comprise free radicals, including singlet oxygen ($^1O_2$), hydroxyl radical ($OH^•$), and superoxide radical ($O_2^{•-}$), as well as non-radical species such as hydrogen peroxide (H_2O_2) partially reduced from atmospheric oxygen (Fig. 8.1a) [5, 6]. The appearance of ROS is hypothesized to have taken place at the same time as atmospheric oxygen molecules about 2.4–3.8 billion years ago and has been paramount to the survival of all aerobic life ever since [7].

A



B

ROS	$t_{1/2}$	Migration distance	Mode of action	Production site	Scavenging systems
Superoxide ($O^{•-}_2$)	1–4 μs	30 nm	Reacts with Fe–S proteins Dismutates to H_2O_2	Apoplast (RBOHs), chloroplasts, mitochondria, peroxisomes, electron transfer chains	SOD, flavonoids, ascorbate...
Hydroxyl radical (OH•)	1 ns	1 nm	Extremely reactive with all biomolecules including DNA, RNA, lipids, and proteins	Iron and H_2O_2 (Fenton reaction)	Flavonoids, proline, sugars, ascorbate,...
Hydrogen peroxide (H_2O_2)	>1 ms	>1 μm	Reacts with proteins by attacking cysteine and methionine residues. Reacts with heme proteins. Reacts with DNA.	Peroxisomes, chloroplasts, mitochondria, cytosol, apoplast	APX, CAT, GPX, PER, PRX, ascorbate, glutathione,...
Singlet oxygen ($^1O_2$)	1–4 μs	30 nm	Oxidizes lipids, proteins (Trp, His, Tyr, Met, and Cys residues), and G residues of DNA	Membranes, chloroplasts, nuclei	Carotenoids and α-tocopherol

Fig. 8.1 Properties and reactivity of ROS [5]. (**a**) Formation of different ROS and reactive nitrogen species from atmospheric oxygen. (**b**) Properties ($t_{1/2}$, migration distance), reactivity (mode of action), formation (typical production systems), and scavenging (typical scavenging systems) of ROS in plant and animal cells. *APX* ascorbate peroxidase, *CAT* catalase, *GPX* glutathione peroxidase, *PER* peroxidase, *PRX* peroxiredoxin, *RBOH* respiratory burst oxidase homolog, *SOD* superoxide dismutase. (Reproduced with permission from [5])

Oxygen free radicals have inherent chemical properties that confer reactivity to different biological targets with stronger reactivity. ROSs are often associated with the principle of oxidative stress, which suggests ROSs cause oxidative damage to lipids, DNA, and proteins [8]. However, there is also the growing recognition that ROSs also serve as important physiological regulators of biological and physiological processes [9–11]. As signaling molecules, ROSs stimulate distinct cell signaling pathways and lead to diverse outcomes depending on their sites of production, levels of reactivities, and potentials to cross biological membranes (Fig. 8.1b) [6, 12]. They were most likely first used by cells as signaling molecules to monitor different metabolic reactions, or to sense unsafe levels of atmospheric oxygen, but have since evolved to regulate almost all aspects of aerobic life in animals, plants, and most eukaryotic organisms. For example, in plants, ROSs were found to regulate development, differentiation, stress signaling, redox levels, systemic responses, cell death, and interactions with other organisms [13–15]. As highly toxic by-products of oxygen metabolism, ROSs are primarily formed in mitochondria, peroxisomes, and chloroplasts, but also at any other cellular compartment that includes molecule or proteins with a sufficiently high redox potential to donate or excite an electron to molecular O_2. They are then detoxified or removed by a wide variety antioxidants and antioxidative enzymes (Fig. 8.1b) [6, 16]. This process of ROS production as harmful metabolic by-products, coupled with ROS removal by cellular antioxidative defense system, occurs constantly in cells to prevent some of the potential cellular damage of ROS that could include protein, RNA, DNA, and membrane oxidation and damage. The cellular antioxidative mechanisms of the cell therefore keep ROS at a basal nontoxic level, and the imbalance between ROS production and ROS scavenging could be used for ROS signaling reactions [17].

8.1.1 The Source of ROSs in Biology: Where the ROSs Produced?

The ROSs are generated exogenously or produced intracellularly either by the mitochondrial respiratory chain (cytochrome-oxidase complex containing NADH-dehydrogenase and ubiquinone) or by metabolism of arachidonic acid, xanthine oxidase, phospholipases, and membrane-bound oxidases [18]. This means that essentially any cell could produce ROSs, provided it contains mitochondria or enzymes involved in redox system [19]. An important consideration for ROS chemistry and biological processes is the specific cellular location where a particular metabolite is generated, because microenvironments can determine what targets these ROSs will potentially encounter in a temporal and spatial manner. The classic subcellular location with localized ROS generation includes mitochondria, endoplasmic reticulum, and the cell membranes [20].

There are two important sources of ROSs, consisting of mitochondria and a family of NADPH oxidases (NOXs). The first major source of ROS is the

mitochondria, which are within most cells and tissues. Recent studies revealed that there are eight sites in mitochondria that produce ROS. The three best characterized subcellular locations are complex I, II, and III. They are all in the mitochondrial respiratory chain, and localized to the inner mitochondrial membrane. Superoxide ($O_2^{\bullet-}$) is the proximal mitochondrial ROS generated by the one-electron reduction of O_2. Complex I, II, and III release superoxide into the matrix of mammalian mitochondria where superoxide dismutase (SOD) rapidly catalyzes dismutation of superoxide to H_2O_2. Moreover, complex III can also release superoxide into the intermembrane space. Superoxide traverses through voltage-dependent anion channels from mitochondria to cytosol and is converted into H_2O_2 by SOD1 [21]. The other key source of ROS, in the form of either $O_2^{\bullet-}$ or H_2O_2, is NOXs and their dual oxidase relatives, which are primarily localized to various plasma membranes [12]. NOX proteins are classically known as important ROS sources of most growth factor- and/or cytokine-stimulated oxidant production. The NOX catalyzes the one-electron reduction of O_2 to $O_2^{\bullet-}$, with NADPH as the electron donor [22, 23].

8.1.2 The Regulation of ROS Production in Biology

Low levels of ROS can activate signaling pathways to initiate biological processes, while high levels of ROS would incur damage to proteins, DNA, or lipids. It means that spatial and temporal regulatory mechanisms must exist to modulate ROS levels in response to oxidative stress [12, 24–28].

Generally, antioxidative enzymes can eliminate ROS. Superoxide is rapidly converted by SOD 1, 2, and 3 into H_2O_2. SODs are mainly located in the mitochondrial intermembrane space (SOD1), the matrix of mitochondria (SOD2), and the extracellular matrix (SOD3). SODs protect aerobic organisms from toxical superoxide that can damage and inactivate proteins.

As H_2O_2 is the by-product of superoxide scavenging by the SOD, there are a wide variety of enzymes that remove H_2O_2, including peroxiredoxins, glutathione peroxidases, and catalase (CAT). The function of these antioxidant enzymes is dependent on the concentration of H_2O_2, their reactivity with H_2O_2, and enzyme in vivo. The regulation of activity and expression levels of these antioxidants occurs by multiple functions and mechanisms in part to modulate ROS levels [29, 30].

Another reactive and damaging ROS is hydroxyl radical ($OH^{\bullet}$), which indiscriminately oxidizes DNA, proteins, and lipids, resulting in genomic instability or irreversible damage of cellular macromolecules. Typically, hydroxyl radicals are formed through reactions with ferrous ions (Fenton-like reaction). Therefore, cells have multiple strategies to maintain homeostasis to prevent the formation of extremely reactive hydroxyl radicals [31–33].

8.2 Biological Effects Induced by Oxidative Stress from Nanoparticles

With the spread and development of nanotechnology and nanoscience, nanomaterials have dramatically increased in biomedical and industrial applications. However, the scientific basis for the cytotoxicity and genotoxicity of most nanomaterials is not comprehensively understood. The influences on ROS are regarded as one main source of toxicity of nanomaterials. The ROSs from nanomaterials have lots of critical determinants, including size, shape, surface charges, surface-containing groups, dissolution, ions release from nanomaterials, light activation, aggregation, mode of interaction with cells, and pH of the medium. Nanomaterials can disturb the balance of ROS, leading to oxidative stress, resulting in cells failing to maintain normal physiological environment and related functions. This may lead to DNA damage, regulate cell signaling, and change in cell motility, cytotoxicity, apoptosis, and cancer initiation.

ROSs played a vital role in all living organism. The cellular signaling pathways, antioxidant defense, and oxidative stress-induced diseases are all known to be associated with the level of ROSs (Fig. 8.2). As mentioned above, there are a variety of ROS-producing pathways by nanoparticles in cells and tissues, including the NADPH oxidase, the mitochondrial, the xanthine oxidase, the cyclooxygenase, and the dioxygenase system. In most of the eukaryotic cells, mitochondria are the major sites for ROS production [16] and a synthetic site of adenosine triphosphate (ATP). During the synthesis of ATP, molecular oxygen is finally reduced to water molecules through a series of redox processes under the action of mitochondrial electron transport

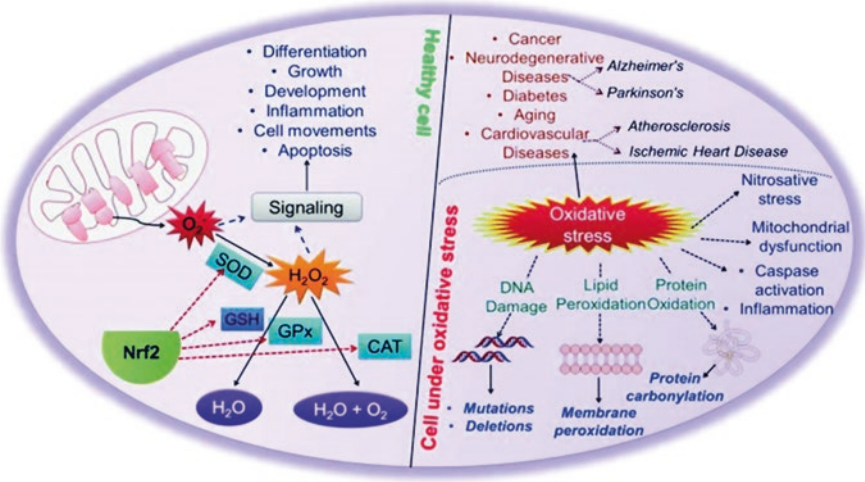


Fig. 8.2 Cellular antioxidant machinery and oxidative stress [8]. (Reproduced with permission from [8])

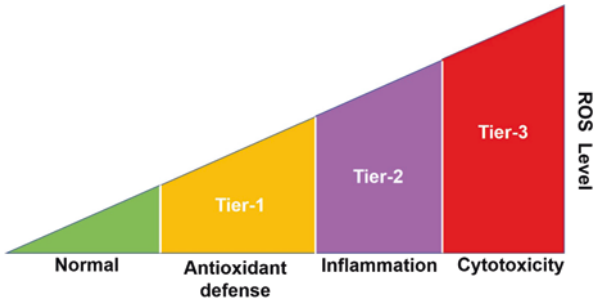


Fig. 8.3 Stratified oxidative stress model [35]. At a lower amount of oxidative stress (tier 1), antioxidant enzymes are induced via transcriptional activation of the antioxidant response element to restore cellular redox homeostasis. As ROS levels increase, this protective response is overtaken by inflammation (tier 2) and cytotoxicity (tier 3)

chain; however, a small portion of oxygen molecules is not reduced. The remaining part of the oxygen molecule is ROS, so ROS is also considered to be a by-product of cellular oxidative metabolism [34]. Generally, oxidative stress with excess ROS is viewed as a dominant mechanism of pathological changes induced by nanoparticles (NPs). Here, we talk about only the nanotoxicology caused by ROS.

Nel proposed a three-layer oxidative stress model of cellular oxidative stress in nanoparticles to explain the toxicity of nanoparticles, as shown in Fig. 8.3, that is, low levels of ROSs induce enhanced antioxidant defense of cells and higher levels of ROSs cause cellular inflammatory responses and ultimately induce cell death at very high levels of oxidative stress [35].

8.2.1 Antioxidant Defense

The production and elimination of active oxygen is maintained at a steady-state level in physiological conditions. In the body, the elimination of oxygen includes the enzymatic antioxidant defense system and the nonenzymatic antioxidant defense system, which is an effective protection mechanism for potential oxidative damage formed by nanoparticle.

In the defense systems, enzymatic antioxidants include superoxide dismutase, catalase, peroxidase, heme oxygenase, glutathione reductase, glucose-6-phosphate dehydrogenation, etc., which are mentioned in the previous chapter. Although these enzymes have different molecular weights, structures, and reaction rates, they all undergo disproportionation reactions to catalyze reactive oxygen radicals. In addition, there are nonenzymatic antioxidant defense systems. It mainly includes vitamin C, vitamin E, lipoic acid, carotenoids, uric acid, flavonoids, and coenzyme Q. These antioxidants are both synthesized in the body and absorbed from natural substances. These antioxidants react directly with free radicals by giving them electrons. The result of the reaction is that these small molecular antioxidants become

new free radical carriers, which are reduced by other substances (such as NADH) and return to a reduced state [36].

In addition, cells have evolved appropriate antioxidant signaling pathways for timely scavenging of excessive ROSs, such as Nrf2/keap1-ARE, PPAR gamma, FOXO, and SIRT [37–40]. For example, Nrf2/keap1-ARE signaling pathway is the most powerful endogenous antioxidant signaling pathway known at present. When exposed to oxidants, Nrf2 dissociates from Kelch-like ECH-related protein 1 (Keap1) and translocates to the nucleus, where it binds to promoter regions of antioxidant enzymes containing antioxidant response elements (ARE) such as Gpx2, NQO1, and GCLC. The mechanism of Nrf2-dependent effect involves the reduction of antioxidant enzyme transcription and damage-related genes, providing protection against oxidation-induced acute tissue injury [41]. Moreover, these pathways can synergistically resist oxidative stress damage and promote cell survival.

With low levels of oxidative stress, cells can start to initiate their own antioxidant reactions. The antioxidant enzymes firstly changed and then the transcription factor Nrf2 activated by the antioxidant response [42], causing a series of changes in antioxidant signaling pathways to regulate the oxidative stress response in cells. In contrast, high levels of ROS production resulting from Nrf2 deletion lead to elevated proinflammatory cytokine levels [43]. Nrf2-deficient mice exacerbate the innate immune-inflammatory response to pathogens, resulting in increased pneumonia and sepsis [11]. Antioxidants increase the survival rate of Nrf2-deficient mice in these sepsis models. Thus, while slightly elevated ROS levels may enhance immune system function, high levels of ROS may promote pathological inflammatory responses.

8.2.2 Inflammation

Inflammation is the defense response of the body to external stimuli. Nanoparticles may cause the release of a variety of inflammatory factors. As gene transcription plays an important role in regulating cells, nanoparticles are involved in the regulation of the expression of many genes, especially those related to the body's defense functions, including adhesion molecules and proinflammatory cytokines. For examples, NF-κB is often involved in cell proliferation and differentiation, as well as inflammation and immune response. Studies have shown that inhibition of NF-κB can alleviate the inflammatory response and fibrosis caused by nanomaterials [44]. There is ample evidence that ROSs are essential second messengers in innate and adaptive immune cells [45]. However, elevated levels of ROS in immune cells can lead to overactivation of the inflammatory response leading to tissue damage and pathology [41]. ROS messengers are needed to maintain the innate immune system. For example, pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) molecules are produced by pathogens, which can induce body injury activating the monitoring receptors (Toll-like receptors (TLR), RIG-I-like receptors (RLRs), and NOD-like receptors (NLRs)) and produce ROS through NADPH oxidase and mitochondria. There are lots of

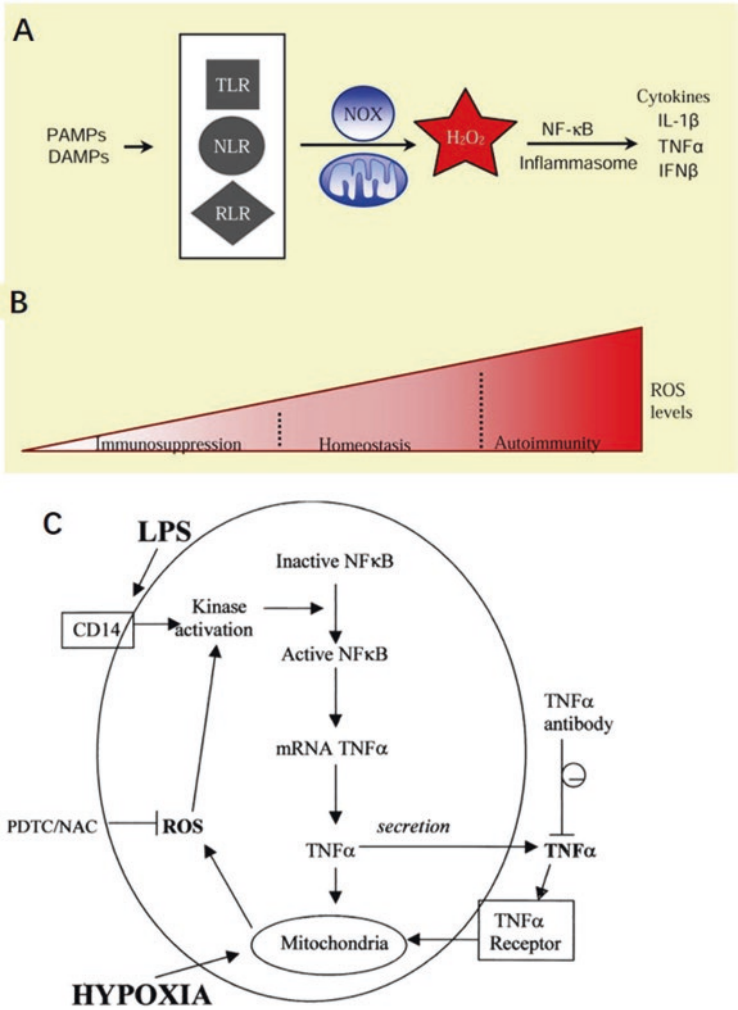


Fig. 8.4 Examples of ROS regulation of inflammation [12, 46]. (**a**) Activation of the innate immune system requires ROS signaling. (**b**) Low levels of ROS maintain a healthy immune system. (**c**) Effects of LPS and hypoxic activation of NF-κB and TNF-α mRNA. (Reproduced with permission from [12, 46])

proinflammatory cytokines, including IL-1β, TNF-α, and IFN-β, which are closely related with ROS. ROSs regulate their release and maintain the innate immune response (Fig. 8.4a). Low levels of ROSs maintain a healthy immune system. Lowering ROS level will inhibit the activation of normal immune response, leading to immune suppression. Increased ROS levels can promote autoimmunity by increasing the release of inflammatory cytokines and the proliferation of specific subsets of adaptive immune cells (Fig. 8.4b).

The earliest studies of ROS in the innate immune system were found by producing NADPH oxidase and mitochondria to produce ROS-activated cellular inflammatory factors in the presence of lipopolysaccharide (LPS). The LPS activates NF-κB and the expression of TNF-α mRNA, and TNF-α increases mitochondrial ROS through an autocrine effect of TNF-α on a cell membrane receptor. Hypoxia induces mitochondrial ROS directly, which subsequently activates NF-κB and TNF-α mRNA expression (Fig. 8.4c) [46]. Studies have found that single-walled carbon nanotubes can activate NF-κB and promote the transfer of NF-κB from cytoplasm to intracellular binding with DNA, starting transcription, resulting in the release of ROSs, oxidative damage to cells, and apoptosis pathway, and leading to cell death [47].

Existing studies have found that NP can increase the levels of some inflammatory cytokines. For example, silica NP has been shown to significantly increase the release of some proinflammatory cytokines. These increases are organ-dependent, which means that systemic administration of NP causes inflammation [48]. It has been reported that the cytotoxicity of three metal oxides (ZnO, CeO_2, TiO_2) has been compared and based on the release of zinc ions, ZnO is the most toxic in bronchial epithelial and macrophage cell lines. Furthermore, ZnO NPs not only generate hydrogen peroxide and superoxide radical, but also cause the formation of IL-8 and tumor necrosis factor-α (TNF-α) in bronchial epithelial cells and macrophages [49]. The same cytokines were also found in the bronchoalveolar lavage fluid of welders exposed to some metal oxide nanoparticles (including ZnO NPs) [50]. This exposure can cause an acute inflammatory response in the lung called metal smog [51].

8.2.3 Cytotoxicity

The toxicity of nanomaterials that damage cells is called cytotoxicity, which is one of the most important toxic effects observed in vitro. Exposure of cells to toxic amounts of nanomaterials can cause a series of changes within the cell, such as cell membrane contraction, rupture, or destruction of intracellular components, leading to apoptosis or necrosis, which can affect cell growth rates. In vitro cytotoxicity assays are commonly used to elucidate acute and chronic toxicity caused by nanomaterials. The benefits of using cultured cell lines are numerous, including reproducibility and ease of handling with test materials. Cultured cells have been shown to have significantly higher sensitivity to nanomaterials [35].

In vitro cytotoxicity assessment involves measuring the proliferation and cellular metabolism of cells exposed to nanomaterials using different assays (such as 3-(4,5-diethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-etrazolium (MTS)). The purpose of these methods is to mimic cellular responses in vitro after exposure to any toxic dose of nanomaterials. However, the in vitro simulated cytotoxicity tests are usually different from the real body; thus it is still necessary to pay attention to more detailed studies. There are publications to demonstrate how the topic of toxicity needs to be discussed [52]. In addition, they

observed that the doses used to obtain in vitro data often lacked relevance with that in vivo. In vivo effects such as dilution, surface changes, and biological interactions are particularly important [53].

The physicochemical properties of nanomaterials are key factors affecting cytotoxicity through ROS, such as the shape, size, surface charge, and chemical modification. The effects of these factors on cytotoxicity of nanomaterials have been systemically reviewed [54].

Small size is a unique property of nanomaterials, which can affect the level of ROSs in cells or tissues. Mirshafa et al. reported that oxidative stress in rat brain tissue induced by alumina nanoparticles (Al_2O_3-NPs) is much larger than that of alumina particles (Al_2O_3-MPs), and Al_2O_3-NPs can significantly induce ROS production in rat brain tissue, what is more, glutathione and potential of mitochondrial membrane decreased. Smaller-sized Al_2O_3-NPs have greater oxidative damage to the brain than Al_2O_3-MPs [55]. Neubauer et al. showed that the ability of Pd-NPs and Ni-NPs to induce ROS production in THP1 cells is significantly dependent on the particle size of nanoparticles. In the range of 4–27 nm, nanoparticles induce strong ROS production, of which 12 nm particle size-induced cells produce the highest level of ROS [56]. Conversely, a report suggested that distribution and toxicity appear to be independent of particle size within the test range [57], but surface charge rather than particle size closely regulates the pharmacokinetics of NPs [58]. This shows the toxic effects of NPs are complex.

Surface cationized nanoparticles are likely to interact with negatively charged cell membranes or nucleic acid species, thereby induce a higher oxidative stress response [59]. Platel et al. have studied the effects of negatively and positively charged polylactic acid-glycolic acid copolymer (PLGA) nanoparticles on the levels of ROSs in different cell lines. Studies have shown that the ROS levels of murine lymphoma cells (L5178Y), human lymphoblasts (TK6), and human bronchial epithelial cells (16HBE) can be significantly increased by positively charged PLGA nanoparticles. However, negatively charged PLGA nanoparticles have less effect on the ROS levels of the above three cells [60]. The result on PLGA nanoparticles is similar with gold nanoparticles, which show that positively charged gold nanoparticles are incorporated more by human umbilical vein endothelial cells (HUVECs) than negatively charged gold NPs, indicating stronger cytotoxicity and oxidation stress reaction [61]. With different surface chemical modifications, the toxicities of NPs are often different even with the same NPs. For example, to employ the polyethylene glycol (PEG) as coating material, the potential toxicity of NPs can significantly decrease by reducing intracellular ingestion and binding interactions with proteins [62]. Taking antibodies as the biomolecules on the surface of NPs, the toxicity of antibody-conjugated quantum dot is much lower than that of unconjugated quantum dot on male Wistar rats [63].

Among the studies on cytotoxicity of nanomaterials, a majority of existing researches focus on the study of endothelial cells. The vascular endothelium cell is the first tissue to contact the nanoparticles before the nanoparticles are delivered to

the target via the blood circulation. The effect of nanoparticles on blood vessels is an important issue that needs to be studied and elucidated. For example, silver (Ag) NPs were found to be taken up by vascular endothelial cells and induce intracellular ROS elevation, which is closely related to the integrity of endothelial cells. Endothelial cell leakage induced by intravenous administration of Ag NPs mediates inflammation of the liver, kidneys, and lungs [64]. Based on this, it was also found that even at noncytotoxic concentrations, an increase in intracellular ROS and CAT activity is a common effect of NPs, depending on the size distribution, composition, and surface chemistry of the NP. This action results in a gap between the endothelial cells that can be rescued by the use of antioxidant [65]. Iron oxide nanoparticles were reported to influence the phenotype and be able to induce endothelial-to-mesenchymal transition in endothelial cells at an acute noncytotoxic dose, although they were rarely taken up by endothelial cells. ROS scavengers can rescue the effect of endothelial-to-mesenchymal transition on HUVEC in vitro and in vivo [66].

With the rapid development of the production and application of nanomaterials, the evaluation of the safety and toxicity of nanomaterials-induced ROS has become a public concern; thus the detection of ROS in nanomaterials is urgently needed.

8.3 Methods of Detecting ROS in Nanomaterials

Because ROSs are easy to produce and may produce toxic effects to biological cells, it is particularly important to accurately detect ROSs. The measurement of ROSs is dependent on the analytic target along with the ROS in question. At the cellular level, specific ROS can be individually assessed from tissue culture, while at the animal level typically the effects of oxidative stress are measured from blood product (e.g., serum or plasma) or from urine samples. Methods for ROS detection can be broadly classified as either direct or indirect. Due to the short lifetimes and typically low concentrations of ROS in aquatic systems, their direct observation is only possible on the sub-millisecond timescale (Fig. 8.1b), with the relatively stable H_2O_2 being an exception. Indirect methods typically involve probes that very rapidly react with ROS to compete with antioxidants and produce stable products, which can be quantified [67–71]. By virtue of introducing additional chemical reactions, all indirect techniques risk perturbing the observed system. Some important aspects to consider when choosing an ROS analysis method include: (1) the sensitivity of the method; (2) the selectivity and specificity of the method for the analyte of interest; and (3) the ability of the method to allow measurements with sufficiently fast time resolution [72]. Additional analytical considerations are availability, robustness, portability (for field studies), the cost of the necessary instrumentation, and in some cases, the cost of the probe molecules. All these methods are mainly divided into three categories.

8.3.1 ESR Technique

Electron spin resonance and paramagnetic resonance (ESR or EPR) are unique techniques that specifically and directly "see" unpaired spins of free radicals. ESR spectroscopy is the most used method for the detection of paramagnetic species. This involves absorption of microwave energy by paramagnetic species in the presence of an external magnetic field resulting in the transition of spin states. ESR spectroscopy is a useful method for studying any materials with unpaired electrons. EPR allows researchers to detect ROS directly and it can also be used to monitor changes in the chemical forms of the oxidizable transition metal ions implicated in ROS generation. ESR spectroscopy stands out from other methods because of its unique ability to detect either short- or long-lived radicals with specificity and sensitivity [73, 74]. For short-lived ROS, the spin-trapping technique involves the reaction with the addition of the free radical to the double bond of a diamagnetic "spin trap". Spin traps are stable, diamagnetic compounds that form longer-lived radical species with transient, very reactive radicals with low half-lives of only 10^{-9} to 10^{-1} s. The paramagnetic spin adducts are stable for minutes or even hours, accumulate in the tissue, and reach a sufficient concentration for detection by ESR. The ESR spectra of the spin adducts are unique and provide a fingerprint for the presence of ROS. Two classes of spin traps are commonly used: the linear nitrones, *N-tert*-butyl-a-phenyl nitrone (PBN) and a-(4-pyridyl-1-oxide)-*N-tert*-butyl nitrone (4-PyOBN); and the pyrroline-based cyclic nitrones, 5,5,-dimethyl-pyrroline *N*-oxide (DMPO), 5-*tert*-Butoxycarbonyl-5-methyl-1-pyrroline *N*-oxide (BMPO), 5-diethoxyphosphoryl-5-methyl-1-pyrroline *N*-oxide (DEPMPO), and 5-ethoxycarbonyl-5-methyl-1-pyrroline *N*-oxide (EMPO). It is important to distinct spin traps and spin probes. Spin traps form covalent bond with the radical by addition reaction, while spin probes are stable nitroxide free radicals. Spin probes have an unpaired electron, which is able to bind to another molecule. The commonly used spin labels include 2,2,6,6-tetramethyl-1-piperidinyloxy, 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO), 2,2,6,6,-tetramethyl-4-piperidone-1-oxyl (TEMPON), 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPOL), 4-amino-2,2,6,6-tetramethyl piperidine-1-oxyl (4-amino-TEMPO), 3-carbamoyl-2,2,5,5-tetra-methyl-3-pyrroline-1-yloxyl (CTPO), and 4-oxo-2,2,6,6-tetramethyl piperidine-d_{16}-1-^{15}N-oxyl (^{15}N-PDT). This technique is not only much more sensitive than other direct detection, but it also allows better identification of the primary radical [75, 76]. The resulting ESR spectrum often exhibits a hyperfine splitting pattern that is characteristic of the trapped radical, and therefore transient radicals that are otherwise undetectable under normal conditions can now be observed [77, 78].

Generally, $OH^{\bullet}$ and $O_2^{\bullet-}$ can react with diamagnetic nitrone spin traps and form a stable free radical (spin adduct). A commonly used spin trap for these two radicals is BMPO. The ESR spectrum of the spin adduct BMPO/$OH^{\bullet}$ shows four lines with relative intensities of 1 : 2 : 2 : 1 and hyperfine splitting parameters of $a_N = 13.56$, $a_H^{\beta} = 12.30$, and $a_H^{\gamma} = 0.66$ (Fig. 8.5a). The ESR spectral characteristics of BMPO/

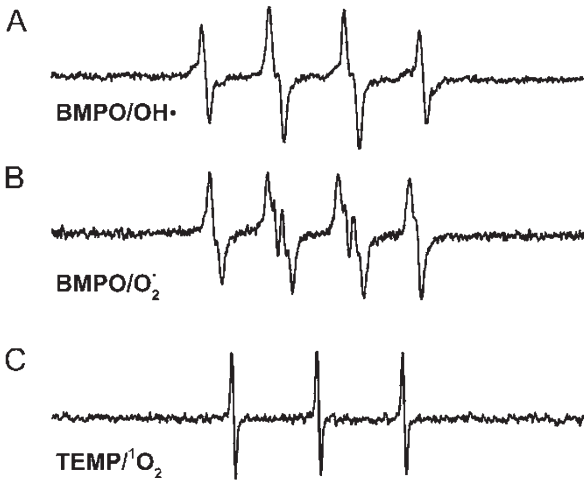


Fig. 8.5 Typical ESR spectra of adducts for (**a**) BMPO/OH•, (**b**) BMPO/$O_2^{\bullet-}$, (**c**) TEMP/$^1O_2$ (TEMPONE)

$O_2^{\bullet-}$ show four lines with relative equal intensities (1 : 1 : 1 : 1), and two hyperfine splitting parameters of a_N = 13.4 and a_H^β = 12.1 (Fig. 8.5b) [79]. Singlet oxygen ($^1O_2$) is another important ROS, and 2,2,6,6-tetramethyl-4-piperidine (TEMP) can be used as a spin trap to specifically capture $^1O_2$ to yield a nitroxide radical (4-oxo-2,2,6,6-tetramethylpiperidine-*N*-oxyl, TEMPONE) with a stable ESR signal. ESR spectra of TEMPONE characteristically have three lines with equal intensities, with a hyperfine splitting parameter of a_N = 16.0 (Fig. 8.5c) [79].

However, there are some limitations for ESR spin trapping during ROS determination in vivo or/and in vitro. The technique requires spin traps that reduce specificity and stability for the measurement. Another major drawback of the method is that spin adducts are only relatively persistent, and their lifetimes, especially in biological environments where they are subject to degradation by several enzymatic systems, are usually too short to allow reliable quantitative measurements. Of course, apart from these chemical limitations of the method, one of the biggest drawbacks is the cost of EPR instrumentation, which is often much greater than typical absorbance or fluorescence spectrometers [80].

8.3.2 Optical Absorption

Some ROSs (such as hydrogen peroxide, superoxide, and hydroxyl radical) absorb in the 230–350 nm region of the UV/Vis spectrum and can be quantified directly at micromolar concentrations by measuring their absorbance [67, 72]. The molar absorption coefficient of H_2O_2 is 0.01 M^{-1} cm^{-1} at 360 nm and gradually increases up to 13 M^{-1} cm^{-1} with decrease in wavelength to 260 nm. However, such a

method is not typically possible because of its limited lifetime, small absorption coefficient, and the presence of other chromophores absorbing in a similar wavelength region [67].

8.3.3 Spectroscopic Probe Technique

Because the ESR technique requires a specialized and relatively expensive ESR spectrometer, alternative methods have been developed for ROS detection with more readily available equipment. The spectroscopic probe methods make use of readily available probe molecules, do not require specialized equipment, and generally afford greater specificity, sensitivity, and lower limits of detection compared to ESR and absorbance-based methods. Spectroscopic detection strategies, including absorbance (UV/Vis), fluorescence (FL), and chemiluminescence (CL), share a common approach with several other techniques for measuring rates of ROS formation and decay in laboratory experiments. These strategies are also compatible with methods such as steady-state kinetic analyses, stopped flow methods, time-resolved laser spectroscopy, flash photolysis, and pulse radiolysis. A spectroscopic probe is a substance that changes its spectroscopic properties (light absorption or emission) upon reaction with ROS. An ideal spectroscopic probe should be highly specific for one kind of ROS form and react with it efficiently, so that it can be used at low concentration without perturbing the studied system [70, 71].

The best and simplest ROS detectors are substances with optical properties that change in reaction with ROS and show some specificity for different ROS species. However, their principal disadvantage is relatively low sensitivity compared to other probes [81, 82]. Ferricytochrome c reduction is a time-honored and accurate method for detecting large amounts of $O_2^{\bullet-}$ released by cells into the extracellular space, which is based on the reduction of ferricytochrome c by $O_2^{\bullet-}$ to ferrocytochrome *c*. This reaction can be followed by the spectrophotometric absorbance at 550 nm [70]. Nitro blue tetrazorium (NBT, 2,2′-di-*p*-nitrophenyl-5,5′-diphenyl-(3,3′-dimethoxy)-4,4′-bisphenyleneditetrazolium chloride) has also been widely used for the detection of superoxide radical. The product of univalent reduction of NBT is tetrazoinyl radical in which its dismutation generates a stable formazan. A number of colorimetric substrates such as tetramethylbenzidine (TMB) and phenol red have also been used in conjunction with horseradish peroxidase (HRP) to measure hydrogen peroxide concentrations. In general, colorimetric means are less sensitive than fluorescent detection methods, but instrumentation costs are significantly lower than those required for fluorescence-based measurements when using tube-based or microplate-based detection methodologies [81].

The fluorescence methodology, associated with the use of suitable probes, is an excellent approach to measure ROS because of its high sensitivity, high spatial resolution, and simplicity in data collection [83–85]. Fluorescence may be measured or observed with a microtiter plate reader, microscope, fluorimeter, or cytometer. Confocal microscopes offer the possibility of additionally observing cellular

topography of ROS production and can provide some degree of specificity through use of various fluorescent probes [84]. A variety of fluorescent probes have been developed recently to detect ROS in order to uncover their unique functions in biological systems. The most widely used fluorescent indicator for superoxide radical is hydroethidium (HE), a two-electron reduced form of the nucleic acid stain ethidium. HE has been employed to detect ROS production during phagocytic respiratory bursts and intracellular oxidative stress. The oxidation of 2,7-dichlorodihydrofluorescein (DCFH) originates 2,7-dichlorofluorescein (DCF), a fluorescent compound ($\lambda_{excitation}$ = 498 nm, $\lambda_{emission}$ = 522 nm) initially thought to be useful as a specific indicator for H_2O_2. However, measurements based on redox-sensitive dyes such as DCFH can be problematic because they depend on dye uptake and lack any specificity toward a particular type of ROS. The advent of protein-based redox sensors like redox-sensitive green fluorescent protein (roGFP) have improved specificity to particular ROS and can be targeted to different compartments within the cells to gather spatial resolution of ROS levels [83]. Fluorescent protein-based biosensors have been developed for the investigation of the ROS in situ in real time. This new generation of live cell fluorescent sensors produces changes in fluorescence in response to alteration in the redox state or with fluctuations in specific target analyte. These sensors are genetically encoded, based on a single fluorescent protein and do not require the addition of any other reagents or cell lysis, making them very amenable to multiplexing.

Chemiluminescent (CL) reactions have been used for their potential increase in sensitivity over absorbance-based detection methods [85–88]. Chemiluminescence (CL) is observed when the electronically excited product of an exoergic reaction relaxes to its ground state with emission of photons, and it can be defined in simplistic terms: chemical reactions that emit light (ultraviolet, visible, or infrared radiation). CL applications in analytical chemistry have obvious potential advantages, such as high sensitivity, wide linear range, simple and inexpensive instrumentation, considerable reduction of background noise, safety, controllable emission rate, and easy computer control. ROSs can generate electronically excited products, which emit the weak CL during their decay to the ground state. Although it is not easy to detect the light emission directly by CL techniques, it can be enhanced by CL substrates. Due to superoxide's brief lifetime and low steady-state concentrations, it is typically measured using highly sensitive CL probe molecules. Successful probes for decay or steady-state measurements must react at rates of at least ten times greater than that of natural superoxide disproportionation. The most widely used chemiluminescent substrate is Luminol and lucigenin. Luminol is the most widely used CL probe for chemical analysis. In the presence of a peroxidase (such as HRP), luminol-derived chemiluminescence has been used to detect cellular superoxide or H_2O_2 production under various experimental conditions. Unfortunately, because lots of species can promote the CL of luminol, this reagent is problematic for the selective analysis [87]. Among the most widely used compounds with higher specificity in their light emission with superoxide is bis-*N*-methylacridinium nitrate (i.e., lucigenin), which could be used at moderate pH [88]. Other compounds used are cypridina luciferin analogues, such as

2-methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo (1,2-α)pyrazin-3-one (MCLA), 2- methyl-6-phenyl-3,7-dihydroimidazo (1,2-α)-pyrazin-3-one (CLA), and 2-(4-hydroxybenzyl)-6-(4-hydroxyphenyl) 8-benzyl-3,7-dihydroximidazole [1,2-α] pyrazin-3-one (i.e., coelenterazine).

Although the use of spectroscopic probes appears to be a simple and easy means for the detection and quantification of ROS production in cellular systems, it should be noted that the techniques of fluorescence, spectrophotometry, and luminometry are less direct and less specific for the detection of free radicals versus ESR [81].

8.3.4 Nanoprobes for ROS Detection

Some drawbacks of spectroscopic probes can be overcome or ameliorated using a method of encapsulating the dye in nanoparticle delivery systems. Encapsulation of these probes by inert nanoparticles, which protect them from nonspecific interactions, provides an elegant solution for delivery into cells. Chemically inert nanoparticle delivery systems are sufficiently small (1–1000 nm) so as to be introducible into cells by standard mechanisms (microinjection, lipofection, and TAT-protein delivery). They are however large enough to encapsulate perfectly relatively large volumes of one or multiple probes, which facilitates a ratiometric measurement of the optical response [69].

Warner et al. reported the ratiometric coumarin-neutral red nanoprobe that can be utilized for detection of the hydroxyl radical. The nanoprobe was prepared by mixing poly lactide-*co*-glycolide (PLGA) nanoparticles, containing encapsulated Neutral Red, and a coumarin 3-carboxylic acid conjugated poly(sodium *N*-undecylenyl-*N*-e-lysinate) that serves as a moiety that is reactive with the hydroxyl radical. The probe was selective for hydroxyl radicals as compared to other ROS including $O_2^{\bullet -}$, H_2O_2, $^1O_2$, and OCl^{-1} [89].

Recent advances in the design and synthesis of carbon nanostructures have provided a novel route for optical biosensor development. Carbon nanotubes generally display semiconductive properties and are therefore photo-luminescent, absorbing radiation and emitting photons at specific wavelengths. One recent study highlights the potential of single-walled carbon nanotubes in the multimodal analysis of intracellular ROS by reporting detection of H_2O_2, singlet oxygen, and $OH^{\bullet}$ [90]. Wu and Zeng et al. developed the multifunctional fluorescent nanoprobe, which is prepared by covalently linking the mitochondria-targeting ligand (triphenylphosphonium, TPP) and boronated fluorescein (PF3) to carbon dots (CDs). In the presence of H_2O_2, the arylboronate moiety in PF3 is converted to a phenol, which triggers FRET from the CDs to fluorescein PF4. The results of a cell imaging study indicate that the nanoprobe can be applied for detecting endogenously produced mitochondrial H_2O_2 in RAW264.7 cells [91].

Alternatively, the detection and quantitation of ROS reaction products can also be accomplished with detector molecules using high performance liquid

chromatography (HPLC), mass spectrometry, and immunochemistry. Other methods of detecting ROS reactions include monitoring lipid peroxidation and oxidative damage to proteins as well as DNA.

8.4 Biomedical Applications of Nanomaterials by ROS Regulation

At present, ROS detection technologies have tended to be diversified. The vigorous research on ROS is not only for avoiding ROS, but also for making use of them in the treatment of human diseases.

8.4.1 Cancer Therapy

Cancer is one of the leading causes of death. From the statistical data [92], there were more than 18 million new cancer cases and 9.5 million deaths worldwide in 2018. Globally, the number of new cases is expected to rise to ~23.6 million per year by 2030. More and more people's life and health are severely threatened by this devastating disease. At present, the conventional ways commonly used in clinical treatment of cancers include chemotherapy, radiation therapy, and surgery. However, such methods bear low cure rates and adverse side effects on patients' physical and mental health. Therefore, the development of new therapeutic strategies with high efficacy and largely diminished side effects in combating cancer is urgently needed for public health. At the cutting-edge field, nanomaterials with unique physicochemical characteristics have received increasing interest for biomedical applications, especially cancer therapy [93]. ROSs have been well recognized as one of the important players, which can be beneficial to kill cancer cells [94]. Recently, many nanomaterials have been reported to generate ROS intrinsically in biologically relevant environments. Nanomedicines based on mediating ROS for cancer treatment caught enormous attention currently. As ROSs are associated with most of the stages of cancer, the therapeutic strategy based on ROS concludes two main categories: drug delivery enhancers and cell death inducers [94]. Nanomaterials triggered the production of ROS mainly through two ways: (1) the photo-irradiation excited the nanomaterials to produce ROS, and (2) the intrinsic ability of nanomaterials to facilitate electron transfer catalytically activated the generation of ROS. Such abilities have been subtly used to develop novel cancer treating modalities, such as photothermal therapy (PTT), photodynamic therapy (PDT), and catalytic reaction therapy (CRT) [95–98].

Photodynamic therapy (PDT) is a promising technique for treating various cancers. The main elements include light, photosensitizers, and oxygen. The fundamental principle of PDT is using light to activate a photosensitizer, leading to the

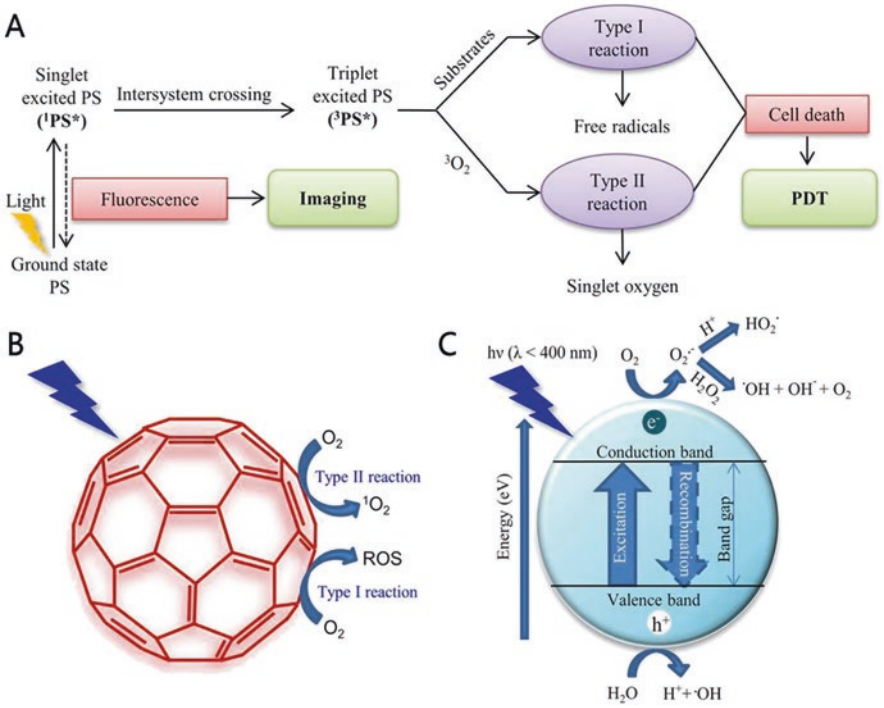


Fig. 8.6 Schematic illustration of a typical photodynamic reaction. (**a**) Two typical ways for photodynamic reaction upon light irradiation on (**b**) fullerene and (**c**) semiconductor nanoparticle [95]. (Reproduced with permission from [8])

generation and release of ROSs at the local site of cancerous body (Fig. 8.6). Though there have been developed several organic photosensitizers (e.g., porphyrins, chlorins, porphyrinoids, and biomolecule conjugates) as candidates for PDT, NPs offer several advantages over traditional treatment options, typically including low toxicity of the NPs in the absence of light irradiation, high efficacy, optimal response to light with varying wavelength, selective and specific accumulation, and deep penetration into the tumors [95]. Various nanomaterials have been prepared and employed as novel photosensitizers, including noble metal NPs, semiconductor NPs, carbon NPs and their derivate, metal organic frames, and others. Noble metal nanoparticles (Au, Ag, Pd, Ti) hold great promise as PDT agents because of their surface plasmon resonance (SPR) enhancing effect on generation of ROS [99–101]. Typically, the gold nanoparticles with tunable SPR from visible to NIR have been demonstrated as PDT agents can selectively kill cancerous cells at varying laser frequency ranges. For example, 15 nm Au nanospheres with citrate coating were reported to destroy the malignant cells upon exposure to laser light, which induced the production of ROSs [101]. The shape and structure of gold NPs can affect greatly the SPR, and consequently the PDT therapeutic efficiency [102]. Semiconductor nanostructures, such as TiO_2 and ZnO, can also produce ROS under irradiation that can be used for reducing tumor growth [103]. Another example is copper sulfide (CuS) NPs, which

is found to induce ROS production by NIR laser irradiation, showing photodynamic effects under laser irradiation and synergistically killing cells [104]. Carbon nanomaterials and their derivatives also have been revealed as PDT agents to produce ROS under irradiation in cancer therapy [105, 106]. PEG-functionalized graphene oxide was as well employed as substrate to load the photosensitizer molecule Chlorin e6, and the product is able to generate singlet oxygen under light excitation and exhibits enhanced PDT efficacy against cancer cells [107]. In addition to the single component structure, hybrid nanostructures consist of different nanocomponents, which provide an effective way to improve PDT cancer therapy [108]. For example, the nanocomposites consisting of $NaYF_4$:Yb/Er upconversion NPs embedded with methylene blue in SiO_2 and conjugated with Au nanorods, and that the integrated structure was confirmed by significantly improving ROS production under NIR irradiation and undergoing efficiency PDT both in vitro and in vivo [109]. The highly toxic ROS damaged the mitochondrial membrane, which could be induced by the cell apoptosis pathway. Note that the photothermal effect coincided with PDT when plasmonic gold NPs are irradiated. Photothermal therapy is an emerging, noninvasive, and effective treatment for cancers; the combination of PDT and PTT has been often designed for a higher efficiency of cancer therapy [110–112].

Without the assistance of light, some nanomaterials can catalyze/trigger specific chemical reactions that can generate abundant ROS in the local biological system and subsequently enable to combat cancers. Based on these findings, versatile catalytic chemical reactions (e.g., Fenton reaction) have been developed as a new strategy for cancer therapy [113]. Such therapeutic modality has higher efficacy and selectivity, as well as low side effects on normal tissues. There are reviews to summarize the very recent studies on nanostructures-triggered in situ catalytic reactions for tumor-specific therapy [96, 113]. For example, iron oxide nanoparticles have been demonstrated to mimic the peroxidase activity and activate the generation of hydroxyl radicals in the presence of hydrogen peroxide through Fenton-like reactions [114]. The highly oxidative hydroxyl radicals can be used for killing cancer cells. One case was reported by fabricated FeO_x-loaded mesoporous silica nanoparticles, which can catalyze the decomposition of H_2O_2 to generate ROS in the acidic microenvironment of lysosomes, thus causing cancer cell death [115]. In another example, by integrating Fe_3O_4 NPs and glucose oxidase into biodegradable dendritic silica nanoparticles, a concept of sequential catalytic nanomedicine for efficient tumor therapy was introduced. The authors found that highly toxic hydroxyl radicals are generated through sequential catalytic reactions to trigger the apoptosis and death of tumor cells [100]. The redox enzyme-like activity of nanomaterials could also be deployed for catalytic therapy of cancers. Nitrogen-doped porous carbon nanospheres were employed as nanozymes that catalyzed ROS generation in a tumor-specific manner, resulting in significant tumor regression in human tumor xenograft mice models [98]. A flower-like MnO_2@PtCo nanozyme was also reported to catalyze a cascade of intracellular biochemical reactions to produce ROS in both normoxic and hypoxic conditions without any external stimuli, resulting in remarkable and specific inhibition of tumor growth [116]. With the fast development of chemistry, materials, and nanotechnology, it offers great

opportunity to fabricate superstructures by assembly of different nanocomponents, which would exhibit integrational functions (e.g., PTT, PDT, CRT) for improving cancer therapy.

8.4.2 Antibacteria

When the nanostructures exposed with microbial (e.g. bacterial, fungal and biofilms), the generated ROS could induce the death of microbial. There are two prevalent mechanisms for ROS-facilitated antibacterial applications.

One is based on the enzyme-like activity, in which nanozymes accelerated the production of ROS in the presence of oxygen or hydrogen peroxides to inactivate bacterial growth. A variety of nanomaterials with peroxidase-like characteristic are listed as promising antibacterial agents. For example, a trace amount of iron oxide NPs could trigger the ROS production and completely kill *E. coli* in a low concentration of hydrogen peroxide. In contrast, the control experiment without NPs only shows a 15% antibacterial efficacy, indicating the important role of iron oxide in triggering ROS-killing strategy [117]. Pd-NPs is found to exhibit facet-dependent oxidase and peroxidase-like activities via generation of ROSs, which endow them excellent antibacterial properties against Gram-positive bacteria. The {100}-faceted Pd cubes are reported to own higher activities to kill bacteria more effectively than {111}-faceted Pd octahedrons. However, the antibacterial activity is reversed in Gram-negative bacteria [118]. Pt hollow nanostructures also exhibit high peroxidase-like activity to catalyze the decomposition of H_2O_2 to hydroxyl radicals. It is demonstrated that Pt nanostructures show excellent bactericidal activity against both Gram-negative and Gram-positive bacteria in the presence of low concentrations of H_2O_2 [119]. This antibacterial activity can be applied to treat wound infections.

Photocatalytic inactivation of bacteria is the other mechanism [120]. Upon irradiation, typically the semiconductor nanomaterials could absorb the light and be excited to create a hole or electron pairs. The separated holes in valence band and electrons in conduction band are highly oxidative and reductive, respectively, which was determined by their energy band positions. The highly active hole and electrons can in turn react with surrounding oxygen-containing molecules or ions to generate ROS, the dominant intermediates for killing bacteria. The photocatalytic properties of titanium dioxide are well known and the earliest example for use in disinfection [121]. The photoexcited TiO_2 was capable of killing a wide range of Gram-negative and Gram-positive bacteria, algae, protozoa, filamentous and unicellular fungi, mammalian viruses, and bacteriophage. The killing mechanism involves degradation of the cell wall and cytoplasmic membrane due to the production of ROSs (including hydroxyl radicals, superoxide, and singlet oxygen) caused by the irradiation of TiO_2 [122]. The antibacterial activity could be enhanced by improving the photocatalytic activity through increasing the charge carrier separation efficiency and production of ROS. It was demonstrated that the formation of ZnO/Au hybrid nanostructures could greatly enhance the photocatalytic and antibacterial activity

toward killing *S. aureus* and *E. coli* under sunlight [123]. Using ESR technique, it is found that the deposition of Au and other metal NPs resulted in a dramatic increase in light-induced generation of ROSs and production of holes and electrons. It has unraveled the important roles of metal type, particle size, and compositions on antibacterial activity of ZnO NPs [124]. Photocatalytic inactivation of bacteria has been one of hot research directions in nanomaterials because of its high antibacterial efficacy and cost-effectiveness. Since 2010, there are more than 2700 research papers found in Web of Science ("photocatalytic + antibacterial"), and more than 100 kinds of nanostructures were tested for their ability to kill bacteria in these studies. Many pathogenic pathogens develop anti-resistance nanomaterials, and the most common of which is for bacteria. The drug resistance of bacteria is a big problem that threatens human health. Owning to the different antibacterial mechanism from conventional antibiotics, the nanomaterials that kill the bacteria through triggering the production of highly toxic ROS may provide the best solution to bacterial resistance.

8.4.3 Prevention of ROS-Related Diseases

Neurodegenerative diseases, include Huntington's disease, Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis, are a heterogeneous group of disorders, which can be characterized by the progressive degeneration of the structure and function of the central nervous system or peripheral nervous system. As chemically reactive molecules, ROSs have been implicated in the pathogenesis of neurodegenerative diseases. Although ROS may not be the immediate cause for neurodegenerative diseases, many evidences suggest that they are likely to exacerbate disease progression through oxidative stress-related processes [125, 126]. Therefore, the regulation of ROS levels may represent a promising treatment option to slow down neurodegeneration and alleviate associated symptoms. It was reported that Mn_3O_4 nanoparticle owns multi-enzyme-like activities, such as glutathione peroxidase, SOD, and CAT. Based on the ROS scavenging activity, Mn_3O_4 NPs exhibit an active role in protecting the cells from 1-methyl-4-phenylpyridinium (MPP+) induced cytotoxicity in a Parkinson disease-like cellular model, suggestive of a new strategy to rescue the biological structures from oxidative damage and thereby possess therapeutic potential to prevent ROS-mediated neurological disorders [127]. After this pioneered work, CuO NPs were prepared with an average size of ~65 nm and with good biocompatibility and multiple enzyme-like activities, which could inhibit neurotoxicity in a cellular model of Parkinson's disease and rescued the memory loss of mice with Parkinson's disease because of the activity of CuO NPs to eliminate ROS [128]. Recently, trimetallic nanozymes possessing multi-enzyme-mimetic activity for clearance of ROS and reactive nitrogen species (RNS) were reported, which can improve the viability of injured neural cell and significantly improve the survival rate, neuroinflammation, and reference memory of injured mice in vitro and in vivo [129]. Benefited by the intrinsic activity of scavenging

ROS, the potential application of hollow Prussian blue nanoparticles in neuroprotection against ischemic stroke primarily by scavenging ROS and RNS was demonstrated. Prussian blue NPs were also reported to not only attenuate oxidative stress, but also suppress apoptosis and counteract inflammation both in vitro and in vivo. This contributed to increased brain tolerance of ischemic injury with minimal side effects [130]. Ceria NPs have also been demonstrated to protect against ischemic stroke by scavenging ROSs and reducing apoptosis [131]. Apart from these neuro-related diseases, the nanomaterials scavenging ROS have also attempted to protect biomolecules or living body against ROS-induced protein oxidation, lipid peroxidation and DNA damages, and inflammations [8, 132, 133].

8.5 Outlook

With the low cost, easy preparation, good physiological stability, high catalytic efficiency, robust antioxidative activity, and biocompatibility, the ROS-producing and scavenging nanomaterials caught special attention, which may represent a novel class of nanomedicine for the treatment of ROS-related diseases and damages with promoting therapeutic outcomes.

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