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Current Progress in Reactive Oxygen Species (ROS)-Responsive Materials for Biomedical Applications

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Abstract

Recently, significant progress has been made in developing “stimuli-sensitive” biomaterials as a new therapeutic approach to interact with dynamic physiological conditions. Reactive oxygen species (ROS) production has been implicated in important pathophysiological events, such as atherosclerosis, aging, and cancer. ROS are often overproduced locally in diseased cells and tissues, and they individually and synchronously contribute to many of the abnormalities associated with local pathogenesis. Therefore, the advantages of developing ROS-responsive materials extend beyond site-specific targeting of therapeutic delivery, and potentially include navigating, sensing, and repairing the cellular damages via programmed changes in material properties. Here we review the mechanism and development of biomaterials with ROS-induced solubility switch or degradation, as well as their performance and potential for future biomedical applications.

Keywords

Biomaterials; Degradation; Reactive Oxygen Species; Solubility Switch; Therapeutic Delivery

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1. Introduction

In the field of biomaterials, much of recent work has been put into developing materials that exhibit specific response to biological parameters under abnormal conditions and deliver therapeutics in a spatially and temporally controlled manner. These materials aim to take advantage of pathological conditions that are often identified with local abnormalities in an array of biological parameters, such as the pH, temperature, protease activities, or redox balance.[1–3] One such biological parameter that is gaining importance recently is reactive oxygen species (ROS) that contribute greatly to the cellular redox state. As the implications of ROS in many diseases are elucidated, ROS-responsive materials are also gaining more importance. Starting with polypropylene sulfide (PPS) developed in 2004, ROS-sensitive materials are still relatively new, but it is an emerging field of studies.

ROS include hydroxyl radicals($OH^{\cdot}$), hydrogen peroxides(H_2O_2), peroxynitrites($ONOO^-$) and superoxides(O_2^-) among others.[4] ROS are produced from several endogenous sources, notably in the mitochondria from an incomplete reduction of oxygen, and NADPH oxidase (NOX) in the plasma membrane and serve an important role in signaling.[5] For example, the cells of the thyroid gland require hydrogen peroxide in order to attach iodine atoms to thyroglobulin in the synthesis of thyroxine. Cell-specific oxygen-sensing cascades involve ROS as secondary messengers and with specific subcellular localizations they help to tailor adaptive responses to varying oxygen availability.[6] All vascular cell types (i.e., endothelial cells, vascular smooth muscle cells, and adventitial fibroblasts) produce ROS continuously at low levels, compared to pathological situations.[7] Low levels of ROS regulate vascular functions by modulating cell growth, apoptosis/anoikis, migration, inflammation, secretion, and extracellular matrix protein production but overproduction of ROS induces vascular pathogenesis, including hypertension, hyperlipidemia, and diabetes.[7] While a moderate level of ROS is involved with normal cell functions, excessive amounts of ROS cause oxidative stress and damage critical components of cells at all levels including DNA, proteins, and lipids by oxidation.[8–10] Oxidized proteins may become dysfunctional, and oxidation in DNA can result in mutations and other deleterious effects. More importantly, a growing body of evidence suggests that chronically increased levels of ROS are accompanied locally in many pathologies such as cancer, atherosclerosis, diabetes, infections, inflammatory diseases, and even in aging process.[11–16] Therefore, ROS can be considered as a target or an indicator that makes the diseased area distinct from its surrounding for treatments. ROS-responsive materials can be used for site-specific delivery of therapeutic and imaging agents, or as the coating material for implant applications that can degrade and release therapeutics in response to ROS *in vivo*.

Here we review the mechanism and development of biomaterials with ROS-induced solubility switch or degradation (Figure 1 and Table 1), as well as their performance and potential for future biomedical applications. We also discuss the current challenges in developing and applying ROS-responsive materials.

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2. Materials with a ROS-induced Solubility Switch

2.1 Polypropylene sulfide (PPS)

The organic sulfides undergo phase transition from hydrophobic sulfide to more hydrophilic sulfoxide or sulfone under oxidative environment (Table 1).[17] Utilizing this property, Hubbell *et al.* discovered polypropylene sulfide (PPS) as a potential oxidation-responsive material for drug delivery applications.[18] This was the first type of oxidation-sensitive biomaterials to be developed. In their study, ABA triblock copolymer comprising hydrophilic polyehtylene glycol (PEG) as A block and hydrophobic PPS as B block was prepared by anionic ring opening polymerization method.[19] This block copolymer was found to self-assemble into the U-shape vesicles in aqueous solution through hydrophilic/hydrophobic interactions, and remained stable in the solution until the particles were oxidized with 10% H_2O_2 to become hydrophilic and dissolve. The responsiveness to oxidation was characterized before and after H_2O_2 treatment by turbidity measurement, NMR spectroscopy and cryo-TEM. These vesicles were found to rapidly destabilize and dissolve within just few hours after the addition of H_2O_2, one of the most prevalent ROS in biological systems. Considering drug delivery applications, lyotropic behavior, drug encapsulation and release behavior of micelles made of diblock copolymers of PEGand PPS were studied by varying chain length of hydrophobic PPS while keeping the same length of PEG.[20] *In vitro* release of immunosuppressive drug cyclosporin A (CsA) at 37 °C from PEG_{44}-b-$PPS_{10,\ 20,\ 40}$ micelles was constant for 12 days.

While PPS was initially found to be responsive to H_2O_2, attempts at rendering PPS sensitive to other ROS have been made. Tirelli *et al.* investigated responsiveness of PPS-b-PEG copolymer micelles towards superoxides.[21] In this study, release of Nile red from PEG-b-PPS micelles was studied in the presence of xanthine oxidase (XO) which was used as an oxidizing enzyme to generate H_2O_2, superoxide and peroxynitrite.[22] The XO-mediated release of Nile Red was found to be time-dependent due to extremely short half-lives of most ROS except for H_2O_2. The release was not observed when XO was used in combination with catalase or superoxide dismutase (SOD). These results were expected as SOD and catalase remove superoxides and hydrogen peroxides in cells.[23] These results also indicated that XO could oxidize the PPS micellar core through H_2O_2-mediated oxidation, while the presence of superoxide appeared to have minimal effect, as the same system showed an even faster release profile in the presence of SOD. In order to overcome this shortcoming, they developed SOD-conjugated PEG-b-PPS micelle system aiming to give sensitivity-albeit indirect one-to superoxide anions. By having the conjugated SOD convert superoxide anions into H_2O_2 to which PPS is originally sensitive, they have developed PPS micelles that are also responsive to superoxide species.

On a similar line of work, Gupta and Duvall *et al.* explored the sensitivity of PPS polymer micelles towards peroxynitrites at a pathophysiologically relevant concentration.[24] In their study, PPS-b-polydimethylacrylamide (PDMA) diblock copolymer was synthesized by thioacyl group transfer (TAGT) and radical addition fragmentation chain transfer (RAFT) polymerization method. The oxidation-dependent drug release from these diblock copolymer micelles was investigated against multiple ROS species such as H_2O_2, 3-

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morpholinosydnonimine (SIN-1), and peroxynitrites. For *in vitro* study, either activated or inactivated RAW 264.7 macrophages were used to create a physiologically relevant ROS-rich environment to induce the release of Förster Resonance Energy Transfer (FRET) fluorophore pair DiI and DiO from micelles. The activated macrophages showed significantly increased release of fluorophores compared to the unactivated counterpart.

Similarly, Reddy *et al.* have synthesized diblock copolymers of PPS and PEG to create self-assembled nanoparticles with a rubbery PPS core surrounded by highly hydrophilic PEG coronas.[25] At diameters of about 20 nm, these nanoparticles were passively internalized into lymph nodes and were shown to disassemble and release the cargo in an *in vivo* mouse model. Since lymphocytes actively employ ROS as signaling molecules to regulate inflammation and as antimicrobial oxidative stress, such ROS-responsive particles showed great potential as vehicles for delivering therapeutics into immune cells for immunotherapy applications or anti-leukemia systems.[26] Indeed, after intradermal injection of these small nanoparticles that were conjugated to antigens, the PPS-based particles were found to robustly stimulate the complement cascade and activate dendritic cells upon ROS-induced disassembly of the particles, generating strong cellular and humoral immunity to the model antigen.[27]

More recently, materials that are both oxidation and reduction-sensitive have been synthesized with oxidation-responsive PPS blocks and reduction-responsive disulfide bonds as linkages. Swartz *et al.* conjugated antigens to PPS-based nanoparticles with reduction-sensitive disulfide linkages.[28] These PPS nanoparticles conjugated to antigens were then tested *in vitro* and *in vivo* for their inductive capabilities on antigen cross-presentation by dendritic cells for vaccine applications. Upon cellular uptake, these particles were subjected to the reducing environment of the cytoplasm where the antigens were freed from the PPS nanoparticles by the reductive cleavage of disulfide bonds. The remaining PPS nanoparticles were oxidized in the late lysosomes to become soluble and then degraded without notable cytotoxic effects eventually. Such delivery of antigens to immune cells proved highly effective for antigen cross-presentation on dendritic cells and stimulation of T-cell activation. Similarly, Cerritelli et al. also fabricated redox-sensitive polymersomes by linking ROS-sensitive PPS block and PEG block with reduction-sensitive disulfides.[29]

Because of the crucial role and prevalence of ROS in immunity and inflammatory diseases as well as aging and cancer, PPS-based oxidation-sensitive polymer schemes will likely continue to be useful for novel drug delivery systems and vaccine applications. In light of the recent connections found between ROS signaling and the cellular response to tissue engineering substrates, PPS-based polymers may also find applications in engineering polymeric scaffolds with properties optimized for inducing positive tissue responses.[30] Ultimately, PPS polymers are easy to synthesize and versatile with useful characteristics such as the switch-like solubility change due to oxidation and superb biological compatibility, making it an ideal material for applications where oxidation sensitivity and rapid oxidation-specific response are sought.

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2.2 Selenium-Containing Block Copolymers

Selenium-based compounds are well-known for their oxidation- and reduction-sensitive nature.[31] Similar to how the sulfide groups in PPS are oxidized and result in a hydrophobic-to-hydrophilic transition, initially water-insoluble selenides become more soluble selenoxides and selenones upon oxidation (Table 1). Additionally, diselenides can be reduced or oxidized leading to the bond cleavage.

Zhang group developed a number of redox-sensitive selenium-containing materials for drug delivery.[32–36] Ma *et al.* developed selenium-containing amphiphilic triblock copolymer (PEG-PUSe-PEG) with a hydrophobic polyurethane block containing selenides and two hydrophilic PEG blocks. This copolymer was found to self-assemble into micelles in aqueous solution and undergo oxidative cleavage in ROS-rich environments.[35] Doxorubicin was encapsulated into micelles and the release behavior in a mildlyoxidative environment was studied in the presence of 0.1% H_2O_2 by volume. The micelles underwent disassembly upon exposure to hydrogen peroxide and showed 72% release of cargo up to 10 hours. Similarly, doxorubicin was loaded into micelles made with PEG-PU block copolymer containing sulfides for comparison, and the release characteristic under the same oxidizing condition revealed inferior release of cargo at 41% in 10 hours. In another study, Ren *et al.* designed and synthesized a new type of amphiphilic poly(ethylene oxide-b-acrylic acid) block copolymers containing selenium as side chains (PEO-b-PAA-Se) to explore new selenium-based oxidation-responsive materials.[33] The hydrophobic selenide groups of PEO-b-PAA-Se underwent oxidation to become hydrophilic selenoxides in a mildly oxidizing condition of 0.1% (v/v) H_2O_2, leading to the disassembly of the spherical micellar aggregates. In 20 hours, micelles were found to dissemble and release all of the payloads. Interestingly, it was found that this oxidation process could be reversed by the addition of vitamin C as reducing agent. For the identification of the disassembly mechanism of PEG-PUSe-PEG, Tan *et al.* examined side chain mechanics of PEG-PUSe-PEG and PEG-PUSeox-PEG using atomic force microscope (AFM)-based single molecule force spectroscopy (SMFS).[34] It was observed that the change from selenide to oxidized selenone considerably altered the amphiphilicity, without changing the single-chain elasticity.

The Se-Se bonds of diselenide undergo cleavage either in the presence of oxidants to seleninic acid or reduced to selenol in presence of reducing environment.[37] Inspired by oxidation and reduction responsive nature of diselenides, Ma and Zhang *et al.* designed and developed another diselenide based block copolymer (PEG-PUSeSe-PEG) containing PEG as a hydrophilic block and diselenide containing polyurethane as a hydrophobic block to make dual redox responsive nanocarriers.[36] The fluorescent Rodamine B was used to study the release of payloads under oxidative and reducing environment from PEG-PUSeSe-PEG copolymer micelles. As oxidants, two concentrations of H_2O_2 (0.1 and 0.01% v/v) were used to study the cleavage of diselenium bond and consequent destabilization of micelles to release the payloads. More than 90% release from micelles was observed after 3 hours even in the lowest (0.01% v/v) H_2O_2 concentration, which indicated excellent oxidative cleavage of the PEG-PUSeSe-PEG micelles. The reduction responsiveness of PEG-PUSeSe-PEG micelles was evaluated in the presence of reductive glutathione (GSH). The micelles were

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found to dissemble completely to release the fluophores in the presence of 0.01 mg/ml concentration of GSH.

2.3 Polythioether Ketal

Among the multi stimuli-responsive materials present today, polythioether ketal is one of the few that take ROS as a stimulus. Almutairi *et al.* have synthesized nanoparticles for protein delivery with polythioether ketal polymer that has a dual stimuli responsive characteristic.[38] In this polymer, the thioether groups provide ROS-sensitivity through a solubility-switch mechanism very similar to that of PPS while ketal groups provide pH-sensitive degradation property (Table 1). Specifically, the hydrophobic and water-insoluble sulfide groups linking the ethyl groups are oxidized in the presence of H_2O_2 to become hydrophilic and water-soluble sulfoxides, conferring the switch-like transition in the overall solubility of the polymer. On the other hand, the ketal groups are known to hydrolyze into biocompatible byproducts such as acetone and diols in mildly acidic environments.[39] Initially, hydrophobic polythioether ketal polymer was first used to make nanoparticles containing either Nile red or ovalbumin by emulsification in excess PBS, using a high-pressure homogenizer. Once these nanoparticles were exposed to ROS, for example, upon cellular uptake by ROS-producing macrophages, the particles were oxidized and solubilized which resulted in a partial release of the cargo due to the swelling of the polymer. However, it was only when the nanoparticles were also exposed to a low pH environment (pH 6.5) as well as ROS in tandem that the degradation of the polymer occurred followed by a near complete release of the payload under one day *in vitro*. Interestingly, even when the pH was kept relatively low at 5, the polymer would not degrade sufficiently without the presence of H_2O_2, presumably due to insufficient access to the pH-sensitive ketal groups in the hydrophobic core. Additionally, in an acidic environment without ROS, only about 20% release of the molecules was observed in the first 4 hours and no additional release occurred in the next 20 hours. This result confirmed that polythioether ketal nanoparticles require both increased ROS levels and acidic environments for successful and sufficient drug release. This extra selective characteristic bestows an “AND” logic gate functionality on polythioether ketal where pH-dependent degradation of the polymer only takes place in the presence of ROS. As such, this type of dual stimuli responsive polymer would be especially suited for minimizing off-target side effects and targeting specifically at inflammatory sites that typically exhibit both higher ROS concentrations and a lower pH.

3. Materials with ROS-induced Degradation

3.1 Materials with Boronic Esters

Dating back a couple of decades, boronic esters have been shown to undergo oxidation-induced degradation and have seen an increase in use as ROS-degradable protecting groups for various applications recently.[40] For example, boronic esters were previously used to conjugate to and hide the active site of matrix metalloproteinase (MMP) inhibitors until the drug molecules reach ROS-rich environments where boronic esters would degrade to reveal the active site of drug molecules.[41] Using a similar principle, imaging agents and anti-cancer drugs have been conjugated to boronic esters for site-specific activations in ROS-rich environments.[42–43]

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Boronic esters undergo oxidation, which eventually results in cleavage (Table 1). Specifically, under oxidizing conditions, the linkage between boronic esters and the material or drug molecules of interest becomes oxidized with an insertion of oxygen. This linkage then undergoes hydrolysis in the presence of water, resulting in cleavage of boronic esters. Moreover, several boronic ester derivatives have been studied among which aryl boronic esters with either ester or ether linkages shows superior degradation kinetics.[41, 44]

In one study, De Garcia Lux *et al.* from UC San Diego developed a new polymer with each monomers incorporating ROS-degradable arylboronic esters and adipic acid.[44] They used oil-in-water emulsion techniques to pack hydrophobic Nile Red into nanoparticles. For comparison, two different polymers were fabricated where arylboronic esters were either linked directly or through an ether linkage. These nanoparticles were subjected to varying concentrations of H_2O_2 for degradation and a consequent release of the payloads. The arylboronic esters with ether linkages proved extremely sensitive to H_2O_2 even at a low concentration (50 μM) while the directly linked arylboronic esters required about 1 mM H_2O_2 for the same degree of release. They also used activated neutrophils to create a physiologically relevant ROS-rich environment *in vitro*, and the nanoparticles with arylboronic esters linked with an ester linkage released twice as much as the particles with directly linked arylboronic esters. In fact, this adipic acid-based polymer with arylboronic esters with ether linkage is one of the most ROS-sensitive materials that have been published to date.

The aforementioned strategy was used to develop a new ROS-responsive dextran material.[45] Here, dextran-a water soluble, biocompatible and FDA approved polymer of glucose-was chemically modified by replacing hydroxyl groups with aryl boronic esters.[45, 46] While dextran is readily soluble in water, the loss of hydroxyl groups from the modification process makes it insoluble in water. This switch in solubility allows for standard emulsion techniques to be used to pack hydrophilic payloads in organic solvents into water-insoluble modified dextran nanoparticles. The modified dextran nanoparticles were exposed to a physiologically relevant concentration (1 mM) of H_2O_2, which resulted in oxidative degradation of arylboronic esters and unmasking of the hydroxyl groups of dextran. Once dextran regained hydroxyl groups, solubility in water was restored as expected and the payloads were released. In summary, the ROS-responsive degradation property of boronic esters have been successfully transduced into a solubility switch. To test the feasibility of using modified dextran-based materials for vaccine applications, ovalbumin(OVA)-loaded modified dextran particles, OVA-loaded poly(lactic-co-glycolic acid) (PLGA) particles, or free OVA were incubated with DC 2.4 murine dendritic cells for 6 hours. While the cell media contained a negligible amount of ROS, particles were exposed to high levels of ROS in the phagosomes upon cellular uptake, which triggered re-solubilization of the dextran particles and a rapid release of ovalbumin in the cytosol. While free ovalbumin added at similar concentrations in media showed no MHC class I presentation on the dendritic cell surface, ovalbumin delivered using oxidation-sensitive modified dextran particles induced robust major histocompatibility complex (MHC) class I presentation within 6 hours and outperformed similarly loaded PLGA particles by 27-fold. These ROS-responsive modified dextran particles could be used as effective antigen

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presenting vehicles to dendritic cells for the activation and proliferation of CD8+ T-cells that will fight against diseases using one's own immune system.

3.2 Silicon

In previous studies, a variety of drugs such as doxorubicin and dexamethasone have been adsorbed to the inner walls of silicon (Si) particle pores for drug delivery applications.[11, 47–48] However, simple adsorption of drug molecules to Si surface often results in a non-specific, rapid burst release of drugs in a matter of hours to few days, which may be less than desirable in cases where specificity and a sustained drug release profile are preferred.

To address this issue, Sailor group from UC San Diego attached fluorescent dye (Alexa Fluor 488) or the anticancer drug (doxorubicin) to the surface and inner pore walls of mesoporous Si particles by the means of Si-C covalent bonding.[49] To covalently attach molecules, Si surface was first modified using microwave-assisted thermal hydrosilylation with undecylenic acid to generate free carboxylic groups. Later, amine group of Alexa Fluor 488 or doxorubicin was coupled to free COOH groups of modified Si surface by standard *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC) chemistry. This resulted in drug release by Si matrix oxidation followed by cleavage of the covalent bonds. While Si-C bonds were stable in aqueous media, the Si matrix underwent oxidation to Si-O-Si in ROS-rich environments. The oxidized Si matrix was further hydrolyzed in aqueous solution to release attached payloads (Table 1). In this study, 1 m SIN-1 was used to generate a physiologically relevant concentration of peroxynitrite ($ONOO^-$). In this condition, the Si microparticles with covalently attached dyes showed a 10-fold increase in fluorescence intensity compared to the particles in PBS over 24 hours, and avoided a rapid burst release at the beginning.

3.3 Material with Proline Oligomers

The free radical-mediated oxidation of free amino acids and peptides has been investigated since 1960s, and its importance has been emphasized recently as it was implicated that an increased level of ROS in numerous pathologies can oxidate various protiens. For detailed literature on protein oxidation, readers are referred to an excellent review by Stadtman.[50] In particular, it was found that amino acid such as aspartic acid, glutamic acid and proline residues in peptides are especially prone to undergo peptide backbone cleavage when oxidized, causing protein fragmentation.[51] Among these three residues, proline was further studied and proved to be cleaved by oxidation (Table 1).[52] On that account, the Sung group from Vanderbilt University used proline oligomers as crosslinkers in fabricating poly(e-caprolactone)(PCL)-based polymeric scaffolds that degrade specifically in response to ROS.[53] Specifically, oligoproline peptides conjugated to PEG_{12} were synthesized and tested for degradability under oxidative environments from metal catalyzed oxidation with 5 mM H_2O_2 and 50 μM Cu(II) at 37 °C. After 6 days of incubation, all proline residues were cleaved away while PEG_{12} molecules remained intact. To Confirm the ROS-degradability of the polyproline peptide crosslinkers, biaminated PEG-$Proline_n$-PEG (n=5–10) was synthesized and crosslinked with 4% PEG- 86% poly(ε-caprolactone) (PCL)-10% carboxylated poly(carboxyl-ε-caprolactone) (CPCL) (% indiccates the molar ratio of

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corresponding unit) by EDC/NHS based coupling between the amine groups of the crosslinkers and carboxyl groups of the polymer backbone. Porous scaffolds were made using salt-leaching method. It was expected that these proline oligomer crosslinkers would be oxidized to form 2-pyrrolidone peptides which results in the crosslinker fragmentation, facilitating the degradation of the overall crosslinked scaffold constructs in the presence of ROS. To determine the ROS-responsiveness, the crosslinked scaffolds were subjected to oxidation in the presence of SIN-1, which produces peroxynitrite and hydroxyl radicals in PBS, as well as activated murine macrophages to mimic a physiologically relevant ROS environment *in vitro*. In both cases, ROS-dependent degradation of the scaffolds was observed.

What is notable in this case is the time frame in which ROS-responsive degradation occurs. While other ROS-responsive materials discussed in this paper are optimized for rapid short-term responses to ROS in a matter of hours to few days, these proline oligomers take few weeks of time to fully degrade. Therefore, this would be especially suitable for tissue engineering and controlled release applications where chronic oxidative stress from an increased level of ROS is expected as in the case of inflammatory response to implants and atherosclerotic lesions.[54] Additionally, the use of naturally occurring amino acids as in this paper may avoid potential biocompatibility issues *in vivo* as opposed to other materials where toxic elements such as selenium are incorporated for ROS-sensitivity.

3.4 Polythioketal

A new class of ROS-responsive synthetic material was developed by the Murthy group for oral delivery of siRNAs for applications in treating gastrointestinal (GI) diseases in which the disease progression is accompanied by a ten- to hundred-fold increase in mucosal ROS concentrations.[55, 56] However, simply using ROS-responsive materials would not be sufficient for this application; oral delivery of drug molecules is often challenging due to the harsh environments that dramatically change throughout the GI tract. Hence Wilson *et al.* developed a new poly-(1,4-phenyleneacetone dimethylene thioketal) (PRADT) polymer that is ROS-sensitive for targeting inflamed intestinal tissues, but equally importantly, is stable in acidic, basic and protease-abundant environments that are analogous to the GI tract. PRADT derives its ROS-sensitivity from the thioketal groups (Table 1). In a superoxide-rich environment, the thioketal groups are degraded into acetone and thiols. Similarly, PRADT is cleaved into acetone and 4-(mercaptomethyl)phenyl methanethiol in oxidizing environments of the diseased and inflamed intestines. The exceptional ROS-specific degradation property of PRADT allowed for successful delivery and release of siRNAs. The siRNAs were kept intact within the particles through most of the GI tract until the particles finally reached the ROS-rich inflamed intestines where the particles were degraded for siRNAs release. In the same study, polythioketal nanoparticles carrying siRNAs for TNF-α were made by an oil-in-water single-emulsion procedure. These particles were orally administered for 6 days into mice that were previously treated with dextran sodium sulfate to induce an inflammatory response in the colon. The biodistribution study showed an effective localization of the particles to the inflamed colon with abnormally high concentrations of ROS. Additionally, the mice treated with the nanoparticles showed intact epithelium and a low level of immune cell infiltration in the colon, and the treated mice also weighed heavier than the control

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group, indicating the restoration of intestinal functions. These positive results indicate successful siRNA delivery to the inflamed intestinal tissues through the highly ROS-specific response of the PRADT nanoparticles. Oral delivery of therapeutics innately possesses a number of desirable properties: non-invasive nature, ease of administration, and reduced costs etc. However, traditional oral delivery of therapeutics for inflammatory GI diseases has been largely ineffective due to improper drug delivery vehicles. These results suggested that ROS-responsive materials with a high stimulus specificity can withstand the harsh environments of the GI tract and serve as vehicles for successful oral delivery of therapeutic agents in numerous inflammatory GI diseases with a pathophysiological level of oxidative stress.

4. Future Challenges

Advanced biomaterials with programmed changes in their composition, shape or structure in response to ROS will have a profound impact on the field of biomaterials and tissue engineering research towards regenerative medicine and other types of applications. The advantages of the technology platform for ROS-responsive biomaterials extend beyond site-specific targeting of therapeutic delivery, and potentially include development of a new tool box to intelligently interact with complex biological signaling underlying a variety of pathophysiological events.

Although significant progress has been made so far in the field, there are several challenges to be addressed for future development. First, it is important to design materials that distinguish between the low levels of ROS from normal cellular activities and the increased levels of ROS from pathologies.[57] Second, ROS-sensitive materials should be biocompatible and must not trigger inflammatory responses becuase activated macrophages and neutrophils can generate a large amount of ROS (respiratory burst) that can act on ROS-responsive materials unintentionally. Third, programmed changes in materials, such as solubility change and degradation, should be controlled at a rate appropriate for pathologies for optimal outcomes. Fourth, the interplay among polymer composition, material properties, and biological effects should be understood thoroughly to guide the design of next-generation ROS-responsive biomaterials. Despite its current challenges, ROS-responsive materials offer a novel treatment avenue and hold great promise in biomedical fields.

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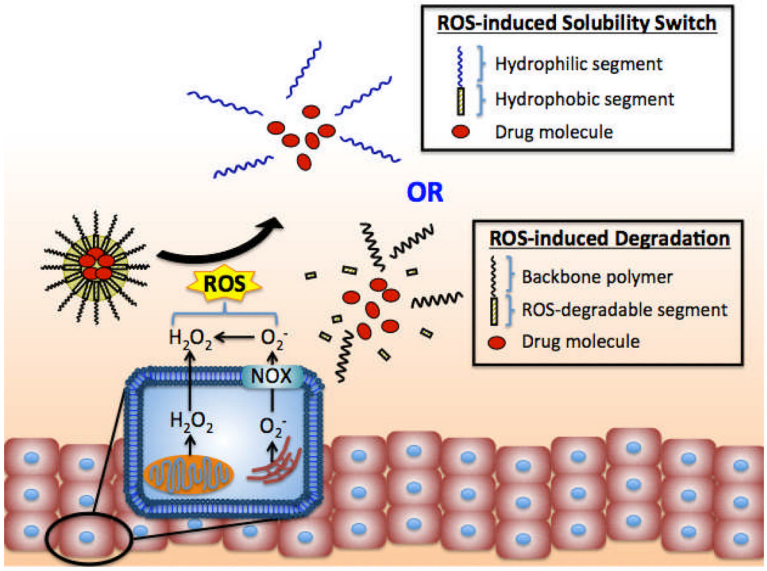

Figure 1.

A generalized schematic diagram of reactive oxygen species (ROS)-responsive drug delivery and drug release via 1) solubility switch and 2) degradation mechanisms.

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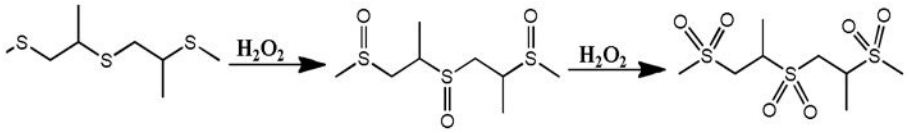
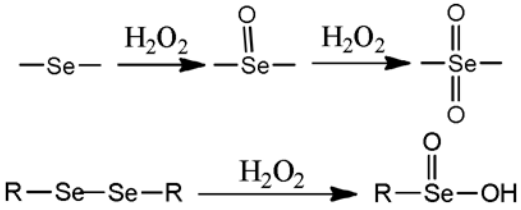
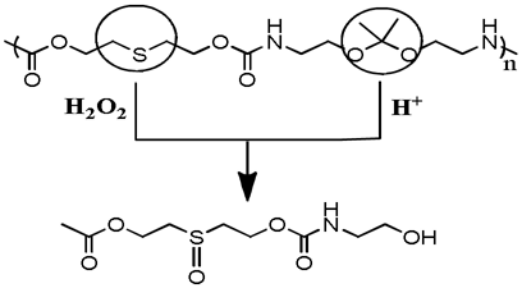
Table 1

Reactive oxygen species(ROS)-responsive polymeric biomaterials and their oxidation and release mechanisms.

ROS-responsive Materials	Chemical Structure and Oxidation	Ref.
< ROS-induced Solubility Switch >		
Poly(propylene sulfide)		[18–21, 24–29]
Selenium-containing polymer		[32–36]
Poly(thioether ketal)		[38]
< ROS-induced Degradation >		

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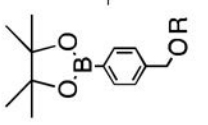
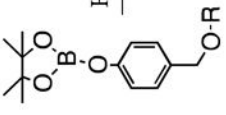
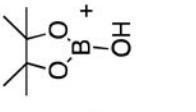
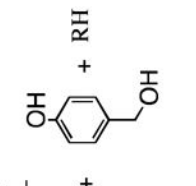
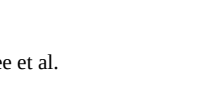
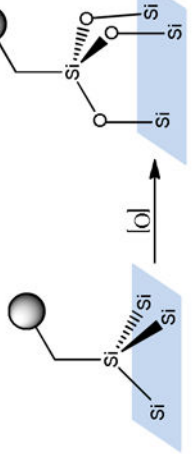
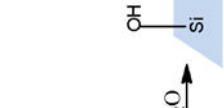
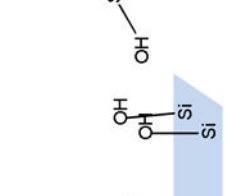
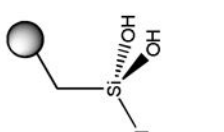
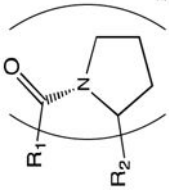
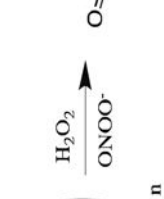
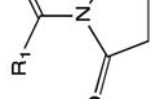
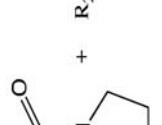
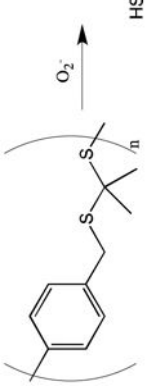
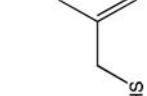
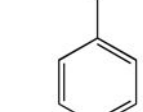
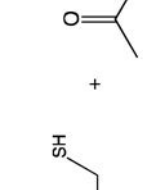
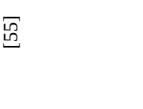
ROS-responsive Materials	Chemical Structure and Oxidation	Ref.
Arylboronic ester- containing polymer	     	[40–45]
Mesoporus silicon microparticles	     	[49]
Oligoproline peptide-crosslinked polymer	      	[53]
Poly(thioketal)	     	[55]