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A Biocompatible Oxidation-Triggered Carrier Polymer with Potential in Therapeutics

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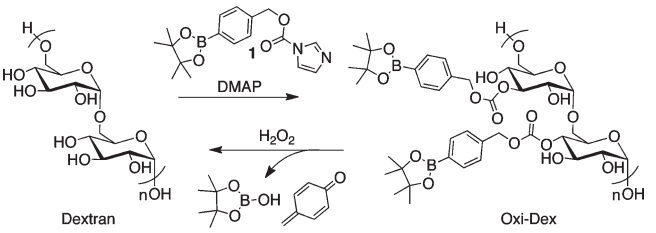
Supporting Information

ABSTRACT: Dextran, a water-soluble, biocompatible polymer of glucose, was modified at its hydroxyls with arylboronic esters to make it soluble in common organic solvents, allowing for the facile preparation of oxidation-sensitive dextran (Oxi-DEX) carrier microparticles. These particles were found to release their payload with a half-life of 36 min at 1 mM H_2O_2, which can be compared with a half-life of greater than 1 week in the absence of H_2O_2. When used in a model vaccine application, Oxi-DEX particles loaded with ovalbumin (OVA) increased the presentation to $CD8^+$ T-cells 27-fold relative to OVA encapsulated in a classical vehicle not sensitive to oxidation. No presentation was observed from cells incubated with unencapsulated OVA. Additionally, Oxi-DEX was found to be nontoxic in preliminary in vitro cytotoxicity assays. Because it is easy to prepare, sensitive to biological oxidation, and biocompatible, this material may represent an attractive new platform for selective delivery applications.

Hydrophobic biodegradable polymers such as polyorthoesters,[1] polyesters,[2] and polyanhydrides[3] have found wide use in the biomedical field as sutures and scaffolds as well as carriers for vaccine applications, gene delivery, and chemotherapeutic agents. Their success is in part due to the fact that they are degradable under biological conditions, making them both biocompatible and resorbable. This degradation typically occurs over the course of several months via surface erosion and hydrolysis of the polymer backbone.[4]

However, for many delivery applications, it is often desirable to release an encapsulated payload rapidly and selectively by taking advantage of a unique chemical environment. While many carrier systems using acid-[5] or reduction-mediated[6] release have been developed, relatively few systems that exploit biologically relevant oxidative conditions for cargo release have been explored. Reactive oxygen species have been implicated in reperfusion injury following cardiac arrest and are also heavily produced in the phagosomes of antigen-presenting cells (APCs), which are critical initiators of the adaptive immune response.[7,8] Recent reports have indicated that the most effective APCs, dendritic cells (DCs), may have phagosomes that are much more oxidizing than they are acidic, having H_2O_2 concentrations of up to 1 mM.[9] Despite these potential areas of use, very few drug delivery systems that are sensitive to biologically relevant oxidative conditions have been designed. At this time, the most promising such systems may have either been tested only at H_2O_2 concentrations far higher than would be encountered biologically[10] or rely on superoxide, a powerful but extremely short-lived cellular oxidant.[11]

Scheme 1. Synthesis and Degradation of Oxi-DEX



Recently, significant advances in sensing oxidative stress have been made by taking advantage of specific chemistry between cellular oxidants such as H_2O_2[12,13] or hypochlorous acid.[14] Here we demonstrate a biocompatible platform for delivery that uses one such reaction to target delivery of therapeutic agents. To accomplish this, we employed a solubility switching mechanism in which a biocompatible, water-soluble polymer already approved for some medical applications was reversibly modified to make it insoluble in water but soluble in organic solvents, thus enabling payload encapsulation. Particles made from the modified polymer could then be returned to their original water-soluble state under the specific conditions that reverse the original modification, with concurrent release of their payload. We chose dextran, a polysaccharide consisting of α(1→6)-linked glucose repeat units, as the polymer because it is biocompatible, biodegradable, widely available, and easily modified. We chose arylboronic esters as the triggering groups because of their facile H_2O_2-mediated degradation at physiological pH and temperature.

Dextran was modified at the hydroxyls using imidazoyl carbamate **1** (Scheme 1). Following attachment of some boronate esters, the modified dextran could be precipitated into water. Once isolated and lyophilized, the new oxidation-sensitive dextran (Oxi-DEX) possessed only limited solubility in organic solvents, including dimethyl sulfoxide, from which it was initially precipitated. We hypothesized that this limited solubility was due to transient cross-linking between polymer strands through exchange of the boronic esters with remaining 1,2-diols on the backbone, which took place upon concentration. The addition of ~10% MeOH to break these linkages restored the solubility of Oxi-DEX in standard organic solvents such as dichloromethane, *N*,*N*-dimethylformamide, tetrahydrofuran, and acetone. Oxi-DEX is only sparingly soluble in MeOH, supporting the hypothesis that

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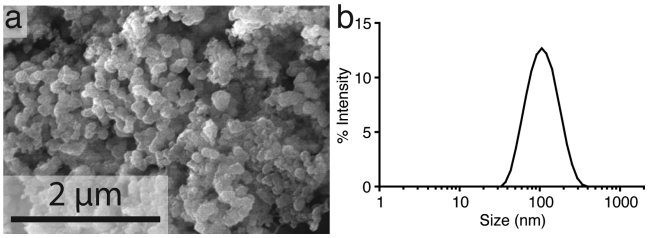


Figure 1. Characterization of particles by (a) SEM and (b) DLS confirmed that particles made from Oxi-DEX were spheroidal with sizes of ~100 nm.

the solubilization is due to the methanolysis of the boronic ester cross-links. Although this yields a mix of pinacol and dimethoxy boronic esters, the desired oxidative degradation reaction should occur in both cases.

Particles could be prepared from Oxi-DEX using standard emulsion techniques.[15] Scanning electron microscopy (SEM) micrographs revealed that these particles were roughly spherical with sizes on the order of 100−200 nm (Figure 1a). This size distribution was corroborated in bulk suspension by dynamic light scattering (DLS) measurements in water, which indicated that the particles had an average diameter of 100 nm. Chicken egg albumin (OVA) was encapsulated in Oxi-DEX particles as a model hydrophilic biomacromolecular payload. Analysis of degraded particle suspensions using fluorescamine revealed that the protein loading was 1.6 ± 0.1 wt %.

At physiologically relevant neutral aqueous H_2O_2 concentrations, the arylboronic esters are expected to be oxidized to phenols,[16] which should then undergo a quinone methide rearrangement, unmasking the hydroxyl groups of dextran. The complete degradation of Oxi-DEX should result in the release of pinacol borate, dextran, and *p*-quinone methide. The *p*-quinone methide should then be trapped by water to form *p*-hydroxymethylphenol (HMP).[17] To confirm degradation, particles were suspended in pH 7.4 phosphate buffer with or without 1 mM H_2O_2. The degradation of particles at 20 °C was observed by measuring light scattering over time (Figure 2a). The suspensions became completely transparent within 2 h in the presence of H_2O_2, while peroxide-free buffer led to only a slight decrease in scattering over 6 h due to particle settling. The degradation was found to follow first-order kinetics with a half-life of 36 ± 1 min.

To investigate how the degradation of the polymer relates to the particle dissolution, NMR spectroscopy was employed. Particles were suspended in 1 mM D_2O_2 at pD 7.4, and the appearance of dextran and other polymer degradation peaks was observed (Figure 2b and Figure S1 in the Supporting Information). Dextran slowly became soluble at roughly the same rate as HMP. The half-life of appearance of each of these molecules was roughly 4 h. On the basis of the relative quantities after degradation, Oxi-DEX contained 1.15 modifications for each anhydroglucose unit. The final quantity of pinacol boronate indicated that 55% of the pinacol boronic ester was removed from the polymer prior to particle formation. This could occur via a combination of hydrolysis on washing, competition from the diols on dextran, and methanolysis upon resolubilization in organic solvent. Interestingly, pinacol borate was released from the particles at a relatively much higher rate than the other degradation byproducts, with a half-life of ~0.5 h. This is similar to the rate of dissolution of particles, which may indicate that

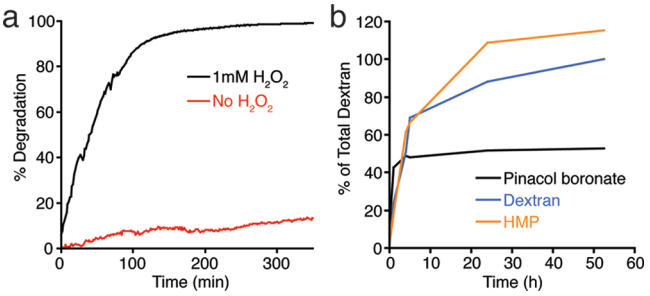
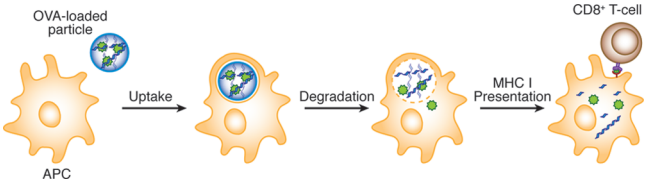


Figure 2. (a) Degradation of Oxi-DEX microparticles with and without 1 mM H_2O_2 as measured by loss of scattering at 550 nm. (b) Appearance of NMR signals from degradation byproducts of Oxi-DEX microparticles incubated in 1 mM D_2O_2.

Scheme 2. Proposed Pathway of Particle Uptake, Degradation, and Presentation



oxidation of the boronic ester is rate-limiting for the degradation of the particles. Alternatively, incomplete aromatic group removal via the quinone methide rearrangement could also be sufficient to resolublize the degrading polymer. It should be noted that no pinacol or pinacol borate was observed over this time in the absence of D_2O_2.

We have previously shown that acid-sensitive particles enhance protein-based vaccine efficacy in cancer treatment by enhancing MHC class I presentation and $CD8^+$ T-cell activation.[18] We wanted to test whether oxidation was also an effective method of increasing MHC I presentation from DCs (Scheme 2). To assess the biocompatibility of Oxi-DEX particles, we compared them to particles prepared from poly(lactic-*co*-glycolic acid) (PLGA), an FDA-approved and widely medically used polymer. In in vitro cytotoxicity assays, we found no significant difference in toxicity between the two materials in both HeLa human cervical epithelial cells and RAW 264.7 murine macrophages (Figures S2 and S3). PLGA has also been successfully used in model microparticulate vaccines.[19] In order to assess the feasibility of using Oxi-DEX-based materials for vaccine applications, OVA-loaded Oxi-DEX particles, OVA-loaded PLGA particles, or free OVA was incubated with DC 2.4 murine dendritic cells. After 6 h of incubation, Oxi-DEX particles led to robust MHC class I presentation of the OVA-derived $CD8^+$ T-cell epitope, SIINFEKL, as measured by the B3Z assay[20,21] (Figure 3). In contrast, no presentation was observed from cells incubated with free OVA at matching protein concentrations. Additionally, Oxi-DEX particles outperformed similarly loaded PLGA particles by over 27-fold. This drastic increase in presentation indicates that these particles may be promising materials for vaccines against tumors and certain viruses where MHC I presentation is crucial for the activation and proliferation of $CD8^+$ T-cells.

In conclusion, we have described the preparation of a biocompatible modified biopolymer carrier that selectively releases

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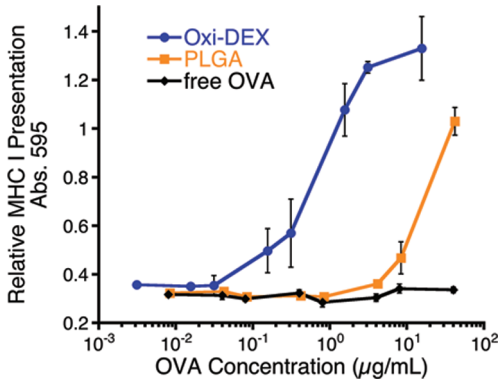

Figure 3. B3Z assay measuring MHC I antigen presentation from DC 2.4 murine dendritic cells pulsed with PLGA or Oxi-DEX particles encapsulating OVA or free OVA.

its payload in the presence of biologically relevant concentrations of H_2O_2. Given the scarcity of extracellular H_2O_2, we expect this material to have fairly selective release behavior, thus complementing the useful array of pH-sensitive or other stimuli-responsive materials. The Oxi-DEX particles also provide a significant improvement in antigen presentation over non-oxidation-degradable materials, indicating the relevance and potential importance of oxidation-selective degradation. This material may also find other worthwhile uses, as, for example, in drug-releasing stents to combat oxidative damage after ischemic events such as heart attacks and strokes.

■ ASSOCIATED CONTENT

Supporting Information. Figures S1−S3, experimental methods, general procedures, and materials. This material is available free of charge via the Internet at http://pubs.acs.org.

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