Aldehyde-Functionalized Magnetic Particles to Capture Off-Target Chemotherapeutic Agents

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ABSTRACT: Drug capture is a promising technique to prevent off-target chemotherapeutic agents from reaching systemic circulation and causing severe side effects. The current work examines the viability of using immobilized aldehydes for drug-capture applications via Schiff base formation between doxorubicin (DOX) and aldehydes. Commercially available pyridoxal-5′-phosphate (VB6) was immobilized on iron oxide nanoparticles (IONPs) to capture DOX from human serum. Leaching of VB6 persisted as a primary issue and thus various aldehydes with anchoring groups such as catechol, silatrane, and phosphonate esters have been studied. The phosphonate group-based anchor was the most stable and used for further capture studies. To improve the hydrophilic nature of the aldehydes, sulfonate-containing aldehydes and polyethylene glycols (PEGs) were investigated. Finally, the optimized functionalized iron oxide particles, PEGylated-IONP, were used to demonstrate doxorubicin capture from human serum at biologically relevant temperature (37 °C), time (30 min), and concentrations (μM). The current study sets the stage for the development of potential compact dimension capture device based on surface-anchorable polymers with aldehyde groups.

INTRODUCTION

Hepatocellular carcinoma (HCC)1 is the third leading cause of mortality from cancer globally.2 Although transplantation is the lone curative procedure for HCC, only 30% of the patients qualify.3,4 The rest of the cases have to undergo systemic5 or intra-arterial6 therapies instead. State-of-the-art treatments such as trans-arterial chemoembolization (TACE), in which the chemotherapeutic agents are administered via a catheter close to the tumor,7 have been shown to improve the survival rate of patients because of the improved dose regiment.8 However, 50−75% of the drug delivered to the cancer using these techniques still passes through the tumor, entering systemic circulation and resulting in severe side effects.9 Furthermore, for common drugs such as doxorubicin (DOX), there is a clinical limit on the cumulative dose that can be administered because of the high potential for it to cause irreversible cardiac failure at cumulative doses exceeding 360 mg.10 In order to improve doses and prevent the off-target chemotherapeutic agents from causing side effects, drug-capture materials and techniques are currently being studied.11−13 So far, DOX capture by adsorption on activated charcoal,14 electrostatic attraction to anionic polystyrene sulfonate resins,11,15 and intercalation with genomic DNA16 have been explored.

Iron oxide magnetic particles are widely used for drug delivery and are generally considered safe for human use.17 Previously, we have shown that genomic DNA-functionalized magnetic particles can be used as a drug-filtering platform. The particles were readily decorated on static rare-earth magnets and directed to desired locations using interventional radiology techniques to capture DOX in an animal model.18 Although DNA-based materials have been shown to have higher rates of capture compared to ion-exchange resins, a key concern with DNA-based materials is the possible DNA fragmentation and unknown effects of foreign DNA in the body.19,20 Therefore, development of drug filters with biologically benign materials is crucial for the success of these drug-filtering materials.

In our quest to find an alternative drug-capture material, we examined the chemical reactions that DOX participated in and the known modes of cytotoxicity of DOX. One of the modes of action that stood out was the cross-linking of amino groups of DNA bases in the presence of cellular formaldehyde and DOX by forming DNA-HN-CH2-NH-DOX linkage (Figure 1).20 Further review of the literature showed encouraging examples of imine formation in aqueous medium and their high stability. For example, imine formed from DOX-NH2 in a pH-induced
drug-release experiment showed reasonable stability at pH 7.4, only releasing less than 10% of DOX even after 96 h. In addition, several aldehydes have been reported to have a large equilibrium constant in water for Schiff base formation even with less-reactive aniline. Finally, aldehyde-containing compounds such as pyridoxal-5′-phosphate (VB6) and 5-formyl-2-furansulfonic acid have been identified to react with aminoglycoside Kanamycin A, an analogous moiety in DOX, in aqueous media to form 100% imine. Encouraged by these precedents, we considered evaluating Schiff base formation between immobilized aldehydes and DOX for drug-capture applications.

Results and Discussion

In a preliminary 1H NMR experiment (Figure S1), a mixture of VB6 and doxorubicin hydrochloride in D2O confirmed the formation of imine (1) in 5 min (Scheme 1). However, a significant amount of DOX existed as a hydrate. Because phosphate groups have been utilized for immobilizing organic compounds on inorganic supports, we decided to directly immobilize VB6 on the surface of iron oxide nanoparticles (IONPs) and use them (IONP-VB6) for drug-capture applications. The particles were prepared by treating them in a solution of VB6 under various loading and washing conditions (Table S1). The initial capture experiments were carried out in phosphate-buffered saline (PBS) solution of DOX (0.1 mg/mL) to imitate the physiological pH and ion concentration. The captured DOX was released when the samples were used for doxorubicin capture from PBS (0.1 mg/mL) experiments at ambient temperature for 30 min showed capture of 3.8 and 4.8 mg of doxorubicin per 100 mg of functionalized IONPs, respectively. However, under similar conditions, both IONP-OSi-CHO and IONP-3F4CPP in PBS in the absence of DOX showed leaching of organics, even though these materials were thoroughly washed with organic solvents and deionized water during their preparation, until no organics could be detected in the washes using UV−vis. Because silatrane and phosphonates are known to bind metal oxide surfaces via various modes, we speculated that the loosely bound phosphate and siloxane molecules came off under the present conditions. To remove these loosely bound molecules, the materials were first treated with PBS at 45 °C for 3 h and then thoroughly rinsed with PBS, water, and EtOH and dried at ambient temperature under vacuum. Afterward, the samples were used for doxorubicin capture from PBS (0.1 mg/mL) experiments at ambient temperature for 30 min resulted in 3.1 and 4.7 mg of doxorubicin captured per 100 mg of functionalized siloxane and phosphate IONPs, respectively. Because the capture capacity was significantly reduced for the siloxane-anchored aldehyde after washing, we decided to pursue further studies with the phosphate-containing aldehyde instead. The capture results of IONP-3F4CPP were

Scheme 1. Imine Formation between Doxorubicin Hydrochloride and Pyridoxal-5′-phosphate in D2O
consistent with three independent preparations. Similar capture performance was also observed with the 3-fluoro isomer. Because of the small size of the fluorine, we speculated a minimal steric effect and a beneficial electron-withdrawing inductive effect to keep the aldehyde group electron-deficient. Thus, further studies were pursued with the ortho-fluoro isomer. Furthermore, key material based on phosphonate anchors described in the manuscript have been characterized using transition electron microscopy, scanning electron microscopy (SEM) with energy-dispersive X-ray, attenuated total reflection FT-IR, and CHN analysis (Supporting Information, Sections 10, 11, 17, and 18, respectively), which show aggregation of the particles during functionalization, the presence of the carbonyl functional group (Figure S13), and increase in carbon content (0.6−2.5%; Table S2).

To deconvolute the effects of adsorption from that of imine formation on the amount of drug captured, a control batch of IONPs was treated under the same aldehyde loading conditions but in the absence of the aldehyde. The capture experiment performed with the IONP-Control at ambient temperature in PBS after 30 min resulted in 2.4 mg of doxorubicin capture, almost 50% as that of IONP-3F4CPP (Figure 2).

Table 1. DOX Capture from Human Serum at Various Concentrations in 30 min at 37 °C<sup>a</sup>

| entry | DOX conc. mg/mL | total % of DOX captured from 10 mL HS | DOX captured (mg) per 100 mg of IONP |
|-------|----------------|--------------------------------------|-------------------------------------|
| 1     | 0.05           | 24                                   | 0.12                                |
| 2     | 0.1            | 43                                   | 0.43                                |
| 3     | 0.2            | 53                                   | 1.06                                |

<sup>a</sup>Average of three experiments.

Figure 2. Capture via adsorption vs imine formation.

Furthermore, the amount captured changed as follows PBS (rt) > PBS (37 °C) > HS (rt) > HS (37 °C). These results show that the capture is directly proportional to the concentration of DOX in solution, which is in line with the Schiff base formation reaction and that binding in HS is significantly lower than that in PBS.

The performance of IONP-3F4CPP was then examined in the presence of human serum (HS) to investigate the antagonistic effects that proteins could have on the surface aldehydes, such as unwanted imine formation, or surface adsorption. We studied capture using 100 mg of IONP-3F4CPP at 37 °C in HS for 30 min at clinically relevant DOX concentration (0.05 mg/mL; Table 1 and Figure 3)<sup>29</sup> and supraclinical concentrations (0.1 and 0.2 mg/mL).<sup>11</sup> The capture amount increased as the concentration of DOX increased: 0.2 > 0.1 > 0.05 mg/mL (Table 1, entry 1−3). Furthermore, the amount captured changed as follows PBS (rt) > PBS (37 °C) > HS (rt) > HS (37 °C). These results show that the capture is directly proportional to the concentration of DOX in solution, which is in line with the Schiff base formation reaction and that binding in HS is significantly lower than that in PBS.

We hypothesized that both surface adsorption of protein and competing protein-aldehyde reactions in HS reduced the capture of DOX at low (0.05 mg/mL) concentrations. In order to improve the capture and mitigate the competing reactions with the protein amines, we envisioned that coating the surface with poly(ethylene glycol) (PEG) could improve selectivity for capture.<sup>29</sup> This approach, known as PEGylation, has been shown to improve the half-life of drug-loaded nanoparticles in blood by avoiding nonspecific protein binding and subsequent clearance by macrophages and monocytes.<sup>30</sup> Furthermore, PEGylation would also improve the wettability of the
functionalized IONPs, as the PEG macromolecules are hydrophilic. In our case, we hypothesized that the PEGs could sterically prevent proteins from interacting with the surface aldehydes, only allowing the small-molecule doxorubicin to approach the surface. As noncovalent-type PEGylation has been studied,31 thus, we started by simply exposing the IONP-3F4CPP to methoxypolyethylene glycols (mPEGs) with various molecular weights to PEGylate the surface. The resulting particles were then tested for DOX capture in HS at 37 °C for 30 min. The results showed that there was an overall increase in the amount of drug captured compared to the non-PEGylated IONP-3F4CPP. It was determined that the amount of doxorubicin captured with respect to the molecular weight of PEG used was mPEG-550 > mPEG-750 > mPEG-200 > no PEG > mPEG-2000 (Figure S12). This was attributed to the higher wettability of the functionalized IONPs and the prevention of protein adsorption. To increase the stability of PEG via covalent binding, a phosphonate-modified tetraethylene glycol (4) was coloaded (10 mol %) with the aldehyde on IONPs to obtain IONP-3F4CPP-PEG-Cl (Scheme 3), which resulted in similar capture (Figure 3) as that of the mPEG-550-exposed material. When the amount of IONP-3F4CPP-PEG-Cl was doubled (200 mg), a further decrease in the DOX concentration was observed (Figure 3).

In order to further increase the capture capacity of the particles, we decided to install both sulfonate and aldehyde moieties, and stable anchoring groups that can be used to design polymers with solubilizing spacers, aldehyde moieties, and stable anchoring groups that can be used to reduce the amount of solid support needed, which will be convenient to be used as devices in in vitro flow models as well as in animal studies. This present work can also be easily extended to any metal oxide surfaces as the anchoring groups used in this study are also commonly employed to functionalize the surface of several metal oxides. For example, biomedical devices made of stainless steel and nitinol can be readily functionalized using this technique. Moreover, the material developed could have applications beyond off-target chemotherapy in areas such as dynamic combinatorial chemistry32 and protein-33 and microorganism-capture34 applications.

**CONCLUSIONS**

In conclusion, we demonstrated that aldehyde-functionalized magnetic particles were able to capture doxorubicin via Schiff base formation, from biologically relevant concentrations and temperature from human serum. PEGylation and sulfonation of the IONPs also appeared to improve the drug capture in human serum. Such aldehyde-functionalized particles could also be extended to capture other drugs with amino groups (−NH₂ and −NHR), such as daunorubicin, epirubicin, and idarubicin. Ongoing efforts are directed toward precisely designing polymers with solubilizing spacers, aldehyde moieties, and stable anchoring groups that can be used to decrease the amount of solid support needed, which will be convenient to be used as devices in in vitro flow models as well as in animal studies. This present work can also be easily extended to any metal oxide surfaces as the anchoring groups used in this study are also commonly employed to functionalize the surface of several metal oxides. For example, biomedical devices made of stainless steel and nitinol can be readily functionalized using this technique. Moreover, the material developed could have applications beyond off-target chemotherapy in areas such as dynamic combinatorial chemistry32 and protein-33 and microorganism-capture34 applications.

**MATERIALS AND INSTRUMENTS**

Commercially available substrates were used as received. Reaction progress was monitored by thin-layer chromatography (TLC) using E. Merck silica gel 60 F254 precoated plates (0.25 mm) and visualized by UV fluorescence quenching, potassium permanganate. Silicycle SiliaFlash P60 Academic Silica gel (particle size 0.040−0.063 mm) was used for flash chromatography. ¹H and ¹³C NMR spectra were
recorded on a Varian Inova 500 spectrometer (500 and 126 MHz, respectively), a Bruker AV III HD spectrometer equipped with a Prodigy liquid nitrogen temperature cryoprobe (400 and 101 MHz, respectively), or a Varian Mercury 300 spectrometer (300 and 75 MHz, respectively) and are reported in terms of chemical shift relative to residual CHCl₃ (7.26 ppm). Data for ¹H NMR spectra are reported as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration). High-resolution mass spectra (HRMS) were acquired from the Caltech Mass Spectral Facility using fast-atom bombardment (FAB+), electrospray ionization (TOF ES+), or electron impact (EI +). Fluorescence measurements were performed using a 96-well plate on a Molecular Devices FlexStation 3 Multimode microplate reader. SEM and EDS measurements were performed on a Zeiss 1550VP field emission SEM equipped with an Oxford EDS module. Transmission electron microscopy (TEM) measurements were performed on an FEI TF30ST transmission electron microscope at Caltech TEM facility. Infrared measurements were performed on a Nicolet iS50 Fourier transform infrared spectrometer equipped with a DuraScope ATR unit. C, H, and N analyses were carried out using a PerkinElmer 2400 Series II CHN elemental analyzer. Unless otherwise stated, reactions were carried out on the bench. Fe₃O₄ (30 nm APS, 99%) was purchased from Nanostructured & Amorphous Materials, Inc. Human serum was obtained from Sigma-Aldrich (Hypo-Opticlear Human Sera). DOX was purchased from LC Labs. All reagents not otherwise mentioned were purchased from Sigma Aldrich and Sera).

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support from the NIH (R01CA194533). The authors also would like to thank Prof. Steven Hetts and Prof. Anand Patel for their valuable feedback throughout the project, Dr. Daryl Yee, Dr. William Wolf, and Dr. Jeong Hoon Ko for proofreading the manuscript, and Dr. Christopher Marotta for proof reading and helping with TEM experiments. The authors also acknowledge the support from the Beckman Institute of the California Institute of Technology for the use of the Molecular Materials Research Center.

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