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# Chemoselective Ligation in the Functionalization of Polysaccharide-Based Particles

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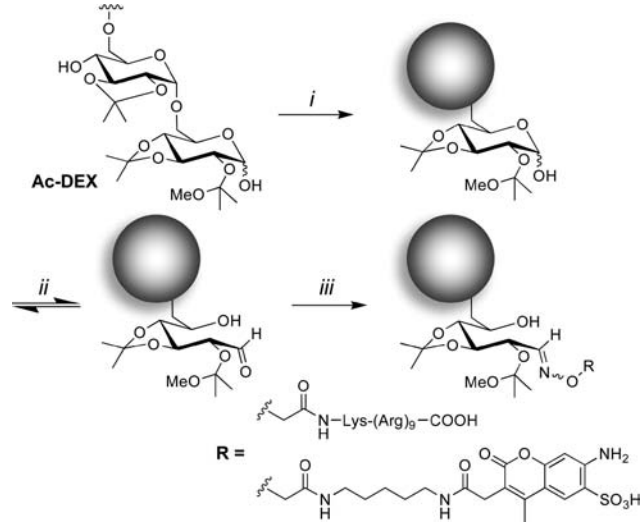
The ability to readily functionalize the surface of colloidal inorganic materials has been essential in the advancement of bionanotechnology.[1] Similar to their inorganic counterparts, polymeric nano- and microparticles have been extensively investigated as vehicles for biomedical applications including gene delivery,[2] vaccines,[3] and chemotherapy.[4] However, there exist few, if any, simple, versatile, and chemoselective strategies for the functionalization of synthetic polymer-based particulate drug delivery vehicles. The *in vivo* behavior and, ultimately, the efficacy of these carriers would almost certainly be enhanced through the functionalization of their surfaces with biologically relevant moieties. Particles made from the polyester poly(lactic-*co*-glycolic acid) (PLGA), a widely studied biodegradable polymer, have been functionalized with agents such as antibodies[5] and aptamers.[6] However, these typical examples utilize functionalization routes based on the synthesis of complicated copolymer conjugates or nonspecific bioconjugation reactions, which may inadvertently modify the encapsulated cargo or functionalizing ligand and consequently alter their activity. Herein we demonstrate a one-step, chemoselective method for the surface modification of polysaccharide-based particles.

We recently developed a biocompatible and pH-sensitive polymer based on acetal-modified dextran (Ac-DEX, Scheme 1), which may offer advantages over polyester-based materials such as PLGA.[7] Due to their pH sensitivity, Ac-DEX particles can selectively and rapidly release their encapsulated payload under mildly acidic conditions including those found in sites of inflammation, tumor tissue, or endocytic vesicles. In contrast to particles made from PLGA, Ac-DEX particles degrade to yield neutral (as opposed to acidic) byproducts, and their rate of degradation can be easily tuned within the time scale of relevant cellular processes. The latter feature has been found to significantly affect the biological activity of the particles, for example, in the case of vaccine formulations.[8]

Given the potential for precisely targeted or otherwise functionalized polymeric nanoparticles, we sought to develop a simple, mild, and selective ligation strategy for the surface modification of Ac-DEX-based delivery vehicles (Scheme 1). Based on prior examples of oxime formation with complex polysaccharides[9] and other small molecule examples,[10] we hypothesized that carbohydrate reducing chain ends present at the surface of the particles could be exploited to form stable oxime conjugates with alkoxyamine-bearing molecules.[11] To test this hypothesis, submicrometer-sized Ac-DEX particles were prepared and treated with aqueous solutions of either an alkoxyamine-functionalized fluorophore or an NHS-ester-functionalized version of the fluorophore to control for the possibility of nonspecific dye adsorption.[12] As shown in Figure 1, use of the

***Scheme 1.*** General Method for the Functionalization of Ac-DEX Particles with Alkoxyamine Reagents[a]




[a] (*i*) Preparation of micro- or nanoparticles via an emulsion/solvent-evaporation procedure. (*ii*) Reducing end equilibrium between hemiacetal and straight-chain forms. (*iii*) Reaction of reducing end aldehyde with alkoxyamine reagents ($R-ONH_2$) to form stable oxime conjugates.

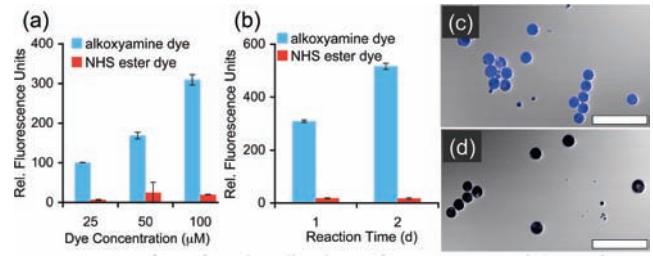



***Figure 1.*** Surface functionalization of Ac-DEX particles using a fluorophore. Particles were treated with an alkoxyamine or control dye at (a) various concentrations for 1 day or for (b) various times keeping the dye concentration constant (100 $\mu$M). Confocal microscopy was used to visualize particles treated with an alkoxyamine (c) or NHS ester control dye (d). Scale bars = 50 $\mu$m.

alkoxyamine-functionalized dye led to both concentration and time dependent labeling of the particles with essentially no fluorescence observed from particles treated with the NHS ester dye, suggesting minimal nonspecific dye adsorption. These bulk measurements were visually confirmed by preparing larger particles which were labeled and examined using confocal microscopy (Figure 1c–d). Importantly, we did not observe any particle degradation or aggregation as a result of these or any subsequent particle functionalization attempts (see Supporting Information).

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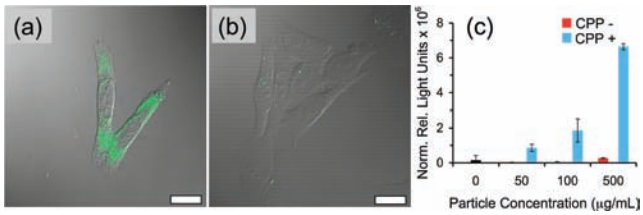


***Figure 2.*** Representative confocal microscopy images of HeLa cells incubated with particles encapsulating FITC-BSA and treated with (a) an alkoxyamine-functionalized CPP or (b) an unfunctionalized CPP. Scale bars = 20 $\mu$m. (c) CPP-modified Ac-DEX particles encapsulating luciferase-encoding plasmid DNA transfect nonphagocytic cells *in vitro*. Relative light unit output was normalized to total protein content in each sample.

We next investigated the possibility of adding therapeutically relevant functionality to the surface of our particles. Although polymeric particles are readily taken up by phagocytic cells of the immune system, uptake and delivery of therapeutic agents to nonphagocytic cells remain challenging. We therefore attempted to functionalize particles with cell-penetrating peptides (CPPs), a class of cationic peptides known to facilitate delivery of cargo across cell membranes.[13] Ac-DEX particles were incubated with solutions of an alkoxyamine-terminated poly(arginine) CPP sequence (K-$R_9$), an unmodified amine-terminated version of the CPP, or buffer only.[14] Particles treated with the alkoxyamine CPP were found to have a higher zeta potential (see Table S1) compared to control samples. Additionally, the CPP loading was measured using fluorescamine, an amine-reactive probe capable of reacting with a lysine residue present in the CPP sequence (Scheme 1),[15] which led us to estimate that there were several thousand CPPs per particle. Based on these data, CPP-modified particles were evaluated *in vitro* for their ability to be taken up by nonphagocytic cells. Ac-DEX particles encapsulating fluorescently labeled protein were treated with solutions of the alkoxyamine-functionalized CPP or control solutions, washed, and then incubated with HeLa cells for 12 h. As shown in Figures 2a−b and S5, fluorescence was only observed for cells incubated with the alkoxyamine-CPP treated particles.

To assess the feasibility of using functionalized particles for the delivery of biologically relevant payloads, we performed proof-of-concept *in vitro* transfection experiments with plasmid-loaded Ac-DEX particles. Blending PLGA with poly($\beta$-amino ester) polymers has been shown to enhance transfection efficiency in phagocytic cell lines, presumably due to a proton-sponge effect.[16] We hypothesized that functionalizing similarly prepared Ac-DEX particles with CPPs would enhance the uptake of these particles by nonphagocytic cells and lead to efficient transfection. Ac-DEX particles blended with 20% (w/w) poly($\beta$-amino ester) polymer (Figure S1), and encapsulating plasmid DNA encoding firefly luciferase as a reporter, were prepared and functionalized with CPPs as described above.[12a] After a 2 day incubation, HeLa cells treated with CPP-modified Ac-DEX particles demonstrated up to a 60-fold increase in expression of the luciferase reporter compared to cells incubated with unmodified particles (Figure 2c), which strongly suggests efficient particle uptake and delivery of the encapsulated DNA.

In conclusion, we have demonstrated a one-step, chemoselective method for the functionalization of polysaccharide-based particles using alkoxyamine reagents. We anticipate that the mild reaction conditions and functional group tolerance of this ligation strategy will enable a variety of materials to be similarly modified with any number of complex bioactive molecules. In addition, this concept could readily be generalized to other biocompatible polymeric particles used commonly in delivery systems through the introduction of functional groups that enable oxime ligation.

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**Supporting Information Available:** Synthetic and experimental procedures, full particle characterization and stability studies, Figures S1−S5. This material is available free of charge via the Internet at http://pubs.acs.org.

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