Design and application of redox polymers for nanomedicine

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Received: 4 February 2018 / Revised: 14 March 2018 / Accepted: 14 March 2018
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Abstract
Reactive oxygen species (ROS), such as superoxide and hydroxyl radicals, cause oxidative stress that strongly affects aging and various diseases. Although various antioxidants have been developed to eliminate ROS, they cause serious problems by destroying important redox reactions in normal cells. We designed redox polymers with antioxidants covalently bonded to them. These polymers, with a self-assembling property, form nanoparticles in aqueous media (redox nanoparticles; RNPs), suppress uptake into normal cells, accumulate at inflammation sites, and effectively prevent ROS-related diseases. As such, RNPs have been found to be effective in preventing diseases involving ROS, such as myocardial and cerebral ischemia-reperfusion injuries, ulcerative colitis, and cancer. Redox polymers have several other applications. We designed redox injectable gels (RIGs), which transform from flowable solution at ambient temperature to gel at body temperature under the physiological conditions. RIGs can be applied for suppression of local inflammation, such as periodontitis. RIGs can also be used in anti-tissue adhesion sprays applied after physical surgery. Redox polymers can also be used as a surface coating of biodevices to make them blood compatible. This review summarizes the synthesis and application of these redox polymers.

Introduction
At the end of the 19th century, methylene blue could be used to stain only the nerve endings of rabbits. The German physician and scientist Paul Ehrlich believed that if a chemical compound could be successfully delivered to its target site, it would be an ideal drug, i.e., nonspecifically spread but accumulated in specific sites, thereby hypothesizing the so-called magic bullets [1]. In the 21st century, a molecular targeted drug called a new drug of dreams became practical, realizing Ehrlich’s idea [2, 3]. Since then, have cancer patients disappeared from the world? The answer is no. In reality, the number of deaths of cancer patients is steadily increasing every year [4]. In addition to further increasing the efficiency of molecular targeted drugs, to lower the patient’s pain and improve the drug’s effect, the development of a new concept of “medicine” has become imperative. In this article, the author discusses the prospective and dramatic development of antioxidative therapy by “making polymers into medicines” as alternatives to low molecular-weight (LMW) synthetic compounds that have been used as mainstream drugs so far.

As with Qin Shi Huang, the first emperor of the Chin dynasty, who was keen for “longevity”, anti-aging is, in general, extremely popular all over the world [5]. Since 1970, it has been noted that reactive oxygen species (ROS) are the cause of various diseases, and subsequent extensive research has shown that ROS are involved in most diseases, including aging [6]. Therefore, various synthetic antioxidants have been developed in addition to natural products, such as vitamin C, vitamin E, and curcumin, to eliminate ROS, but there are few effective antioxidants. In 2007, Bjelakovic and colleagues examined 68 randomized studies from the 385 articles published so far and summarized the following results for the antioxidants used in more than 230,000 clinical trials [7].

i. The increased risk of death from beta carotene is 7%, vitamin A uptake 16%, and vitamin E intake 4%.
ii. The effects of vitamin C and selenium ions need to be further investigated.
That is, taking antioxidants daily is statistically more harmful in regard to health. In the mitochondria of healthy cells, a large amount of ROS are generated through the electron transport chain. Small molecular antioxidants enter normal cells and their mitochondria and affect the important redox reactions involved in electron transfer chains, thereby causing serious adverse side effects. As ROS are “double-edged swords” like this, it is not possible to administer a sufficient amount of LMW antioxidants; that is, these antioxidants leave a low or even no therapeutic window. We designed an alternate antioxidant nanomedicine. This is a covalent conjugate of a conventional antioxidant and a polymer with a moderate molecular weight, which can be metabolized.

Specifically, as shown in Fig. 1, a nitroxide radical (TEMPO) having catalytic ROS scavenging ability was covalently conjugated with an amphiphilic block copolymer possessing a self-assembling ability [8]. The amphiphilic redox polymer self-assembles in an aqueous medium to form nanoparticles (redox nanoparticles; RNPs).

**Physicochemical properties and safety of antioxidant nanoparticles (RNPs)**

The redox polymers we have designed are an amphiphilic block copolymer consisting of a hydrophilic poly(ethylene glycol) (PEG) segment and a hydrophobic poly(chloromethylstyrene) (PCMS) segment, in which a 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) is covalently conjugated to the chloromethyl group in the PCMS segment (Fig. 1) [9]. Since this redox polymer has a molecular weight of approximately 10,000, it does not easily cross normal cell membranes and mitochondrial membranes and, thus, does not inhibit ROS-mediated normal electron transfer chain and other redox reactions in the mitochondria. Since this redox polymer has both a water-soluble segment and an insoluble segment, it self-assembles in aqueous media to form nanoparticles. Here is a comparison of this RNP with 4-hydroxyl-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL, as a model of an LMW antioxidant), used on zebrafish. When 3 and 30 mM TEMPOL are added to the diet of zebrafish, the fishes die completely after 5 days and half a day, respectively. In contrast, in the case of RNPs, even at a TEMPO concentration of 30 mM, the zebrafish survival rate was 100% even after 5 days, as shown in Fig. 2a [10]. Figure 2b shows the results of staining zebrafish mitochondria with fluorescent dye (JC-1). Although healthy zebrafish glow red, almost no light is emitted by zebrafish treated with TEMPOL, indicating that...
mitochondria are almost completely destroyed by TEMPO. In contrast, in zebrafish treated with RNPs, mitochondria emit strong red fluorescence, indicating their healthy condition. In experiments with rodents, such as mice and rats, it has been shown that RNPs do not show remarkable organ damage or acute toxicity, particularly when administered through routes such as oral, intravenous, and subcutaneous [11, 12]. Based on these findings, polymeric antioxidants, especially nanoparticles, can be regarded as safe nanomedicines that can be used to avoid the strong side effects associated with LMW antioxidants.

As shown in Fig. 1, there are two types of RNPs, which are formed from different block polymers. In one, TEMPO is conjugated to the block polymer via an ether linkage, while in the other, TEMPO is conjugated via an amino linkage [13]. Nanoparticle prepared from block polymers with ether linkages (abbreviated as RNPN) forms stable particles, with diameters of 30–40 nm, regardless of the surrounding pH. Nanoparticle prepared from block polymers with amine linkages (abbreviated as RNPN) forms stable particles, with a diameter of 30–40 nm under physiological conditions, while they disintegrate under low pH due to the protonation of the amino groups in the core. The disruption of RNPN in the low pH region is confirmed by the lower scattering intensity in dynamic light scattering (DLS), measured at pH 7 or lower, as shown in Fig. 3a [14]. The disintegration of RNPN can also be confirmed by electron spin resonance (ESR) measurements. As described in Fig. 3b as well, the ESR spectrum of the nitroxide radicals of RNPN shows broad peaks at neutral and alkaline pH conditions, indicating restricted motion of the nitroxide radicals due to their confinement in the solid core of the nanoparticle. Conversely, a sharp triplet signal is observed under acidic conditions, indicating that the exposed nitroxide radicals are moving by disintegration [15].

The behavior of disintegration in response to pH changes is observed in vials using DLS and ESR measurements. The pH-triggered disintegration of RNPN was also confirmed in vivo using a mouse model. Figure 3c shows the ESR spectra of blood and kidney homogenates after intravenous injection of RNPs from the tail vein into the renal ischemia-reperfusion mouse model [14]. pH-insensitive RNPN shows a broad ESR spectra in the blood and kidney. pH sensitive RNP also shows a broad ESR signal in the blood but shows a distinct triplet signal in the kidney, which indicates the disruption of RNPN in the pH-lowered kidney, with inflammation associated with reperfusion. Almost no example has been reported in which the pH-responsive nanoparticles actually function in vivo in this way.

Therapeutic effects of RNPs

In the above section we showed that RNPN does not destroy the redox environment in normal cells, and it disintegrates in response to a decrease in the environmental pH. In the acidic region, nitroxide radicals are exposed to the outside of RNPs, so it is anticipated that it will eliminate excessive ROS at the site of inflammation. It is known that when the inflow of blood into the organ stops and, subsequently, the flow recovers after a while (ischemia-reperfusion), the oxygen concentration rises rapidly after the reperfusion, where a large amount of ROS is produced, and the resulting oxidative stress can cause serious organ damage. Effective antioxidant activity of RNPN is anticipated because it disintegrates and exposes nitroxide radicals in the kidneys that have become inflamed and have lowered pH due to ischemia-reperfusion. Figure 4a, b shows the levels of kidney superoxides in the ischemia-reperfusion mouse model [14]. The ROS level increased significantly after
reperfusion compared with that in the control mice. Intravenous administration of LMW TEMPO did not decrease the ROS level in the kidney. In contrast, intravenous administration of RNP\textsuperscript{N} after reperfusion significantly decreased the ROS level in the kidney. It should be noted that RNP\textsuperscript{O} did not decrease the ROS level as much as RNP\textsuperscript{N} did; this is proof that the exposure of nitroxide radicals from the RNP\textsuperscript{N} core in the inflamed site increased its ROS scavenging ability.

Since RNPs effectively eliminate the ROS excessively generated at inflammatory sites, they can suppress oxidative damage to the organs. Malondialdehyde (MDA) is known as an indicator of lipid oxidation of cell membranes and is significantly increased after the renal ischemia-reperfusion treatment. As seen in Fig. 4c, however, it is reduced to the control level by the administration of RNP\textsuperscript{N}. It is interesting to note that RNP\textsuperscript{N} also decreases the level of inflammatory cytokines, such as IL-6 (Fig. 4d). Thus, it can be concluded that RNP\textsuperscript{N} suppresses the amplification of local inflammation by suppressing the rapid increase in the ROS level. In this way, RNPs can recover the function by preventing ROS-induced oxidative damage to the organs. As shown in Fig. 5a, b, the blood creatinine and blood urea nitrogen (BUN) levels drastically increase after renal ischemia-reperfusion of mice, while after the tail-vein administration of RNP, it recovers to the control level, confirming that kidney function is greatly improved by this treatment (Fig. 5c).

With the aging of the population, ischemia-reperfusion injury is becoming a serious problem in various organs, such as in the kidney, as well as in the brain, heart, and mesenteric capillaries. We have confirmed that RNPs are highly effective in cerebral [16], myocardial [17], and mesenteric capillary [18] ischemia-reperfusion injuries, promising to be a new antioxidant nanomedicine.

**RNPs for chronic diseases**

We have thus shown that RNPs are effective against severe acute diseases, such as ischemia-reperfusion injury. To evaluate RNP effectiveness against chronic oxidative stress disease, oral administration tests were carried out. As described above, we have designed two kinds of RNPs: RNP\textsuperscript{N} that disintegrates in response to a decrease in pH and RNP\textsuperscript{O} that does not disintegrate, regardless of the
environmental pH. Since RNP$^O$ does not disintegrate, regardless of the pH change, it is not taken into the blood stream, instead localizing in the gastrointestinal tract after oral administration. Contrarily, RNP$^N$ disintegrates in the acidic stomach, is absorbed from the mesentery, and circulate in the blood. Therefore, the pharmacokinetics of these two RNPs administered orally are completely different.

The cause of ulcerative colitis (UC) is not clearly understood, but the inflammation of the colon continues for a long time; it is designated an intractable disease [19]. RNP$^O$ was used for treatment of UC. Figure 6a, b shows the drug accumulation tendency in the colon mucosa of mice [20]. TEMPOL does not occur in this area due to its absorption in the blood stream and/or metabolization in the small intestine. Oral administration of polystyrene latex particles of different sizes confirmed the size-dependent accumulation in the colon mucosa. It was confirmed that accumulation of RNP$^O$ in the colon mucosa was at much higher levels than that of polystyrene latex of the same size, which is probably due to the higher dispersion stability of RNP$^O$ possessing a core-shell structure. Figure 6c shows the results of blood uptake. LMW TEMPOL is rapidly detected in the blood. Especially in the UC mouse model, the substance uptake capacity increases (because of a leaky gut), so a large amount of TEMPOL is observed in the blood. In contrast, RNP$^O$ is not detected in the blood at all, even in the UC mouse model. Thus, it was confirmed that despite RNP$^O$ accumulating in the mucous membrane, it did not migrate into the blood but was localized in the gastrointestinal tract. Since RNP$^O$ is located in the colon mucosa for an extended period, it can continuously scavenge ROS in the inflamed colon area. Since the colon of the UC mouse model is shortened due to inflammation, its length was measured after drug treatment. The colon length of the UC mice treated with RNP$^O$ is almost completely identical to the length of the control mice, in contrast to that of the mice treated with TEMPOL, which have not recovered completely, as shown in Fig. 6d, indicating suppression of the inflammation by RNP$^O$ due to the continuous elimination of ROS in the colon mucosa. In addition, the survival of the UC mouse model is significantly increased by the RNP$^O$ treatments (Fig. 6e). It is clear, therefore, on the basis of these findings, viz., accumulation, long retention of RNP$^O$ in the colon mucosa, and continuous elimination of ROS, that RNP$^O$ effectively suppress colon inflammation in the UC mouse model.

Although various causes of dementia, including Alzheimer’s disease, have been proposed [21], the mechanisms behind this condition have not yet been confirmed.
Regardless of the mechanisms, the symptoms are almost the same, viz., specific proteins such as amyloid β and tau accumulate in the brain, causing plaque and fibrosis, which induces oxidative stress [22]. That is, it is reported that metal ions, such as iron and copper, chelate to these fiberized proteins and induce Fenton’s reaction with hydrogen peroxide to form hydroxyl radicals, which cause damage to the surrounding neural cells. None of the known antioxidants show sufficient effectiveness in treating this condition.

In contrast to RNP⁰, the pharmacokinetics of RNP⁰ administered orally are very different. As shown in Fig. 7a, orally administered RNP⁰ disintegrates in the acidic stomach and is absorbed into the blood from the intestine [11]. As seen in the figure, the amount of antioxidant polymer absorbed into the blood is not very high, being 5–10% of the dose, but the polymer remains in the blood for a long time. Because the antioxidant polymer constituting the RNP⁰ is a polycation having a molecular weight of approximately 10,000, it is absorbed slowly into the blood stream from the mesentery; in addition, it circulates for an extended period by binding with proteins in the blood.

When considering the use of antioxidants to treat dementia and Alzheimer’s disease, we hypothesize that, in addition to toxicity, the rapid metabolism and excretion of LMW antioxidants are responsible for most of the obstacles faced during effective treatment. That is, although it is reported that the blood–brain barrier is destroyed in dementia and Alzheimer’s disease, it is extremely difficult...
for LMW antioxidants, with rapid metabolism and excretion, to be taken into the brain. As described above, the oral administration of RNP\textsuperscript{N} is anticipated to have an effect on cognitive impairment because its duration of blood circulation is extremely long. Figure 7b shows the uptake of redox polymers into the brain after oral administration. As anticipated, it is confirmed by both ESR and RI measurements that a small but definite amount of antioxidant polymer is taken into the brain after oral administration. The senescence-accelerated mouse (SAMP8) is known to accummulate a large amount of amyloid β in the brain and have deficits in learning and memory [23]. In the latency period of the Morris water maze test, SAMP8 mice take approximately 1 min, while senescence-accelerated resistant mice (SAMR1; possessing normal aging) take 25 s, as shown in Fig. 7c. Thus, due to deficits in learning and memory, SAMP8 mice require a longer latency period compared with the SAMR1 mice. This symptom did not improve in the SAMP8 mice even with the daily administration of TEMPOL for 4 weeks. In contrast, the oral administration of RNP\textsuperscript{N} led to a significant recovery of the symptoms. Indeed, the latency period after 4 weeks of oral

Fig. 7 Delivery of redox polymers to the brain after oral administration of RNP\textsuperscript{N}. a The biodistribution of RNP\textsuperscript{N} was determined using \textsuperscript{125}I-labeled RNP\textsuperscript{N}. The percentage of radioactivity in the blood was determined by comparison to the injected total radioactivity (n = 5). b The biodistribution of RNP\textsuperscript{N} in the brain delivered via oral administration using \textsuperscript{125}I-labeled (open circle) and ESR measurement (closed circle) (n = 5). c Therapeutic effect of RNP\textsuperscript{N} on cognitive dysfunction.

Fig. 8 Protective effects of RNP\textsuperscript{N} on the survival of C57BL/6 J mice after whole-body irradiation (WBI) with X-rays. Eight-week-old, female C57BL/6 J mice (n = 10) were subcutaneously (s.c.) injected with RNP\textsuperscript{N} (200 mg/kg) 24 h before irradiation (7.5 Gy). a Hemorrhagic lesions in C57BL/6 J mice 2 weeks post-irradiation. Representative organs and tissues of X-ray irradiated mice. Red arrows indicate hemorrhagic regions. Scale bar, 1 cm. b Kaplan-Meier survival curves of mice injected with 200 mg/kg RNP\textsuperscript{N} (solid blue line) at 24 h are plotted in comparison with irradiated control (solid black line). The confidence limit (95%) for each survival curve is also shown (dashed lines); n = 10. Reproduced by modifying ref. 12 from Elsevier (2017)
administration of RNP^{N} became almost similar to that of the SAMR1 mice. In this way, RNP^{N}, which disintegrates with decreases in pH and is taken into the blood, has a highly ameliorative effect on cognitive-deficit mice, such as SAMP8, based on its long retention in the blood. Since the blood retention is so long, the redox polymer accesses the cerebral blood vessel for a long time and is gradually taken into the brain. It should be noted that the extremely low toxicity of the redox polymer enables an increased duration in the blood circulation without any damage to the rest of the body. RNP^{N} also shows similar effects in a transgenic Alzheimer mouse model (Tg2576), showing its promise as a new Alzheimer’s nanomedicine [24].

Development of a radioprotection agent

The main damage to the human body upon radiation exposure is the scission of genes, followed by the induction of cellular apoptosis [25]. The generation of ROS by ionizing decomposition of water also causes serious damage to the body [26]. As stated above, due to their size, RNPs have remarkably long retention in the blood and organs compared with the retention of LMW antioxidants such that the radiation protection effect continues. When a mouse is exposed to a 7.5 Gy X-ray, bleeding is observed in all the organs, as shown in Fig. 8a [27]. This strongly indicates that ROS produced by X-ray exposure enter the blood stream and destroy the vascular wall cells of each organ by oxidation. Even if LMW NH_2-TEMPO is administered subcutaneously 24 h before, bleeding hardly stops and little effect is observed. In contrast, when RNP^{N} is administered subcutaneously 24 h before, bleeding is not observed from 37.5 to 15 °C.
organs, and the high effect of RNP is confirmed. As a result, the survival of mice was prolonged, as shown in Fig. 8b. It is concluded that LMW antioxidants are not suitable as radioprotective agents because of their extremely fast metabolism in addition to their strong side effects. Therefore, the nanoadsorbent RNP may pioneer new fields for safe radioprotective agents.

**Redox injectable gels (RIGs) for local antioxidative treatments**

As polymeric antioxidants exhibit such a high blood circulation tendency and do not destroy the normal redox reactions of healthy cells and tissues, they can be applied for various uses as a high-performance antioxidant, as stated above. To expand the applications of our polymer antioxidants, we designed injectable gels possessing ROS scavenging activity.

Jeong et al. [28] reported that an A–B–A type triblock copolymer, consisting of hydrophobic A segments and a hydrophilic B segment, forms flower-type micelles in aqueous media and converts to hydrogels with increasing temperature. This is because the PEG chain loop formed in the shell layer of flower micelles dehydrates (hydrophobizes) as the temperature rises and aggregates. A triblock copolymer (PMNT-b-PEG-b-PMNT) was newly designed in place of the block copolymer (PEG-b-PMNT), constituting the RNP shown in Fig. 1 [29]. As shown in Fig. 9a, this PMNT segment is a polyamine having one amino group in each repeating unit because TEMPO (as an antioxidation moiety) is covalently conjugated at a side chain via an amino group. A mixture of this polyacation-b-PEG-b-polycation and a polyanion, such as poly (acrylic acid), forms flower-type micelles, the driving force of which is the formation of the polion complexes (PICs, see the figure). The size of the flower micelle measured by the DLS method is approximately 80–90 nm and is monodisperse regardless of the composition (Fig. 9b). From the rheological analysis, this PIC flower micelle solution has a very low G" (storage elastic modulus) of 10⁻² Pa or less at room temperature or lower and almost no viscosity at room temperature or lower under these concentrations (ca. 25–30 mg/mL). It also shows a value lower than G" (loss modulus), indicating a liquid state with almost no viscosity (Fig. 9c). With increasing temperature, the elastic moduli rise abruptly at approximately 28 °C, and G" exceeds G′, which means that the solution turns to a hydrogel state (redox injectable gel; RIG) [30]. The final elastic modulus of the gel is greater than 1 kPa at 37 °C.

It should be noted that the modulus of the amphiphilic triblock copolymer micelle of PLGA-b-PEG-b-PLGA is reported to be approximately 0.1–1 Pa at 37 °C [31]. We believe that this high viscoelasticity of this RIG is due not only to the stiffness of its polymer chain but also to its different gelation mechanisms. That is, since this flower micelle was formed by an electrostatic interaction as the

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**Fig. 10** Morphometric bone measurement. Quantitative measurements of alveolar bone were performed by comparing the distance from the cement-enamel junction (CEJ) to the alveolar bone crest (ABC) at seven palatal sites on three molars on the left side of the maxilla. Pg: *Porphyromonas gingivalis*. n = 6. *P < 0.01. Reproduced by modifying ref. 32 from Elsevier (2016)

**Fig. 11** Prevention of postoperative tissue adhesions in talc-induced model mice. a Image of RIG spray on organ surface. b Abdominal tissue adhesion scoring system (score 0: no adhesion, score 1: single band adhesion from organ to abdominal wall, score 2: two band adhesion from organ to abdominal wall, score 3: three band adhesions from organ to abdominal wall, and score 4: more than three band adhesions from organ to abdominal wall). *P < 0.05 compared with control, *P < 0.05 compared with RIG. n = 6. c Histological assessment of peritoneal membrane by Masson trichrome (MT) staining. Fibrosis and collagen deposition (blue) were observed on the peritoneal tissue (red). The arrows indicate the thickness of the peritoneum. Scale bars = 100 μm. Reproduced by modifying ref. 35 from Elsevier (2015)
driving force, the coagulation force of the core decreases when the temperature rises under high ionic strength, and the micelle partly collapses to form crosslinking with other micelles via electrostatic interaction of the collapsed chains. In fact, even with decreasing temperature of the formed gel, the gel does not collapse (irreversible gelation). This is in contrast to the PLGA-b-PEG-PLGA gel, which forms a reversible gel via the hydrophobic interaction described above such that the gel dissolves as the temperature decreases.

The flower micelle (ca. 80 nm) thus prepared can be injected as a mildly viscous solution and exhibits in situ thermo-irreversible gelation under physiological conditions. As one of the important characters of RIGs, in vivo non-invasive imaging and quantitative evaluation were achieved for monitoring the residual amounts of the nitroxide radicals using L-band and X-band ESR instruments, respectively. In contrast to TEMPO, that disappeared from the injected sites in less than 1 h after administration to mouse hind paws, the RIG can be seen for more than several hours by the ESR imaging, as shown in Fig. 9d, e. From the quantitative analysis using X-band ESR (Fig. 9e), we have confirmed that it persists for up to 1 week [27]. Furthermore, we evaluated the protective effect of the RIG using a mouse carrageenan-induced arthritis model. We found that the RIG inhibits neutrophil infiltration and cytokines production by eliminating excessive ROS, leading to the suppression of hyperalgesia activity. Therefore, RIGs, which can locally eliminate ROS for extended periods, can be applied to suppress local inflammations such as periodontitis.

Periodontal diseases are oral inflammatory diseases caused by bacterial infections. Antimicrobial agents are employed to treat periodontitis, but these LMW drugs diffuse rapidly. Therefore, they cannot remain in periodontal pockets for a long time, and their effect is limited. In addition, because there are thousands or more different types of bacteria in the mouth, these antibiotics are not always completely effective against all strains of bacteria. The continued use of antimicrobial agents also leads to the development of resistant bacteria, necessitating caution while using such agents. In periodontal diseases, inflammation is caused in the periodontal pocket in this way, and we have formulated a strategy to use antioxidants because a large quantity of ROS is generated here. Since the RIG converts to a hydrogel after entering deeper within the periodontal pockets, it remains there for a long period of time, and its antioxidant effect is continued for extended periods. When infected with periodontal disease, the number of osteoclasts increases and the absorption rate of alveolar bone increases. Therefore, an RIG was administered into the periodontal pockets of rats infected with Porphyromonas gingivalis (Pg), and the alveolar bone loss was evaluated by X-ray microCT-imaging. Figure 10 shows the CT image and results of alveolar bone resorption [32]. The alveolar bone of the Pg-infected rat is absorbed statistically faster compared with that of the control. nRIG has no TEMPO, that is, it shows an injectable gel with no ROS scavenging ability and therefore has no effect at all. In contrast, it is confirmed that the RIG suppressed alveolar bone resorption almost to the control level. Thus, the RIG exerts a large effect in the treatment of local chronic inflammation. Since the RIG uses a core-shell type nanoparticle, various drug types, such as proteins, peptides [33], and local anesthetics [34], can be enclosed and slowly released locally, thus further expanding its application. The RIG can also be applied as a spray type injectable gel.

Tissue adhesion after surgical operations causes various serious complications, such as ileus and infertility. For example, following the birth of the first child by Caesarean section, the second child cannot be delivered naturally, and Caesarian section must be employed. In such a case, if there...
are strong tissue adhesions, both the mother and baby face danger. Even the most advanced endoscopic or robotic surgery has limited field of vision, and bleeding outside the visual field causes unexpected tissue adhesions, causing serious problems. To prevent such tissue adhesion, biodegradable films are commercially available; however, their handling is difficult, and their effect is not always sufficient.

We evaluated the effectiveness of an RIG as a tissue anti-adhesive agent by applying it as a spray, as shown in Fig. 11a. An adhesion model was prepared by opening the mouse abdominal cavity and sprinkling talc particles on the organ surface [35]. In the adhesion score evaluation (Fig. 11b), there was no effect at all with TEMPO and almost the same for nRIG and commercially available biodegradable films. In other words, nRIG covers organ surfaces, and it is possible to use nRIG to physically prevent adhesions to the same extent as that provided by biodegradable films. In contrast, the RIG shows a much higher tissue anti-adhesion effect, which indicates that in addition to physical separation effects, ROS elimination contributes significantly to adhesion inhibition. Since the antioxidant flower micelles (RIG) thus prepared form a hydrogel under the physiological environment, we can develop a variety of applications, such as eye drops for dry eyes, viz., apply an antioxidant hydrogel on the corneal surface to effectively eliminate oxidative stress and retain a high water environment for an extended period.

Silica-loaded redox nanoparticles (siRNPs)

With the aging of the population, the number of patients with renal failure has increased, and more than 300,000 patients are undergoing dialysis in Japan [36]. Most of the patients have blood taken from their body and undergo hemodialysis (HD) to purify blood via extracorporeal circulation using dialysis membranes. However, in the HD method, since the blood and the dialysis membrane are in direct contact with each other, the blood is seriously damaged. For example, since HD completely destroys the remaining kidney function, not only the blood purification ability but also renal physiological functions (e.g., production of erythropoietin) are lost. HD also increases the risk of stroke and myocardial infarction. In contrast, in peritoneal dialysis (PD) that stores dialysate in the peritoneal cavity and removes unnecessary toxins in the blood via the peritoneum, there are fewer obstacles than in HD. However, PD has other large problems. One is the high glucose concentration of the dialysate to increase its osmotic pressure for improving the toxin extraction efficiency. The high glucose concentration in the dialysate generates ROS, which oxidize the peritoneum itself and cause encapsulating peritoneal sclerosis (EPS) [37]. The other problem is that the exchange capacity is low, so it is necessary to frequently change the dialysate, which often induces infection. To reduce the frequency of dialysate exchange and to reduce the risk of EPS, we have designed a new antioxidant nanoadsorbent. That is, as shown in Fig. 12a, b, a nanoparticle with both high ROS-scavenging capacity and high adsorption capacity was designed by encapsulating silica into the core of RNP [38].

This silica-containing redox nanoparticle (siRNP) not only stabilizes the crosslinking of nanoparticles by silica but also functions as a nanoadsorbent, as silica adsorbs LMW compounds. When this siRNP is mixed with the peritoneal dialysate, the thickness of the peritoneal membrane is reduced in the peritoneal membrane-inflamed mice, which are prepared by chlorhexidine gluconate (HC) treatment, as shown in Fig. 12c [39]. It is suggested that ROS scavenging by siRNPs decreases the inflammation of the peritoneal membrane well. Placing siRNPs in the peritoneal cavity of a renal failure model mouse (nephrectomy) effectively reduces BUN and creatinine levels in the blood compared with those obtained using commercially available dialysates.
thereby confirming the high toxin removal effect of the siRNP (Fig. 12d). In this way, if the dialysate exchange capacity can be increased by using siRNPs, dialysate can be exchanged once or twice a day at home, thereby remarkably reducing the risk of infection; this, as such, promises to be an ideal dialysis method. Since siRNPs thus prepared possess high adsorption capacity due to the entrapped silica, in addition to the ROS-scavenging power, we can develop a variety of applications for siRNPs, such as an oral DDS carrier, viz., siRNPs may solubilize non-water-soluble drugs in the core and release them slowly in the intestinal mucosa. The siRNP itself scavenges ROS to suppress drug-induced adverse effects such as diarrhea [40].

Design of a redox polymer surface for high-performance biodevices

As mentioned above, the problem with HD is that blood comes in direct contact with the dialysis membrane, blood is activated, and the activated blood damages the whole body via the circulatory system. Since ROS are one of the main players in this activation process, it is an effective practice to eliminate ROS at the interface of the blood and membrane (Fig. 13a). Therefore, we used our redox polymer (TEMPO-conjugated polymers) to cover the surface of the device in contact with blood, and as a result, almost no blood coagulation was observed, as shown in Fig. 13b, c [41]. Although it is known that ROS are involved in blood coagulation, it is not well known whether ROS scavenging alone completely prevents coagulation like this. While various polymers have been designed aimed at infinitely suppressing protein adsorption [42], this approach of developing a blood-compatible surface using a redox polymer that eliminates ROS is completely different from the existing approaches, and this polymer is expected to emerge as a new biodevice surface-treatment material.

The ROS-scavenging surface is also effective in cell culture. For example, during trypsin treatment of cell culture systems, the glutathione level decreases and the cells are subjected to strong oxidative stress [43]. In addition, various oxidative stresses, such as culturing at an oxygen concentration higher than that in vivo and in contact with air, are applied under the present circumstances. Excessive ROS generated during cell culture disrupts the oxidation–reduction potential of the mitochondria and changes the morphology of the cell. Conventional small molecule antioxidants, such as vitamins, enter into the cell and inhibit the normal redox reaction [44]. Coating the cell dish with our redox polymer differs from using conventional LMW antioxidants in that only the extracellular
excessive ROS are eliminated in the former case. We confirmed that elimination of excessive ROS during cell cultivation does not change the membrane potential of mitochondria and retains the active state of the cell.

The redox polymer and the CD34 antibody were co-immobilized on the surface of a cell culture dish, and hematopoietic stem cells were separated from the cells collected from a mouse fetus (Fig. 14a). As shown in Fig. 14b, c, the separated cells can maintain the CD34 activity for a long time; this approach is thus expected to emerge as one of the cell-culture techniques in regenerative medicine technology [45].

**Conclusion**

It has long been known that 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), a famous and stable nitroxide radical compound used as a probe for ESR spectroscopy, has a strong antioxidant function. However, even if its antioxidant capacity is strong, it destroys normal intracellular redox reactions and damages normal tissues, which is a serious problem associated with using LMW antioxidants. Our approach is to covalently bind TEMPO to polymers and suppress its uptake into normal cells, thereby solving the problems associated with conventional antioxidants; our approach has been shown to be highly effective against various oxidative stress diseases (Scheme 1).

Since the late 19th century discovery that acetylation of salicylic acid markedly reduces its side effects, organic synthesis has become the center of drug development and is now leading to the present drug development. In recent years, proteins have been developed as drugs, and a paradigm shift in the development of target drugs is occurring. However, the development of proteins as drugs incredibly increases their cost. Basing drugs on high molecular weight compounds dramatically alters their pharmacokinetics, which is in sharp contrast to that of the conventional LMW compounds that nonspecifically spread throughout the body. Nanomedicines based on polymer drugs are different from the conventional organic drugs and protein drugs, and their use in developing a new field of medicine is highly anticipated (Fig. 15). The author concludes this review in
anticipation that our antioxidant nanomedicine will further emerge as one of the pillars of the new drug development field.

Acknowledgements This article is a contribution of the author, who was awarded the Award of the Society of Polymer Science, Japan (2017). The author would like to express his sincere gratitude to the Society of Polymer Science and to all the associated people. The data described here are the findings of studies conducted in collaboration with his students and colleagues in his laboratory at the University of Tsukuba, as well as with many other collaborators, including medical doctors. The author also thanks them for their continuous support. Most of the work described here was supported by a Grant-in-Aid for Scientific Research S (25220203), the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References


Yukio Nagasaki was born in 1959. He received a B.S. and Ph.D. degrees in Engineering School of Science University of Tokyo in 1982, and 1987. Since 1987, he was working Science University of Tokyo as Research Associate, Assistant Professor, Associate Professor and Professor. In 2004, he moved Graduate School of Pure and Applied Sciences, University of Tsukuba. He hold concurrent posts of Adjunct Professor,Master’s School of Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, and Principal Investigator, Center Research in Isotope and Environmental Dynamics (CREID), University of Tsukuba. During last 30 years, he engaged in materials science especially in the field of biology, pharmaceutics, and medical science. Especially, he was focusing on biointerface, drug delivery system, and nanomedicine. He published more than 200 scientific papers. He received the excellent Ph.D. thesis award from Inoue Foundation of Science in 1989, Young Researcher Award from Polymer Society, Japan in 1993, SPSJ Mitsubishi Chemical Award from Polymer Society, Japan in 2010, The Award of The Japanese for Ulcer Society (2014), The Award of the Japanese Society for Biomaterials (2014), The Nagai Award from The Japan Society of Drug Delivery System (2015), and The Award of the Society of Polymer Science, Japan (2017).

Selected Publications


Graphical Abstract

Amphiphilic block polymers having nitroxide radicals (TEMPO) self-assemble in water to form nanoparticles. This is a new nanomedicine that avoids the adverse effects of conventional antioxidants, which destroy the intracellular redox environment, and makes it possible to treat various oxidative-stress-related diseases such as cerebral or cardiovascular ischemia-reperfusions, Alzheimer’s disease and cancer. In addition, we have designed a gelation function in these materials and developed them as a material for anti-tissue adhesion agents and periodontal diseases. Additionally, we succeeded in enhancing cultured cell functions using an antioxidant biointerface.
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