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Review

Recent advances in self-assembling redox nanoparticles as a radiation protective agent

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Abstract: The search for potent radiation-protective drugs for clinical use continues. Studies have, so far, focused on targeting the neutralization of radiation-generated reactive oxygen species (ROS) to protect the cells against the deleterious effects of exposure to ionizing radiation. However, the development of efficacious radioprotective drugs, which are mostly low molecular weight (LMW) compounds, is mainly limited due to their inherent toxicity and rapid excretion from the body. Thus, researchers reformulated these LMW compounds into nano-based formulations. This review discusses recent advances in the use of self-assembling redox nanoparticles as a new group of radioprotective agents. The copolymer micelle (herein referred to as redox nanoparticles; RNP) contains an active part, amino-TEMPO, that effectively scavenges radiation-induced ROS in the body, as demonstrated *in vivo*. With the use of nanoparticle-based technologies, optimized

formulations of these LMW ROS-neutralizers lead to the significant reduction of its toxicity, high bioavailability and longer blood circulation, which consequently resulted in its notable enhanced efficacy (for example, increased survival rate, reduced radiation-induced syndromes and organ damage) against the damaging effects of ionizing radiation. Consistent with the available data on the use of RNP and other nano-based radioprotective agents, it can be concluded that the inherent ROS-targeting activity of a drug intended for radiation protection is as vital as its bioavailability in the specific tissues and organs, where the short-lived ROS are produced during radiation exposure. In this review article, we summarized the current status of the development of radioprotective agents, including our self-assembling radioprotective agents.

Keywords: self-assembling redox nanoparticles; nitroxide; reactive oxygen species; ionizing radiation; radiation protection; radioprotective agent; biodistribution; blood circulation of nanoparticles

1. Introduction

Radiation is part of our daily lives and comes from natural and/or manmade sources. Among the many types of radiation, ionizing radiation, coming from radioactive materials (gamma rays) and electrically operated devices (X-rays), is of primary concern in terms of its possible risks and health hazards. Gamma rays and X-rays are major types of ionizing radiation, which has enough energy to remove electrons from the orbit of an atom, turning the neutral atom into a radical or an ionized species [1]. The use and applications of ionizing radiation in medicine and diagnostic imaging have become one of the major sources of manmade radiation. These medical exposures, together with natural radiation and radioactivity in the environment, largely contribute to the accumulated human exposure to ionizing radiation [2]. The exposure of cells and their components to ionizing radiation rapidly results in the generation of reactive oxygen species (ROS), such as hydroxyl radical (•OH) and ionized water (H2O+), including hydrogen radicals (H•) and hydrated electrons (eaq^{-}) [3]. Also, secondary ROS products of ionizing irradiation, such as superoxide ($O2•^{-}$) and hydrogen peroxide (H2O2), form within picosecond (10-12 s) [4,5]. The generation of these ROS consequently damages the cells by directly destroying the genetic material, producing DNA lesions, strand breaks, and crosslinks [6–8]. Additionally, in biological tissues, radiation ionizes and destabilizes water molecules. As described just above, this process of water radiolysis results in the generation of reactive radical species that eventually react with nearby molecules, and subsequently, produce ROS that indirectly damage cells and tissues [2,5]. Among the ROS, hydroxyl radicals are known to be the most cytotoxic, especially when located near the DNA. ROS and other free radicals are very reactive due to their unpaired electron that reacts with the DNA molecules, causing their damages. It is important to note that hydrogen peroxide is also toxic to the DNA. These free radicals cause the indirect effects of ionizing radiation that damages the DNA resulting in the functional impairment of the cell or its death [2,9]. Because water constitutes around 70% of the cell, the majority of radiation-induced damages are the result of indirect effects [2]. Thus, the neutralization of ROS, especially hydroxyl radicals, is essential for protecting the cells against the deleterious effects of exposure to ionizing radiation [10].

In 1998, Krishna [11] and his team conducted a comprehensive study on nitroxides; among the 73 compounds (30 nitroxides, 23 hydroxylamines, and 30 amines) examined, they observed that the

positively charged nitroxides, 4-amino-2,2,6,6-tetrametylpiperidine-1-oxyl (amino-TEMPO or NH_2-TEMPO, Figure 1), which are LMW antioxidant compounds, possess the highest radioprotective efficacy.

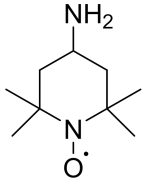

Figure 1. Structure of TEMPO.

Nitroxide compounds containing an amino group possess an effective radioprotection activity compared to unsubstituted base compounds and other nitroxides. This observation is in line with the most effective thiol-based radioprotectors that require the presence of an amino group [11]. However, LMW antioxidants, including LMW nitroxides, have disadvantages associated with their use and efficacy due to their toxicity and rapid excretion, as demonstrated by previous studies [12–14]. For example, the short half-life of amifostine (Figure 2) limits its clinical use. Its size, daily dosing, toxicity on the administration route, and its cost represent major limitations [15].

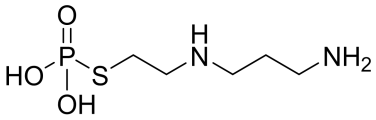

Figure 2. Structure of amifostine.

When an LMW antioxidant enters the body, it immediately diffuses to all the nearby tissues and organs, regardless of their route (oral, intravenous, or subcutaneous), and is subsequently excreted from the body, thereby reducing its therapeutic efficacy due to rapid excretion. Moreover, non-specific tissue diffusion of LMW antioxidants causes toxicity and leads to serious side effects in humans, as shown by some radioprotective drugs, including WR-2721, WR-3689, bioactive lipids, and some immunomodulators [14–16]. These side effects of LMW antioxidants are a serious concern as they jeopardize their use in the radiation protection of healthy cells and tissues.

To overcome previously cited drawbacks, these LMW antioxidants can be encapsulated into a nanoparticle-based self-assembling system, resulting in significant improvement in its biodistribution and longer blood circulation time, thereby improving its effectiveness in protecting the healthy cells against the damaging effects of ionizing radiation [17,18]. Another strategy consists of covalently conjugating the antioxidant to the hydrophobic segment of an amphiphilic block copolymer, and further formulating the corresponding nanoparticles. With this technique, leakage of the antioxidant from the core of the nanoparticle is, thus, prevented [19].

In this review, we discuss recent advances and summarize significant findings regarding the use of self-assembling redox nanoparticles and other nano-based antioxidants as radiation protective agents focusing on studies conducted *in vivo*.

2. Inorganic nanoparticles as radioprotective agents

Before we discuss organic self-assembling radioprotective agents, let us briefly summarize other statuses of the development of radioprotective agents. The ability to effectively scavenge the free radicals brought about by irradiation is the primary role of radiation protective agents. Recent results focused on this mechanism by developing nanoparticles for radiation protection, and examples of these include graphdiyne, bismuth selenide, and cerium oxide nanoparticles, to name a few [20–28].

Bovine serum albumin (BSA)-modified graphdiyne nanoparticles (graphdiyne-BSA NPs) were recently evaluated for their free radical scavenging ability, and their application for radioprotection, both in cells and animal models, has been investigated. Graphdiyne (Figure 3), which consists of a strong π-conjugated structure and reactive diacetylenic bounds, has a high free-radicals scavenging activity [18,20,21].

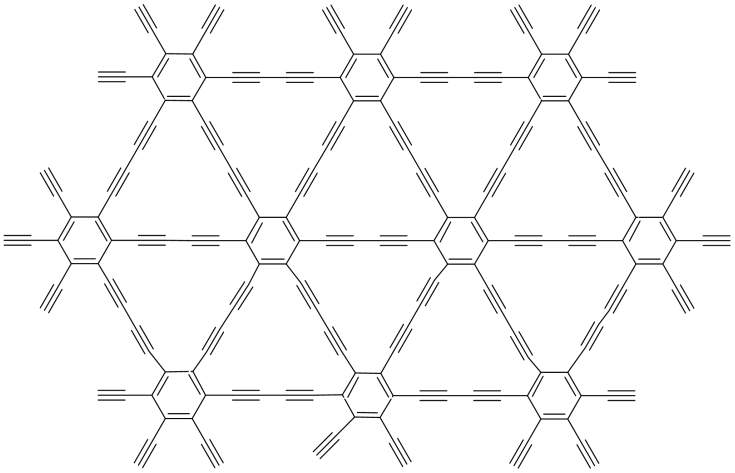

Figure 3. Structure of graphdiyne [20].

Results obtained with such graphdiyne-BSA NPs indicated that these NPs effectively reduced the free radicals, decreased radiation-induced DNA damage in cells, improved the viability of irradiated cells, and decreased the radiation-induced DNA damages in the bone marrow of mice [20,21].

Bismuth selenide (Bi_2Se_3) was recently used for applications in photothermal and radiation therapy [22,23]. Moreover, this material is known to be oxidized in contact with blood and to be able to clear the free radicals during its blood interaction [24]. For these reasons, its use as a radioprotective agent was explored mainly under nanoparticles' forms [25,26]. In these studies, the surface of Bi_2Se_3 NPs was biocompatibilized with poly(vinyl pyrrolidone). Results obtained within these studies showed that Bi_2Se_3 NPs increased the survival of the irradiated mice. Bi_2Se_3 NPs were also found to minimize DNA and bone marrow nucleated cell damages, and helped in the recovery of radiation-induced damages on white blood cells and platelets. Further, the authors concluded that Bi_2Se_3 NPs behave as free radical scavengers and induce the increase in superoxide dismutase (SOD) and the decrease in malondialdehyde (MDA) levels. *In vivo*, it was shown that Bi_2Se_3 NPs have not significant toxicity and lead to an increase in survival of mice exposed to high energy γ-rays [25,26].

The growing interest in the use of cerium oxide nanoparticles (CeO_2 NPs) was also noted in recent years [4,27–29]. The use of CeO_2 NPs as a potential radioprotector relies mainly on their antioxidant properties. Kadivar et al. [28] tested the protective effects of cerium oxide nanoparticles against radiation-induced acute lung injuries in rats. The results obtained by this group showed that CeO_2 NPs could protect the normal cells against radiation-induced damage. The CeO_2 NPs decreased the incidence of tissue collapse and neutrophil aggregation in rats when given with CeO_2 NPs before irradiation. It was also shown by other groups that the use of CeO_2 NPs protects various types of tissues against irradiation damages [27].

Besides the above-mentioned radioprotectors, and as briefly described in the Introduction, TEMPO and its derivatives (Figure 1) have also been investigated as powerful ROS scavengers [11,30–32]. However, as all low molecular weight (LMW) molecules, the TEMPO derivatives are rapidly eliminated and/or degraded upon administration whatever the route used. To improve the biodisponibility of TEMPO, Li et al. have linked the amino-TEMPO to a α-thiol,ω-carboxylic acid poly(ethylene glycol), HS-PEG-COOH, through an amide bound, and then grafted this HS-PEG-CONH-TEMPO onto the surface of gold NPs (Figure 4) [32].

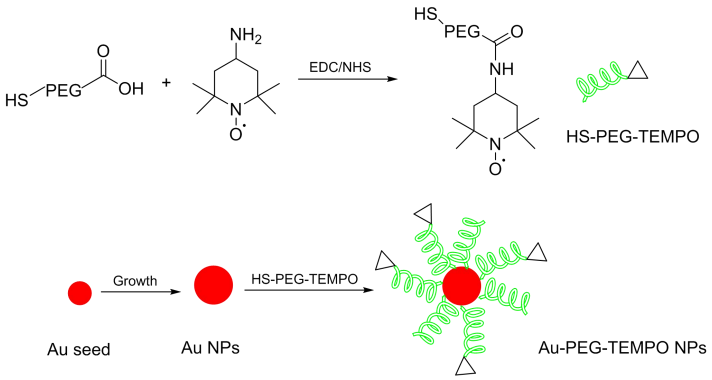

Figure 4. Synthesis of Au-PEG-TEMPO NPs [32].

These authors further evaluated their ROS-scavenging effects and their influence on osteogenic and adipogenic differentiation of human bone marrow-derived mesenchymal stem cells (h-MSCs). Various types and formulations of inorganic nanoparticles with diameters ranging from 10 to 200 nm were tested *in vivo*. Table 1 summarizes the recent studies on the use of such inorganic nanoparticles in the radiation protection of normal cell.

The use of nanotechnology in delivering radioprotective drugs significantly improved their blood circulation time and biodistribution/tissue residence time. In most of the studies conducted, the nanoparticles were administered several hours (1 to 24 h) before exposure to ionizing radiation [20–34]. Intravenous, intraperitoneal, and subcutaneous routes are preferred, mainly to escape the harsh environment of the gastrointestinal system and to deliver a significant amount of nanoparticles into the blood. The data obtained from these studies revealed that delivering the nanoparticles directly into the blood via the above-mentioned routes significantly improved the biodistribution of the nanoparticles in the body; this is essential in protecting the tissues from deleterious damages (for example, organ degeneration and cellular vacuolation) due to exposure to ionizing radiation. The presence of an effective ROS scavenger at the site of ROS production is

required to effectively neutralize them and reduce their damaging effects on tissues and organs. Also, the increased bioavailability of the nanoparticles in the blood resulted in the effective protection of the hematopoietic system, which is most affected by radiation exposure. These effects collectively, significantly improved the survival rate of the irradiated mice. When administered orally, the nanoparticles mainly protected the gastrointestinal tract and were observed to be associated with improved intestinal absorption, resulting in the inhibition of ROS-induced apoptosis of the intestinal cells [21]. However, the toxicity and metabolism of these inorganic nanosized materials should be carefully investigated.

3. Design of organic nanoparticle-based radioprotective agent

To limit possible toxicity linked to the use of inorganic nanosized materials, nanocarriers constituted by organic materials have been considered as potential nanocarriers for LMW antioxidants [35–40]. Indeed, LMW antioxidants, such as curcumin and nitroxide compounds, have a low biodisponibility due to their low solubility and rapid degradation under physiological conditions, thus limiting their applications as a protective drug. Solutions have therefore been proposed to improve the efficiency of such LMW antioxidants without using inorganic nanocarriers and/or inorganic antioxidant compounds. Several biocompatible nanovectors encapsulating LMW antioxidants have been studied (Table 2).

In this context, Nosrati et al. [35] have evaluated the radioprotective effect of curcumin (Figure 5) conjugated to albumin-based nanoparticles.

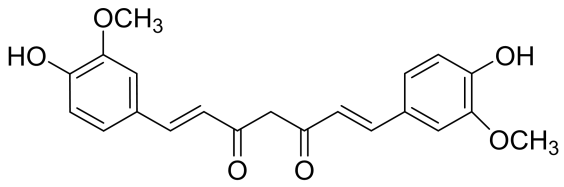

Figure 5. Structure of curcumin.

In their study, the curcumin was covalently bonded to bovine serum albumin (BSA) nanoparticles leading to spherical nanoobjects with a diameter around 174 nm (PDI = 0.19), and a slightly negative surface charge. Nosrati et al. showed that curcumin-grafted BSA nanoparticles have good hemocompatibility and no cytotoxicity *in vitro*. Moreover, a higher radioprotective effect of curcumin-grafted BSA nanoparticles has been observed *in vitro* on HFF-2 cells having been exposed to X-ray irradiation (8 Gy). In *in vivo*, they showed that their curcumin-grafted BSA nanoparticles were well tolerated by healthy mice, and allowed a significant increase in the survival rate of mice irradiated by X-ray, and concluded that curcumin grafted BSA nanoparticles have a promising radioprotective effect [35].

Table 1. Inorganic nanoparticles as radioprotective agents.

Materials	Size (Diameter)	Mode of Administration	Drug Dose Tested *In Vivo*	Blood Bioavailability	Observed Biodistribution	Radiation Protective Effects *In Vivo*	Ref.
Bovine Serum Albumin modified Graphdiyne (GDY-BSA)	30–40 nm	Oral Injection	12.5 mg/kg	NR	< 24 h in the Gastrointestinal tract	Protected the gastrointestinal tissues of mice by inhibiting the ROS-induced apoptosis.	[21]
Cerium oxide nanoparticles (CNP)	10–30 nm	Biweekly Intraperitoneal Injection for 2 weeks before or after IR	0.00001 mg/kg	NR	NR	Decreased the incidence of tissue collapse and neutrophile aggregation in rats receiving CNP before radiation and protected the normal cells against radiation-induced damage.	[28]
Selenium-Containing Nanoparticles (PVSe)	~184 nm	Intraperitoneal Injection 2 h before IR	10 mg/kg	< 72 h	Rapid distribution in major organs within 6 h and remained in some major organs even at 72 h after intravenous injection.	Showed better radioprotective efficacy *in vivo* and longer circulation time with a half-life of ~10 h than the small molecule drug; Its radioprotective effects is attributed to its ROS-scavenging ability, thereby inhibiting radiation-induced apoptosis.	[33]
polyvinylpyrrolidone-functionalized untrathin two-dimensional niobium carbide Mxene nanosheets (Nb_2C-PVP)	~150 nm	Intravenous Injection 24 h before IR	20 mg/kg	>48 h	4–48 h in almost all tissues after i.v. injection	Increased Mice Survival; Promotes Hematopoietic Recovery; Protects against IR-induced Multiple Organ Degeneration; Reduced radiation-induced ROS production.	[34]

Note: NR, not reported; IR, ionizing radiation.

Table 2. Organic nanoparticles as radioprotective agents.

Materials	Size (Diameter)	Mode of Administration	Drug Dose Tested *In Vivo*	Blood Bioavailability	Observed Biodistribution	Radiation Protective Effects *In Vivo*	Ref.
Curcumin conjugated albumin-based nanoparticles (BSA-CUR)	~40 nm	Intravenous Injection 4 h before IR	0.125 mg/mouse	NR	NR	Increased survival rate of irradiated mice.	[35]
Melanin Nanoparticles (MN)	~80 nm	Intraperitoneal Injection 30 min before & after IR	50 mg/kg	NR	NR	Protects against oxidative stress and DNA damage induced by ionizing radiation.	[36]
Chitosan-Oligofucoidanpoly saccharides Nanoparticles (C-FP)	190–230 nm	Oral Injection	20 mg/kg	NR	Improved intestinal absorption	Prevents and treats radiation-induced intestinal injury; Prevents radiation-induced lipid peroxidation and restores intestinal enzymatic and non-enzymatic antioxidant status; Enhanced antioxidant properties.	[37]
poly(ε-caprolactone)-b-poly(ethylene glycol)-b-poly(ε-caprolacton e)/WR-1065 nanoparticles (PCEC/WR-1065)	183 nm	Oral Injection 1 h before IR	300 mg/kg	NR	Mainly localized in the intestines 0.5–4 h after oral injection	Provide localized protection in the intestines by neutralizing the IR-generated free radicals and increased the survival rate of the irradiated mice.	[38]
Redox Nanoparticles (RNP)	20–40 nm	Subcutaneous Injection 24 h before IR	200 mg/kg	> 24 h	24 h p.i., RNP was determined at significant amount in most of the major organs and was detected in other tissues.	Increased survival rate of irradiated mice; Reduced radiation-induced organ damages and bone marrow depletion; Reduced the radiation-induced life-shortening in mice.	[39,40]

Note: NR, not reported; IR, ionizing radiation.

Melanin, a high molar mass pigment, is known to have a radioprotective activity [36]. Rageh et al. prepared melanin nanoparticles modified or not with poly(ethylene glycol) (PEG), and evaluated their radioprotective effects *in vitro* and *in vivo* concerning oxidative damages induced by γ-irradiation. Melanin nanoparticles (MNPs) with a diameter of around 80 nm showed a radioprotective effect *in vitro* on irradiated CHO cells. Melanin nanoparticles have been intraperitoneally injected into mice before and after irradiation, and the effects have been followed in the heart, kidneys, and liver. Results showed that these melanin nanoparticles protected the cardiac tissue and the hepatocytes whether administrated before or after irradiation. Rageh et al., thus, concluded that melanin nanoparticles can protect mice against oxidative stress and ionizing radiation-induced DNA damage. Also, they concluded that MNPs treatment after irradiation may be useful to prevent side effects on tumor-adjacent tissues caused by long-lived radicals and oxidative stress in cancer radiotherapy [36].

Like several radioprotective molecules, amifostine, and its thiol-free metabolite WR-1065, cannot be orally administrated because of their very bad gastric stability leading to a very low biodisponibility. These radioprotective molecules have thus been encapsulated into nanovectors to improve their biodisponibility after oral administration. In this context, Lin et al. [38] have synthesized, in a first step, a terpolymer constituted by a central block of PEG linked to two blocks of poly(ε-caprolactone) (PCL) at each end through the reactive oxygen species (ROS) sensitive thioketal bounds (Figure 6).

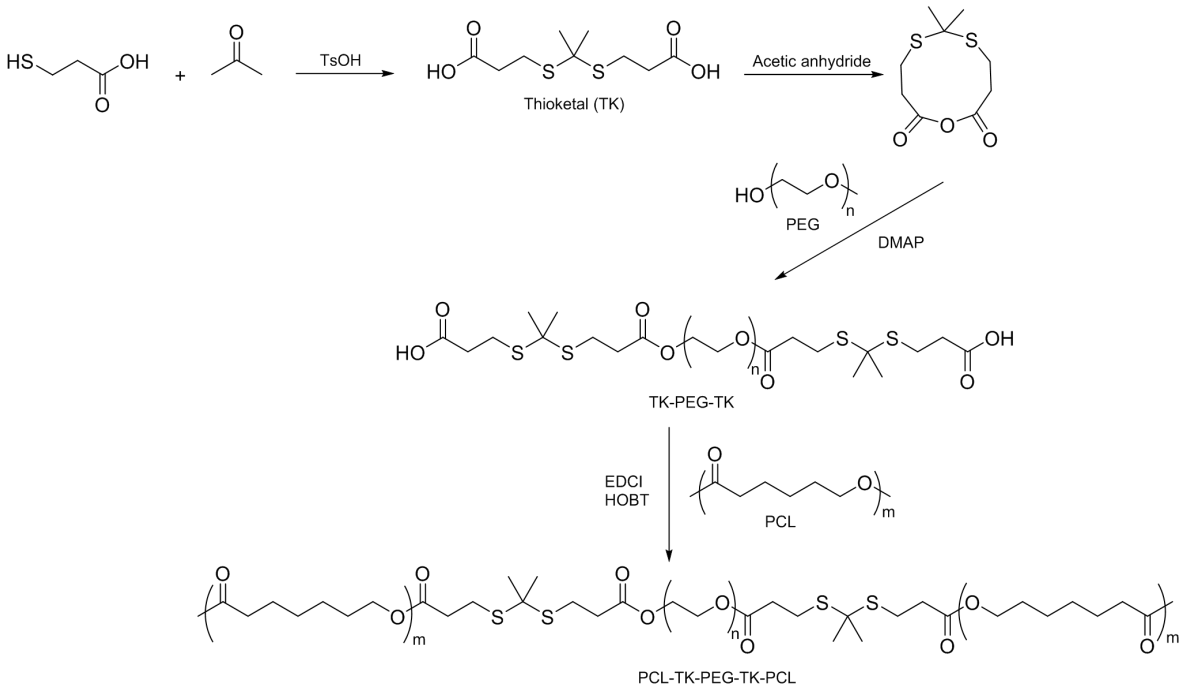

Figure 6. Synthesis of terpolymer having the ROS-sensitive linker TK.

In a second step, WR-1065 molecules were loaded into nanoparticles constituted by the previously synthesized ROS-sensitive terpolymer. These authors further evaluated the cell uptake, the oral effectiveness, and the *in vivo* biodistribution of the PEG-PCL nanoparticles encapsulating the WR-1065 [38]. A drug loading efficiency of about 20% was observed for these nanoparticles with a diameter of about 180 nm. Despite a burst effect with almost 35% of WR-1065 release within

the first 5 hours, the authors concluded: i) on the sustained release of WR-1065 over 25 hours of incubation at pH 7.4, and ii) the difficulty to release WR-1065 in absence of ROS. In parallel, they showed that: iii) the thioketal bounds were efficiently degraded by ROS, and iv) the WR-1065 loaded nanoparticles were internalized by Caco-2 cells *in vitro*. Results of *in vivo* studies showed that the WR-1065 loaded nanoparticles allowed a good radioprotective effect, protection of the hematological system and main organs, and improvement of survival days of irradiated mice after oral administration [38].

Our group has been working on the design of an amphiphilic block copolymer, poly(ethylene glycol)-*b*-poly(methylstyrene), PEG-*b*-PMST, possessing NH_2-TEMPO as a side chain of the repeating units in the PMST segment, and PEG-*b*-poly[4-(2,2,6,6-tetramethylpiperidine-1-oxyl)aminomethylstyrene] (PEG-*b*-PMNT). The PEG-*b*-PMNT block copolymer forms a core-shell type self-assembling polymer micelle in aqueous media (Figure 7).

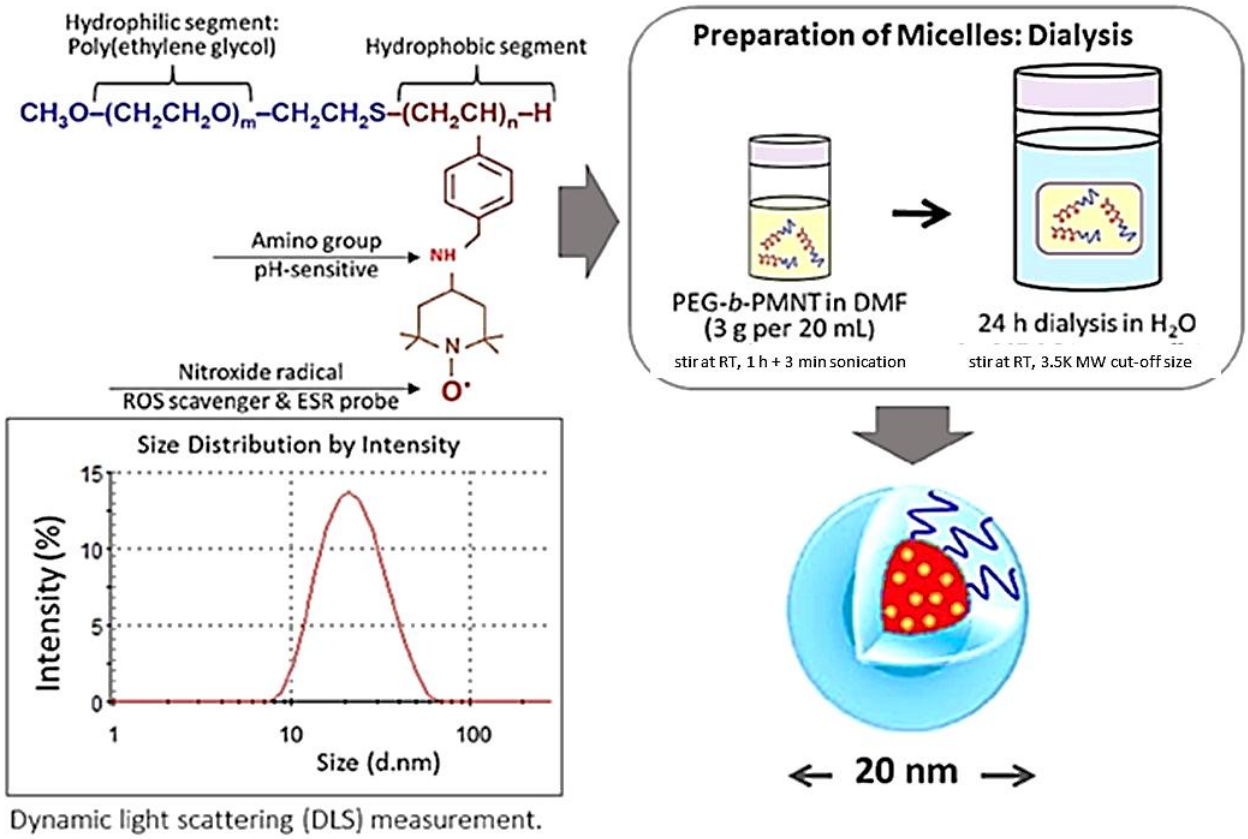

Figure 7. Design and preparation of nitroxide radical-containing redox nanoparticles (RNP^N). Monodispersed micelles were obtained after dialysis, as measured using dynamic light scattering (DLS) analysis. Adapted with permission from reference [39], Copyright © 2017 Elsevier Ltd.

The hydrophilic PEG layer forms the shell, and the hydrophobic PMNT segment forms the core of the micelle having a size equal to several tens of nanometers. The prepared PEG-*b*-PMNT copolymer micelle (herein referred to as redox nanoparticles; RNP) contains an active part, amino-TEMPO that effectively scavenges ROS in the body, as demonstrated *in vivo* [12,19,39,41]. The block copolymer synthesis process was modified to control its composition and increase

productivity. It should be noted that RNP is not a simple drug delivery system made by physical entrapment of the antioxidants amino-TEMPO in the core of nanoparticles. The covalent conjugation of amino-TEMPO to the amphiphilic block copolymer is one of the important steps to avoid possible leakage of LMW antioxidants from the nanoparticle. The self-assembling formulation of RNP is another important point because of smooth excretion after collapsing of nanoparticles, which can avoid possible long-term, toxic effects.

Results from our previous studies showed that RNP has very low to no toxicity when injected subcutaneously into mice at its maximum injectable dose (300 mg/kg); there were no signs of any inflammatory reactions and systemic toxicity *in vivo*. Hematological analysis revealed that all blood biomarker levels were within normal ranges [39]. When administered orally (300–900 mg/kg), the mouse survival rate was 100% even several months after its administration [42]. Also, despite long-term oral treatment of mice with RNP, there were no toxicities observed in the major organs of the mice examined [43–45]. The low toxicity of RNP compared with that of its LMW counterpart (nitroxides and other antioxidants) is due to its size and the compartmentalization of nitroxide radicals in the core of RNP [45]. Our previous study established that the characteristic size and core-shell structure of RNP prevented its internalization into the cell, thereby preventing mitochondrial dysfunction, primarily caused by the rapid internalization of LMW compounds [12]**.** The detailed toxicity profile of TEMPO has been reported previously [46,47], and results from these studies determined that the maximum tolerable dose of TEMPOL when given intraperitoneally is 275 mg/kg. Mice survival was monitored for 30 days after the intraperitoneal administration, and no mice died when they were injected with <400 mg/kg. However, a seizure was observed in the injected mice due to neurotoxicity. These results limit the use of nitroxide radicals. In contrast, RNP prevents these toxicities due to the confinement of the nitroxide in its core and the gradual release of the redox polymer. Also, the effective dose of RNP *in vivo* against ionizing radiation was found to be 200 mg/kg, which is equivalent to only 60 mg/kg of nitroxide, i.e. more than four times less than its known maximum tolerable dose (275 mg/kg).

The rapid preferential renal clearance and diffusion of LMW nitroxides across the body were prevented by the self-assembling RNP. Moreover, the polymeric micelles were observed for a relatively long period after intravenous injection in mice with renal injury [45]. It is noteworthy that the route and time of drug administration are important to achieve the beneficial and optimum effects of radioprotective drugs; we observed significant radiation protection when the nanoparticles were injected subcutaneously into mice 24 h before irradiation. As a result of the covalent encapsulation of the nitroxide NH2-TEMPO into the core of the biocompatible polymeric micelles, the bioavailability was increased significantly, and rapid clearance and systemic toxicity were prevented [39,40]. As shown in Figure 8, the efficacy of the RNP is attributed to the following characteristics: i) unique pH-responsiveness, as observed by Yoshitomi et al.; they found that the self-assembled RNP disintegrates at pH below 7.0 due to protonation of the amino groups in the hydrophobic core of the nanoparticles' [45]; ii) low toxicity as the size (20–40 nm), where nanoparticles prevent their cell internalization and does not interrupt the regular redox reactions in the cells [12,19,44]; iii) superior biocompatibility of PEG [poly(ethylene glycol)], a hydrophilic segment of the amphiphilic block copolymer that makes RNP colloidally stable; iv) effective antioxidant activity of NH2-TEMPO that effectively scavenges ROS; v) long blood circulation time

due to the covalent encapsulation of nitroxide in the nanoparticle core that allows its gradual metabolism.

Electron spin resonance (ESR) spectroscopy studies revealed that RNP retains its core-shell structure when administered intravenously or subcutaneously; the nitroxide radicals were found to be located in the core of the self-assembling polymeric micelles (RNP) in the bloodstream. The formation of the polymeric micelles in the blood prevents its rapid renal elimination, resulting in its long blood circulation time [39,45]. Finally, vi) high bioavailability extends the ability of RNP to scavenge radiation-induced ROS, primarily hydroxyl radicals, particularly those distributed in the blood and tissues. The long blood circulation time of RNP after the subcutaneous injection has been confirmed. The nanoparticles peaked in the blood 3 h post-injection (p.i.), and remained in circulation for more than 24 h p.i. These findings are in sharp contrast to the rapid clearance of its LMW counterpart NH2-TEMPO; the nitroxide radicals peaked at 5 mins p.i., and were undetected in the blood at 60 min p.i. A significant amount of RNP was detected in most organs p.i. These findings are vital for radiation protection because the effective scavenging of ROS at the required site is crucial in protecting the cells and organs from the damaging effects of ionizing radiation. The efficacy of any radioprotective drug is centered on its ability to scavenge ROS produced by the ionizing radiation, primarily the removal and neutralization of the hydroxyl radicals distributed in the cells and tissues. The nanoparticles developed by us were effective in providing tissue protection against hydroxyl radicals [39].

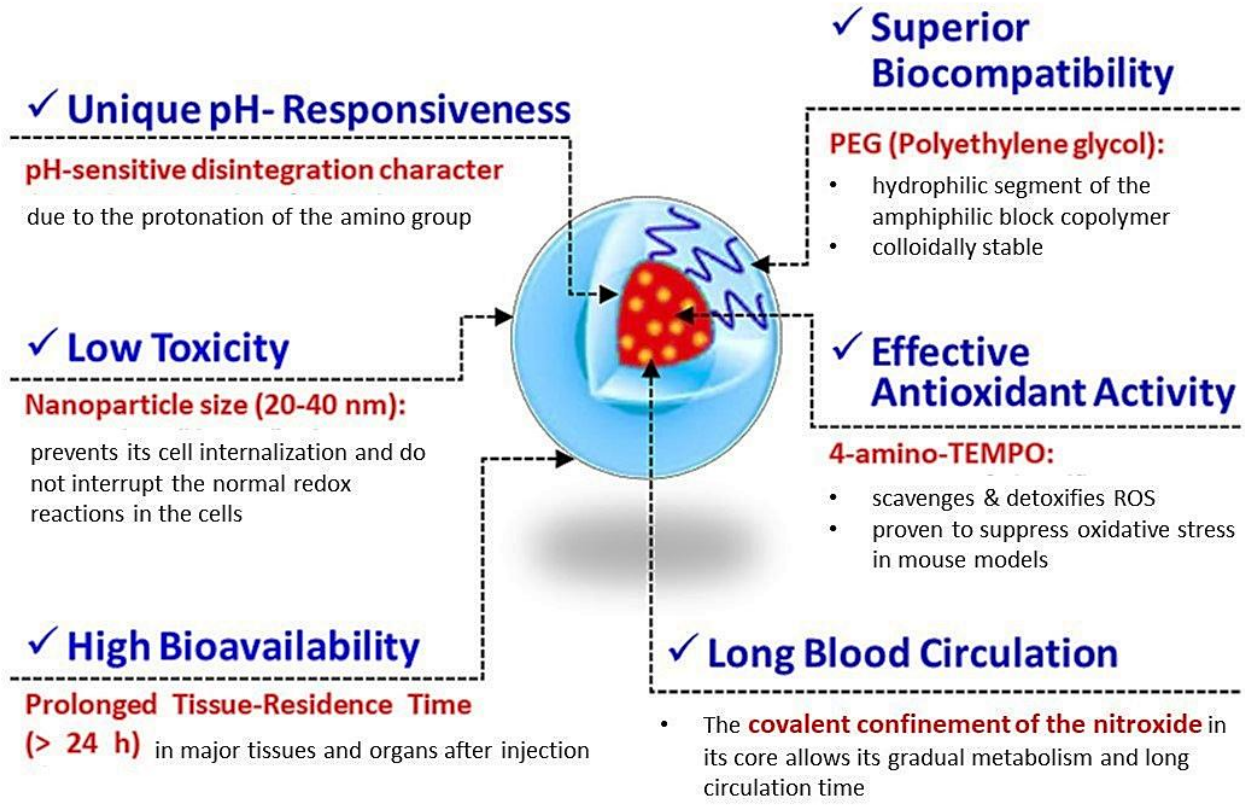

Figure 8. Unique characteristics of the redox nanoparticles.

The unique characteristic of RNP allows its gradual absorption into the bloodstream after oral administration, resulting in high bioavailability for a longer period. At low pH conditions, RNP dissociates due to the protonation of its hydrophobic core, freeing the redox polymer. Subsequently, the redox polymer gradually absorbs into the blood (Figure 9) and circulates in the body [42,43]. The radiation protection efficacy of RNP, when administered orally *in vivo*, is currently under study.

The effective design, very low toxicity, high bioavailability, and effective ROS scavenging abilities of the new nanomedicine prompted us to test its efficacy as a radiation protective drug. Using C57BL/6J mice, we demonstrated the ability of RNP to protect the mice against radiation exposure syndromes and death after whole-body irradiation. RNP, when injected subcutaneously to mice 24 h before irradiation, protected the mice against radiation-induced weight loss, and significantly increased survival after whole-body irradiation. Close examination of the mouse organs revealed that RNP prevented the radiation-induced hemorrhagic lesions and septicemia, reduced cell and organ degeneration, and protected the lymphoid organs against apoptosis and severe damage [39].

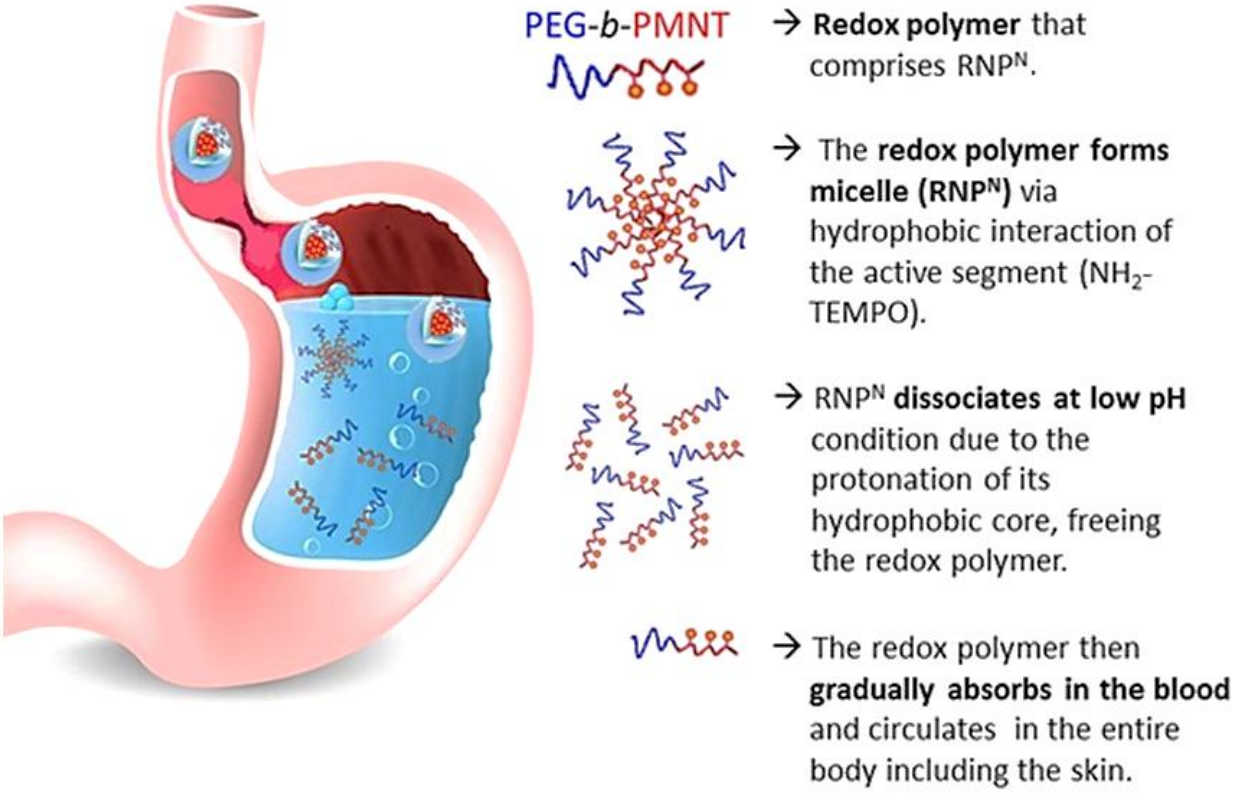

Figure 9. Biodistribution of RNPN via oral route: graphical illustration of the oral delivery of RNPN and its absorption into the body and the skin area. The unique characteristics of RNPN allow the gradual absorption of the redox polymer into the bloodstream after oral administration, keeping its bioavailability high for its effective ROS scavenging effect. Adapted with permission from reference [42], Copyright © 2017 Elsevier Ltd.

The efficacy of RNP against radiation-induced life-shortening was also evaluated *in vivo*. Mice were treated (s.c. injection) with the nanoparticles 24 h before exposure to ionizing radiation (6 Gy), and were kept for over one and a half years (555 days) after irradiation to complete the reported lifespan of the mouse strain (C57BL/6J) used in the study. The results revealed that RNP significantly reduced the life-shortening effect due to exposure to ionizing radiation. Further examination of the control and treatment groups showed that the nanoparticles reduced the occurrence of radiation-induced disorders. Microscopic examination of the bone tissues of the treatment groups showed that the nanoparticles protected the bone marrow from radiation-induced cell death [40].

Recently, we have found that post-administration of RNP prevented severe adverse effects for the radiation treatment against cancer-bearing mice, although nanoparticles are known to accumulate in the solid tumor (so-called enhanced permeation and retention (EPR) effect). [48] Depletion of ROS in the tumor microenvironment did not reduce therapeutic efficacy, but significantly reduced systemic side effects. We concluded that the main point is to reduce the resistance of cancer cells by eliminating ROS in solid tumors.

4. Conclusions

Neutralization of ROS generated in cells and tissues after exposure to ionizing radiation is crucial for the action of radioprotective agents. Several LMW antioxidants have been developed and examined for this purpose. Most of these LMW radioprotective agents have proven their effectiveness in ROS scavenging *in vitro*, but failed in efficacy testing *in vivo* due to their rapid excretion and inherent cellular toxicity. These limitations hampered the development of new and effective radioprotective agents for clinical use. Unless the side effects of these antioxidants are eliminated or minimized, they are unsafe to administer to healthy people. RNP is a promising approach because it maintains the redox homeostasis of normal cells and tissues due to its characteristic size and biocompatibility. With the use of nanoparticle-based technologies, optimized formulations of these LMW ROS-neutralizers led to significant improvement in their bioavailability and longer blood circulation time, consequently improving their efficacy *in vivo*, increasing survival rate, reducing the incidence of radiation-induced syndromes, and organ collapse.

The data gathered by our group and other laboratories on biodistribution studies led to an important conclusion that the intrinsic ROS-targeting activity of a drug intended for radiation protection is as vital as its bioavailability in the specific tissues and organs where ROS are produced during radiation exposure, specifically when neutralizing the short-lived radiation-induced ROS. The ability of RNP to scavenge ROS, primarily hydroxyl radicals, is attributed to its high bioavailability and longer circulation time in the blood and tissues [39,42]. These characteristics are very important since the generation of ROS due to ionizing radiation occurs rapidly, approximately in 10^{-10} to 10^{-6} seconds [49]. Moreover, results obtained by several studies demonstrated that the route and time of administration of the nanoparticles should be seriously considered to achieve the desired effects and radioprotective efficacy. Available data suggest that administration of radioprotective drugs before exposure to ionizing radiation (for example, 1 to 24 h before irradiation) is crucial in achieving its protective, and other beneficial effects.

Future studies are warranted to test the radioprotective efficacy of these nanoparticle-based antioxidants when administered over a sustained period for the prevention and treatment of radiation

injuries and syndromes. Most of the recent studies on nano-based radioprotective agents have the limitation of single dose and administration, and are administered minutes or hours before a short period of irradiation. Also, the efficacy of these nano-based agents should be tested for subjects with a prolonged period of radiation exposure for cases where continuous protection against radiation is needed (for example, radiation/nuclear emergencies and space travels). Concerning the use of nanoparticle-based radioprotective agents, it is of high importance to have efficacy without compromising the other regular functions in the body and causing any side effects, including cellular dysfunctions and behavioral, cognitive, and locomotor toxicity, even when used for a prolonged period; these positive outcomes will lead to its safe and effective clinical use.

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Conflicts of Interest

We declare no conflicts of interest.

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