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Spirocyclic acetal-modified dextran as a flexible pH-sensitive solubility switching material

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ABSTRACT

Bioresponsive polymers can enable the development of more effective drug delivery vehicles and medical materials. Acetal-modified polysaccharides allow pH-triggered solubility switching in a versatile and effective manner, but prior work has been limited to a combination of acyclic methoxyisopropyl (MIP) and cyclic isopropylidene acetals. We describe here the preparation and characterization of Spirocyclic Acetal-modified Dextran (SpAc-Dex), which comprises dextran decorated with cyclopentyl, cyclohexyl, or cycloheptyl acetals (SpAc5-, SpAc6-, and SpAc7-Dex respectively). A library of materials with varying acyclic and cyclic acetal content was synthesized, and organic-soluble materials were formed into microparticles and assessed for degradability and cytocompatibility. At high levels of modification SpAc5-Dex degraded most quickly and SpAc7-Dex degraded most slowly. SpAc6-Dex features lower degrees of substitution but spans a wide range of degradability. These materials were found to be non-cytotoxic, and may find future use in biomedical applications.

KEYWORDS dextran bioresponsive biocompatible degradable acetal

Introduction

As medical treatments become increasingly precise and personalized, demand has grown for technologies that enable control over the timing and location of therapeutic action.[1] Biocompatible polymeric materials have proven to be versatile building materials for these tools, allowing the production of stents, and cell scaffolds, and slow release drug depots.[2–5] The use of polymers can improve the efficiency of drug delivery, reduce dose-limiting toxicity, and make the administration of drugs more effective.[6,7] To be ideal for these applications, these polymers must meet certain standards: first, the material must be inherently non-toxic; second, it must have a clear path for degradation to prevent deleterious bioaccumulation; third, it must be able to target or otherwise accumulate in a tissue of interest; and fourth, it must be able to release a drug in a controlled manner over a specific duration.[5,8,9] Finally, it is ideal if payload release should be controllable by external environmental cues such as temperature, pH, oxidation, or the presence of enzymatic activity.[10,11]

Because of the great promise they hold for improving the state of biomedical technology, a vast array of biomedical polymers has been developed and evaluated. Polyesters, polyorthoesters, and polyanhydrides account for the largest share of these polymers owing to superior processing properties, good biocompatibility, and passive hydrolysis under biologically accessible conditions.[12] These polymers have been engineered to encapsulate and control the release a variety of hydrophilic and hydrophobic compounds.[13] Responsive elements have also been incorporated into many of these passively degrading materials, often leading to cleavage along the backbone of the polymer, which results in small molecule byproducts after complete degradation.[14] Although ideal in many cases, degradation along the backbone of a polymer generates a heterogenous

mixture of smaller polymers, oligomers, and small molecule byproducts, which can complicate engineering of drug release.

Degradation that leads to a switch in polymer solubility is an alternative strategy that avoids some of these issues. In this strategy, a water-soluble polymer is chosen as a backbone and is modified with degradable hydrophobic modifiers. Degradation of these modifying groups regenerates the original backbone and restores aqueous solubility. This strategy has proven to be especially effective using the biopolymer dextran.[15] Dextran is a biocompatible and non-toxic biologically derived α-glucan polymer that is easily modified due to the abundance of equatorial hydroxyl groups and the mostly linear nature of the polymer.[16] Dextrans saw early use in synthetic blood substitutes,[16] and are already widely used in the medical industry,[17] making it an affordable and an easily accessible polymer to modify. Acetal-modified dextran (Ac-Dex) in particular has shown promise in the area of drug delivery, and especially in immunotherapy settings.[18] Ac-Dex is generated through reaction of dextran with 2-methoxypropene (2MP). The resulting mixture of cyclic isopropylidene and acyclic methoxy isopropyl (MIP) acetal modifications determines the properties of the product polymer.[19,20] Because acetal hydrolysis is acid-catalyzed, acetal-modified polymers revert to unmodified polymers under slightly acidic conditions.[21] Decreased pH is associated with intracellular endosomes, tumor microenvironment, immunesurveillance, and tissue damage, making acetals attractive groups for triggered degradation in response to these biological environments.[21–24]

One of the major benefits of Ac-Dex is its facile tunability. Its properties are dependent on the degree of modification with MIP and acetonide acetals, which changes over time during the preparation process. Initially acyclic acetals form through the acid catalyzed addition of an enol ether to a hydroxyl on the polymer backbone. These acyclic acetals can continue reacting with the

polyhydroxylated polymer to form cyclic acetals through acid catalyzed transacetalation (Figure S1). Depending on the duration of reaction between dextran and 2MP, the acetal content of the resulting Ac-Dex can vary widely.[18] Because cyclic acetals are inherently slower to hydrolyze than analogous acyclic acetals, the degradation rate of Ac-Dex can be controlled through reaction time.[19,21] Hydrolysis of an acyclic MIP acetal releases one molecule of acetone, and one molecule of methanol. In contrast, hydrolysis of a cyclic isopropylidene acetal releases only a single molecule of acetone. Complete hydrolysis of all acetals unveils the unmodified water-soluble dextran molecule.[18]

Although Ac-Dex is easily prepared with a wide range of pH-triggered degradation rates, not all Ac-Dex materials have uniformly favorable properties. When it has a low overall degree of substitution (DS) and a high proportion of acyclic acetals, Ac-Dex degrades quickly at reduced pH, but also has significant degradation at neutral pH. These quickly degrading Ac-Dex materials are also often less soluble in organic solvents, and therefore difficult to solution process. Consequently, there is a tradeoff in preparing materials with optimal triggered degradation and ideal processability. Additionally, all Ac-Dex materials possess high acetal coverage (~70% of hydroxyls covered),[18] and so degradation entails the release of potentially undesirable quantities of acetone and methanol.

To address the limitations presented by this tradeoff, the acetal group can be examined to understand its effect on the polymer's properties. It has been shown that cycloalkylidene acetals possess a range of degradation rates bracketing those of isopropylidene acetals.[25,26] Cyclopentylidene and cycloheptylidene acetals generally degrade more rapidly than isopropylidene acetals, and cyclohexylidene acetals are expected to be much slower to degrade. We hypothesized that we might access polymers with a broad range of degradation kinetics and

materials properties through use of cyclic enol ethers in place of 2MP during the modification of dextran. We additionally hypothesized that the increased size of the modifying groups would lead to solubility switching at lower degrees of hydroxyl modification.

Materials and Methods

Materials: Chemicals were purchased from Sigma Aldrich, and Acros Organics. NIH3T3 cells were purchased from ATCC and biological reagents were purchased from Fischer Scientific. Nuclear magnetic resonance (NMR) experiments were performed on a Bruker Avance 400 Hz spectrometer. Infrared Spectroscopy was performed on Bruker Alpha FTIR-ATR spectrometer. Dynamic Light Scattering was performed on Malvern Zetasizer ZS. Absorbance and fluorescence measurements were made using a Molecular Devices SpectraMax M5 multimode plate reader. Unless otherwise stated, all reactions were carried out under an inert atmosphere of argon.

Synthesis of enol ethers (**1-3**)*:* Enol ethers were each prepared using an adapted method from Gasman et al.[27] Generally, each ketone (100 mmol) was combined with trimethyl orthoformate (13.1 mL, 120 mmol) in a flask on ice. After cooling, p-toluene sulfonic acid (0.952, 5 mmol) was quickly added and the reaction was allowed to stir overnight. Fractional distillation (180 mm, glass helices) simultaneously promoted elimination of the dimethyl acetal intermediate and allowed purification of the enol ether product. Methanol and excess trimethylorthoformate were distilled off before collecting enol ethers at elevated temperatures.

1-methoxycyclopentene (**1**)*:* Collected at 125 °C at atmospheric pressure (1.8 g, 18%, 70% purity). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.47 (s, 1H), 3.62 (s, 3H), 2.40 – 2.29 (m, 4H), 1.90 (p, J = 7.5 Hz, 2H). (Figure S2).

1-methoxycyclohexene (**2**)*:* Collected at 142 °C at atmospheric pressure (6.3 g, 56%, 57% purity). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.63 (t, 1H), 3.52 (s, 3H), 2.06 (t, J = 3.6 Hz, 4H), 1.79 – 1.47 (m, 4H). (Figure S3).

1-methoxycycloheptene (**3**)*:* Collected at 66 °C and 25 torr (5.8, 46%, 93% purity). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.73 (t, J = 6.7 Hz, 1H), 3.46 (s, 3H), 2.28 (m, 2H), 2.08 (m, 2H), 1.72 (m, 2H), 1.60 – 1.48 (m, 4H). (Figure S4).

Synthesis of Spirocyclic-Acetalated Dextran (SpAc-Dex) (**4-6**). Dextran (1.00 g, 10 KDa, 6.17 mmol of AGU) was dissolved in DMSO (10 mL) over the course of 15 min at rt. The desired enol ether (37.0 mmol), was added to a dry reaction vial and the dissolved dextran was added to the vial. Acid catalyst (0.002 mmol) was added in a single portion to initiate the reaction. At selected intervals (0.25, 1, 4 and 24 hr) roughly even-sized aliquots of the solution were removed and the material was precipitated into water (35 mL, adjusted to pH 8 with NEt_3). The material was isolated via centrifugation at 15,000 x g for 15 minutes; the supernatant was discarded and the pellet was resuspended in pH 8 water. The sample was centrifuged in this manner twice more, and the resulting pellet was lyophilized overnight to yield a white powder. Each SpAc-Dex sample was characterized with IR and ^{1}H NMR (Figures S5-S10).

SpAc5 (**4**): Pyridinium p-toluene sulfonate (PPTS) was used as an acid catalyst.

SpAc6 (**5**) Camphorsulfonic acid (CSA) was used as an acid catalyst.

SpAc7 (**6**) PPTS was used as an acid catalyst.

Analysis of polymer composition by degradation. Particles were added to a clean NMR tube and suspended in D_2O. DCl (37% in D_2O, 1 drop) was added and the suspension was allowed to react at rt until fully dissolved. The resulting products were analyzed via ^1H NMR to determine the composition of the polymer:

$$DS_{100}\ acyclic = \frac{[MeOH]}{[Dextran]}$$

$$DS_{100}\ cyclic = \frac{[cyclic\ ketone]}{[Dextran]} - DS_{100}\ acyclic$$

Relative concentrations were measured using integrations normalized to an internal standard, 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS), and then divided by proton count. (Figure S11-S13).

Single Emulsion Particles. Microparticles were formed using a protocol adapted from Bilati et al.[28] (Figure S14) A sample of polymer (50 mg) was dissolved in DCM (1 mL) and added to an aqueous solution of poly(vinyl alcohol) (PVA, MW = 13,000 – 23,000 g/mol, 87-89% hydrolyzed) (2 mL, 3% w/w in PBS), then emulsified by sonication (Branson Sonifier 550) for 30 seconds at 40% power alternating between 0.7 s on and 0.3 s off. The resulting solution was poured into a lower concentration PVA solution (10 mL, 0.3 % w/w in PBS) and let stir for 4 hours to remove any remaining organic solvent. The particles were transferred into 50 mL centrifuge tube and spun down at 10,000 x g for 10 minutes. Particles were washed by resuspending in basic water using a

benchtop vortexer, then centrifuging using the same settings. After washing twice, the pellet was lyophilized, yielding a white powder (~30 mg, ~60%).

SEM Imaging SpAc-Dex. Particles were suspended in basic water (adjusted to pH 8 with NEt_3, 0.25 mL, 0.5 mg/mL). A drop of this suspension was placed on a silicon wafer and allowed to dry for 15 minutes before excess moisture was removed using a kimwipe. Particles were sputter coated with 5 nm of Au and imaged at 10 kV in secondary electron mode. Microparticle sizes were measured manually using the FIJI distribution of ImageJ,[29,30] averaging at least 100 individual particles.

Bulk Microparticle Degradation. Particles were suspended at a concentration of 2 mg/mL in either 0.3 M acetate buffer (pH 5) or PBS (pH 7.4) and incubated at 37°C under agitation using shaking incubator (Eppendorf Thermomixer R, 1400 rpm). Aliquots (120 μL) were taken at various time points and centrifuged (11,900 x g, 10 min) and supernatant was removed and stored at 4°C for later analysis.

Quantification of particle degradation via BCA Assay. Soluble carbohydrate was quantified through reducing end activity using a bicinchoninic acid (BCA) assay (Pierce) according to manufacturer protocols. Briefly, samples (50 μL) were diluted with PBS (0.3 M 100 μL) in a 96-well plate, then the BCA reagent solution (150 μL) was added. Absorbance was measured at 562 nm after 30 min at room temperature.

In situ monitoring of particle degradation by NMR. Approximately 3 mg of selected polymer was added to a clean NMR tube containing acetate-d3 buffer (Cambridge isotopes, 10 mM in D_2O, pD 5). Spectra were collected every 10 minutes at a temperature of 37°C.

DOSY experiments. DOSY NMR was performed on SpAc-Dex polymers dissolved in DMSO-d6. Diffusion constants were calculated and 2D plots were generated using TopSpin Dynamic Center (Bruker, US).

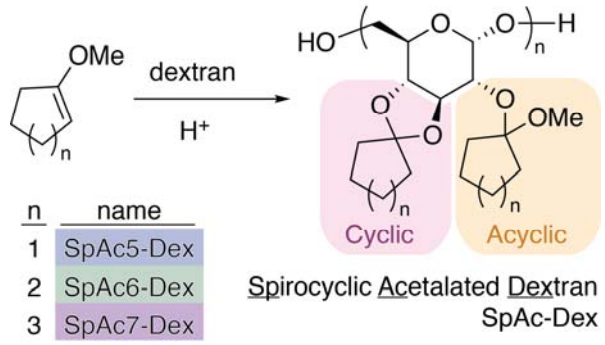
Cell culture. Cells were cultured according to ATCC guidelines: NIH3T3 cells were cultured at 37 °C under an atmosphere containing 5% CO_2. Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM, ATCC) supplemented with 10% Bovine Calf Serum (ATCC) and subcultured using TrypLE Express (Thermo Fisher) every 2 days to maintain below 80% confluence.

alamarBlue Assay. Cell viability was measured according to manufacturer's protocols. Briefly, cells were added to a 96 well plate at a concentration of 6,000 cells/100 µL and allowed to adhere for 1 h. Particle suspensions were prepared with concentrations of 1000, 500, 250, 125, 62.5, 31.3, 15.6 µg/mL in media. 100 µL of the microparticle suspension was added to the cells and allowed to sit for 10 h (SpAc5 and SpAc6) and 20 h (SpAc7). Media was removed and 100 µL of new media and 10 µL of alamarBlue (Thermo Fisher) were added to the cells. Plates were read for fluorescence (λ_{ex} 560 nm, λ_{em} 590 nm) after 18 hours.

Results and discussion

Acetal-modified dextrans are generally prepared through acid-catalyzed addition of enol ethers. Although they are not commercially available, cyclic enol ethers were synthesized from cyclic ketones according to literature precedent.[27,31] Three enol ethers, methoxycyclopentene, methoxycyclohexene and methoxycycloheptene were reacted with dextran from *Leuconostoc mesenteroides* in the presence of an acid catalyst (Scheme 1). The resulting product polymer was precipitated into basic water from DMSO indicating significant modification of backbone hydroxyl groups. Although methoxycycloheptene and methoxycyclopentene reacted quickly in the presence of PPTS, methoxycyclohexene reacted slowly under the same conditions. This could be overcome by use of a stronger acid, CSA, in place of PPTS to promote robust reaction. The cyclic enol ethers contained dimethoxycycloalkane impurities as synthesized; control experiments were performed to exclude their role in acetal modification, and no reaction was observed in the absence of enol ethers. To differentiate them from other acetal-modified dextrans, we named the product polymers spirocyclic acetalated dextran, or SpAc-Dex, with SpAc5, SpAc6, and SpAc7 representing the products of 5-, 6-, and 7-membered enol ethers, respectively. Although direct characterization by NMR proved difficult due to broad overlapping resonances, clear evidence of both cyclic and acyclic acetals could be acquired through analysis of hydrolysis byproducts. Polymers were suspended in D_2O, and hydrolysis through addition of catalytic DCl yielded dextran, methanol, and a cyclic ketone (Figure S12-S14). Since acyclic acetals release both ketones and methanol upon hydrolysis, while cyclic acetals release only the ketone component, the molar ratio of these degradation products could be compared to determine the amount of acyclic and cyclic acetal coverage.

Scheme 1. Preparation of SpAc-Dex.



To characterize the effect of varying acetal composition, a library of SpAc-Dex materials was prepared by aliquoting samples throughout the reaction at various time points, yielding 14 characterizable samples. Each sample was analyzed by NMR for degree of substitution (DS_{100}, modifications per 100 anhydroglucose units) of cyclic and acyclic acetals. As the reaction progresses, acyclic acetals are formed before being converted into cyclic acetals (Figure 1). Although each reaction appears to follow a similar overall trajectory, there are significant differences between the different SpAc-Dex materials.

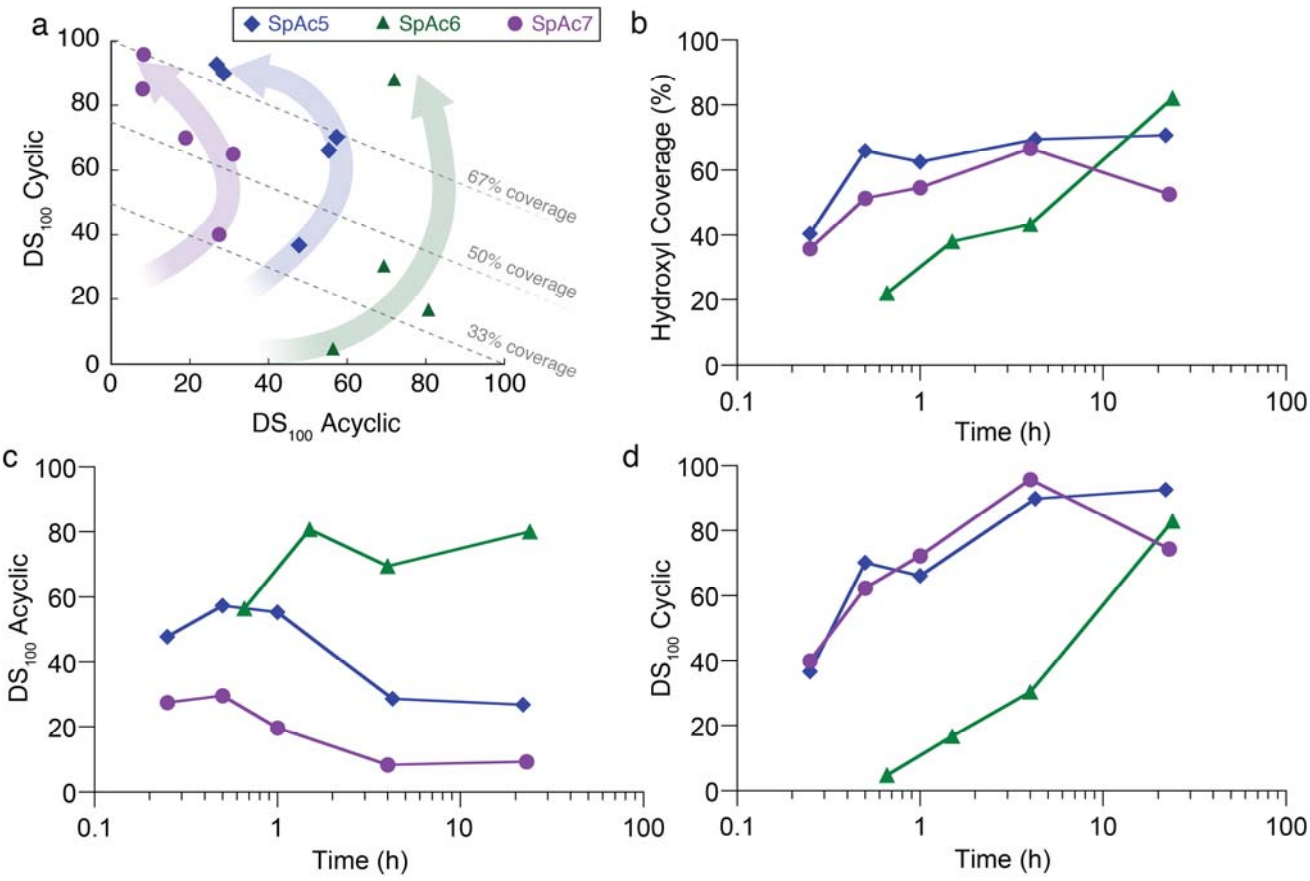


Figure 1. Composition of SpAc-Dex samples changes over the course of an acetalation reaction. a) Composition of SpAc-Dex samples collected, where arrows indicate approximate reaction trajectory. b) Percentage of hydroxyls modified as acetals generally increase over time. c) Acyclic acetal subsitution generally decreases after an early maximal coverage. d) Cyclic acetal substitution increases over time.

As their preparation proceeds, SpAc5-Dex and SpAc7-Dex become water-insoluble when 35-40% of hydroxyls are modified. At these levels of coverage, 40% of AGUs possess cyclic acetals for both materials. As the reaction proceeds, acyclic acetals convert into cyclic acetals, yielding nearly complete cyclic acetal substitution within 6 h. Overall coverage decreases between 6h and 24 for SpAc7-Dex, which is attributed to either partial hydrolysis by adventitious water or

methanolysis by methanol generated from acyclic-cyclic transacetalation. In contrast to SpAc5- and SpAc7-Dex, SpAc6-Dex becomes water-insoluble at 20% hydroxyl coverage, and possesses high acyclic acetal coverage throughout the reaction. Cyclic acetal content increases over the life of the reaction, but without concomitant loss of acyclic acetals. Like cyclic acetal content, hydroxyl group coverage reaches its maximal value within the first hour for SpAc5- and SpAc7-Dex, but continually increases over 24 h for SpAc6-Dex. Overall, all three SpAc-Dex material types produce a range of cyclic and acyclic substitution distributions; SpAc5- and SpAc7-Dex react more quickly, have low degrees of acyclic substitution, and generally possess a narrower range of DS_{100} values; in contrast, SpAc6-Dex reacts relatively slowly, maintains higher acyclic acetal coverage throughout the reaction, and produces materials that cover a wider range of substitutions.

Characterization of polymer through degradation product analysis relies on the absence of small molecule impurities that might come from incomplete purification or premature degradation. To confirm that methanol and cyclic ketones are absent from the undegraded polymers, Diffusion Ordered Spectroscopy (DOSY) NMR was performed (Figure 2a and S15-S17).[32,33] In contrast to gel permeation chromatography (GPC), this technique allows the measurement of diffusion constant for each individual resonance in an NMR spectrum.[34] Diffusion rates for resonances corresponding to acetals and to dextran are comparable, indicating that they are attached, and not present as a mixture. The only other compounds observed in the sample were H_2O and DMSO present in the solvent. To confirm the absence of degradation byproducts from the sample, a "co-spot" experiment was performed: methanol and cyclic ketone were added and DOSY was reacquired. As expected, new resonances were observed with faster diffusion than the polymer (Figure 2b).

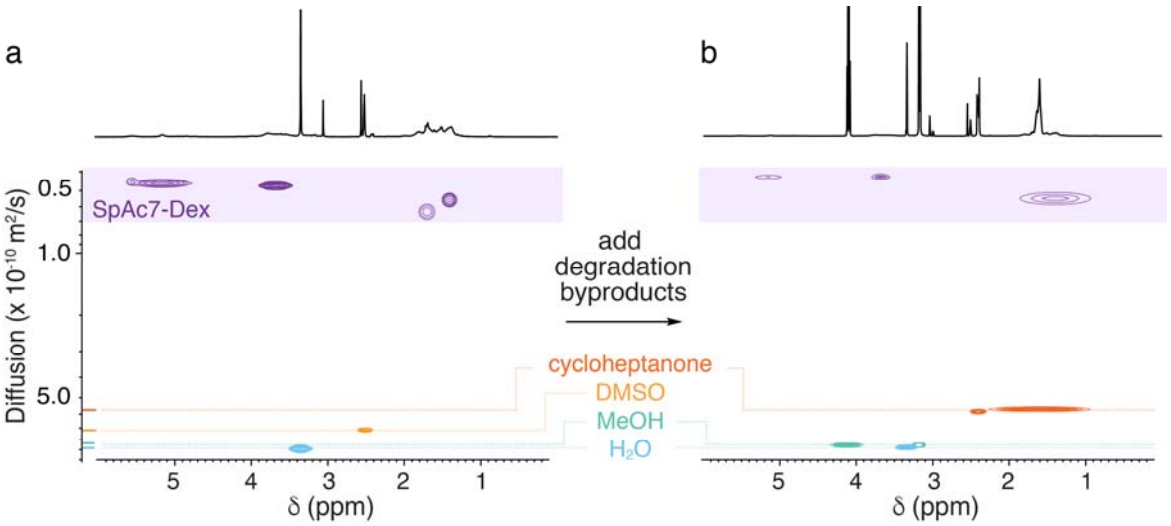


Figure 2. Diffusion ordered spectroscopy confirms absence of degradation byproducts in SpAc-Dex polymer samples. a) SpAc7-Dex (DS_{100} 30 acyclic, 62 cyclic) has consistent diffusivity across all resonances. Small molecules at 2.5 ppm and 3.3 ppm are assigned as DMSO and H_2O, respectively.[35] b) Adding of MeOH and cycloheptanone introduces new resonances with distinct diffusivities.

The solubility switching mechanism for payload encapsulation and release requires that candidate polymers be both water-insoluble and highly organic soluble. SpAc-Dex polymers were screened for solubility (Table S1). In addition to DMSO, all SpAc-dex samples with greater than 40% coverage are soluble in DCM and chloroform. SpAc7-dex is insoluble in acetone and ethyl acetate, while SpAc5 and SpAc6 with high hydroxyl coverage are relatively more soluble in these solvents. Encouraged by the favorable solubility properties of SpAc-Dex materials, material processing was evaluated. Microparticles were synthesized using a standard single emulsion solvent displacement technique using DCM as the dispersed phase and polyvinyl alcohol as a surfactant.[28] Particles were analyzed by scanning electron microscopy (SEM). SEM micrographs clearly show discrete spherical microparticles with sizes of 220 ± 120 nm, indicative of favorable

solution processability (Figure 2a). Bulk material characterization by dynamic light scattering revealed that microparticles fall into a single size population with an average size of 321 ± 92 nm (Figure 2b and Table S2).

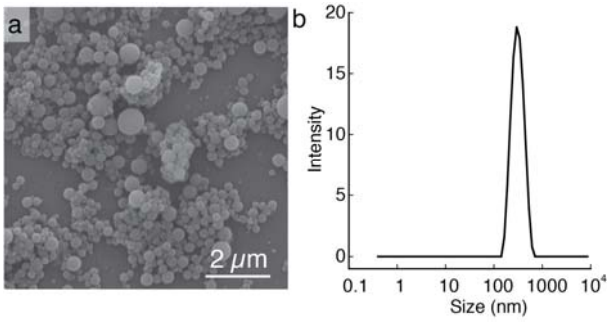


Figure 3. Microparticle characterization. a) Scanning electron micrographs of SpAc6 DS 69% acyclic and 30% cyclic show well dispersed spherical microparticles. b) Dynamic light scattering data indicates single population of relatively narrow dispersity of microparticles

The pH-triggered degradation of SpAc-Dex microparticles was next characterized in aqueous suspension. Because they are inherently heterogenous, the rate of their degradation is not only dependent on the lability of pendant acetals, but is also dependent on diffusion of water into the microparticle, and diffusion of byproducts out of the polymer matrix. The degradation of microparticles was analyzed by monitoring the release of soluble reducing sugars (Figure 4, S18-S20). Microparticles were suspended in pH 5 or pH 7.4 buffer at 37 °C, and aliquots were taken over the course of 280 h. Undegraded microparticles were removed from aliquots by centrifugation, and the supernatant was assayed using a bicinchoninic acid (BCA) copper reduction assay. All SpAc-Dex samples were stable for > 200 h at pH 7, but had widely varying rates of degradation at pH 5. SpAc5-Dex samples with low cyclic DS_{100} degraded the fastest with half-lives of 22.2 ± 4 h for SpAc5 (DS_{100} 55 acyclic, 66 cyclic) and 9.8 ± 2.4 h for SpAc5 (DS_{100} 57 acyclic, 70 cyclic). In line with published rates of degradation,[19] Ac-Dex microparticles with

higher levels of substitution (DS_{100} 76 acyclic, 75 cyclic) were found to degrade significantly more quickly (Figure S21). SpAc6 had similar degradation rates at 17.3 ± 1.3 h for SpAc6 (DS_{100} 69 acyclic, 30 cyclic), its lowest acetal coverage sample. SpAc7 was slow to degrade with its quickest half-life being 40.8 ± 6.8 h. Although SpAc7-Dex microparticles might be expected to degrade at a rate similar to SpAc5-Dex microparticles based on similar rates of removal in the context of organic protecting groups, it was instead very slow to degrade.[25,36] This may explained by observations that carbohydrates can strongly influence the degradability of cyclic acetals by influencing changes in bond angle in the transition state.[36] Another possible explanation for this difference could be the influence of hydrophobicity on overall degradation:[37] decreased water concentration increases the likelihood that the oxocarbenium hydrolysis intermediate is recaptured by dextran, thus slowing the overall reaction. The importance of this reversibility has been well described for acetals of trans-1,2-cyclohexane diol.[38]

We compared the half-life of degradation of each characterized material with its cyclic and acyclic composition. Although the rate of degradation varies significantly between SpAc5- and SpAc7-Dex, both appear to be controlled solely by cyclic acetal content. Increasing DS_{100} of cyclic acetals correlates with slower degradation at pH 5. Conversely, decreasing DS_{100} of acyclic acetals correlates with slow degradation, indicating that the hydrolysis of acyclic acetals does not limit the rate of hydrolysis. In contrast, increased DS of both cyclic and acyclic acetals on SpAc6-Dex microparticle correlates with slowed degradation. This may indicate the rate of hydrolysis of methoxycyclohexyl acetals is sufficiently slow to have an impact on particle degradation, while methoxycyclopentyl and methoxycycloheptyl acetals hydrolyze quickly relative to their cyclic counterparts.

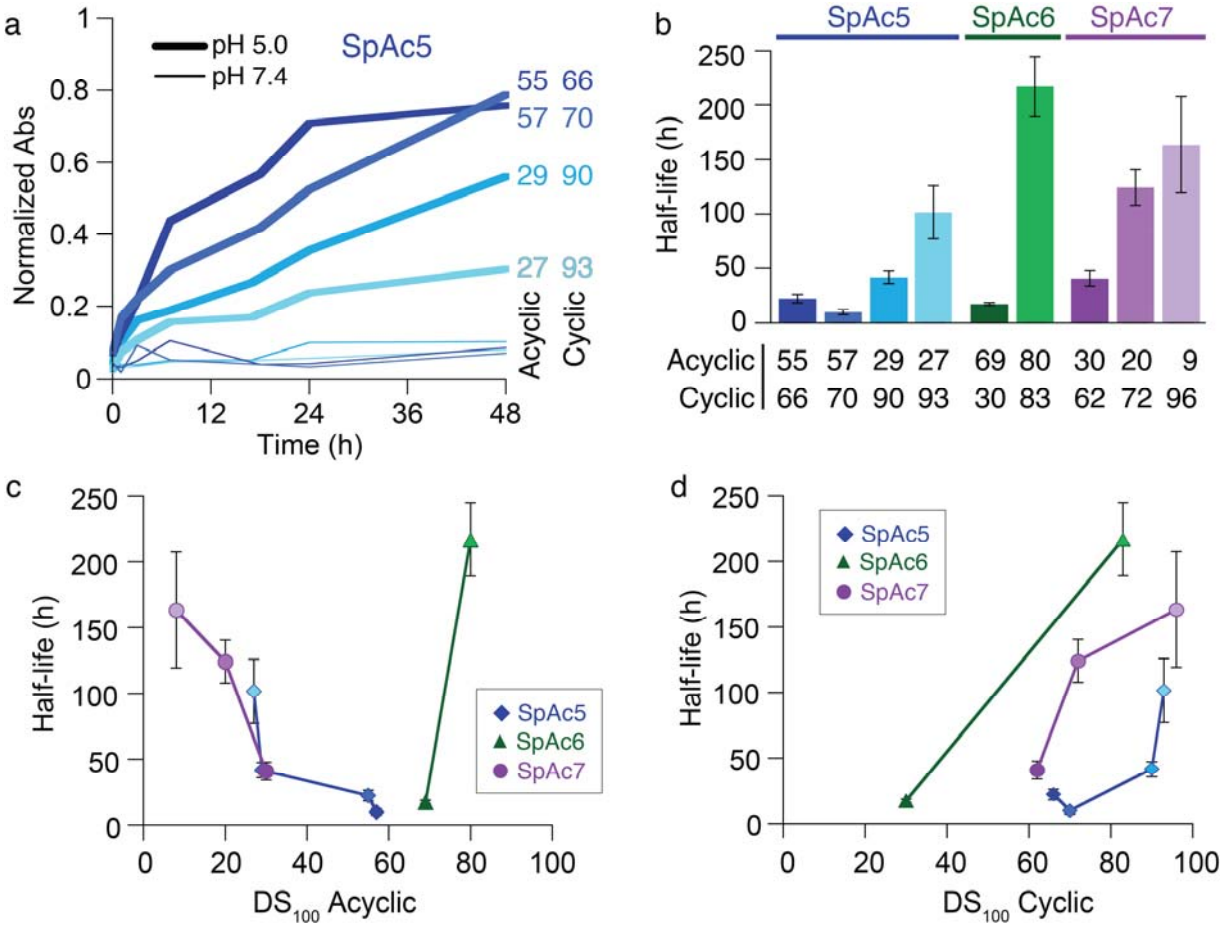


Figure 4. Characterization of microparticle degradation rate. Presence of reducing sugars in supernatant of degrading microparticles measured by a BCA copper reduction assay.

To further explore the role of acetal type the degradation of SpAc-Dex materials, microparticle degradation was carried out in deuterated buffer and monitored by in situ NMR (Figure 5). The microparticles themselves produce very little NMR signal, but the appearance of their degradation byproducts in solution could be measured and compared. The ratio of byproduct at any given time reports the relative cumulative amount of cyclic and acyclic acetal degraded. The acyclic and cyclic acetals on SpAc5-Dex hydrolyze at roughly equal rates initially, and then diverge as acyclic acetals are depleted and cyclic acetals continue to degrade. Acyclic acetals degrade somewhat

more quickly than their cyclic counterparts in SpAc7-Dex, but similarly levels off as their hydrolysis nears completion. In contrast to SpAc5- and SpAc7-Dex, the acyclic acetals on SpAc6-Dex degrade much more quickly than cyclic acetals. The rapid degradation of acyclic acetals on SpAc6-Dex places doubt on the possibility that acyclic acetals play a limiting role in bulk microparticle degradation. Instead, hydrolysis of cyclic acetals likely limits the degradation of all three SpAc-Dex materials.

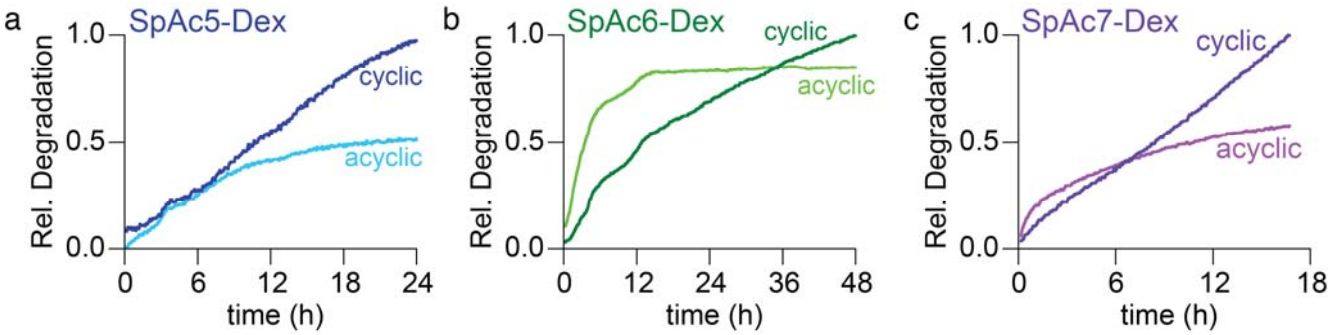


Figure 5. In situ NMR of SpAc-Dex degradation. Tracking release of methanol and cyclic ketone from dextran reveals the relative rate of cyclic and acyclic hydrolysis.

Dextran is known to have excellent biocompatibility,[39] and modified biomaterials based on dextran can often be expected to also have good biocompatibility. The biocompatibility of SpAc-Dex materials was measured in vitro using NIH3T3 murine fibroblast cells. Cells were incubated overnight with varying concentrations of microparticle suspensions and cell viability was quantified using the alamarBlue resazurin reduction assay (Figure 6). Up to a concentration of 1000 μg/mL, SpAc-Dex microparticles had no significant effect on cell viability, indicating that the particles have very low inherent toxicity. The major products of degradation are all well-characterized and are known to be well tolerated at low concentration. Although methanol has a reputation for toxicity when taken in large doses, the EPA reference dose for chronic exposure is 2 mg/kg/day,[40] which corresponds to 17 mg/kg of microparticles at most, and is a larger dose than

likely for most biomedical applications. In the event that greater biocompatibility is necessary, ethyl enol ethers could be prepared and used in place of methyl enol ethers during the synthesis of SpAc-Dex materials.

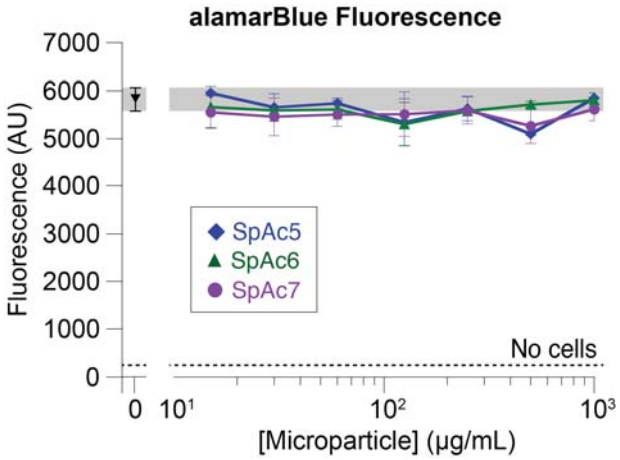


Figure 6. Microparticle cytocompability measured using Almar Blue. No toxicity was observed up to 1000 µg/mL.

Conclusions

We have identified and explored a straightforward method for expanding the palette of acetal-modified dextrans based on conversion of cheaply available ketones into alkyl enol ethers. Although one might expect cyclopentylidene acetals to degrade significantly faster than isopropylidene acetals based on prior work with protected glucofuranoses,[36] we find the opposite to be the case. At similar levels of modification SpAc5-Dex degrades more slowly than Ac-Dex. Similar discrepancies have been observed measuring the hydrolysis of various acyclic acetals under physiologically relevant pH values.[41] The likely explanation for this difference is due to the electronic environments around the acetals, but might also be influenced by the unusual geometry imposed by forming a cyclic acetal with a trans diol on a glucopyranose ring. Cyclopentylidene and cycloheptylidene acetals are known to degrade at similar rates as protecting groups,[42] but we

find that SpAc7-Dex materials degrade very slowly relative to similarly substituted SpAc5-Dex materials. This difference may be the result of the particular structural qualities of a cycloheptylidene acetal of an anhydroglucopyranose. It may be that the steric requirements of this cyclic acetal might inhibit access to the geometries necessary to degrade. Another possibility is that the increased hydrophobicity of cycloheptyl acetals diminishes the ability of water to intercept the oxocarbenium intermediate formed during cyclic acetal hydrolysis, increasing the likelihood that a hydroxyl from dextran adds to reform an acetal.

Interestingly, SpAc6-Dex materials feature both nearly the fastest and the slowest degradation. This may be explained by the relatively slow reactivity of cyclohexylidene acetals. The acetal modification reaction requires stronger acidic conditions to proceed and SpAc6-Dex materials are the only ones where acyclic acetal substitution is positively correlated with increased degradation half-life. Another potentially important contributing factor to the hydrolytic stability of the slow-degrading material is that SpAc6-Dex is able to achieve higher overall hydroxyl coverage than SpAc5-Dex or SpAc7-Dex materials. This coverage is similar to what is typically observed in Ac-Dex materials, but the added steric bulk of cyclohexylidene groups relative to isopropylidene groups may in part account for the difference in degradation rate. While it seems likely that both acetal reactivity and hydrophobicity play a role in controlling material degradation rate, further study is necessary to disentangle these possibilities. Irrespective of the reason, this difference highlights that the specific acetals used for the acetalation of dextran have a large impact on the behavior of the derived material.

The amount of acetal modification necessary to render dextran into an organic processible polymer varies considerably depending on the acetal. Ac-Dex materials feature a high degree of modification, and require > 70% of hydroxyls to be covered before a water-insoluble, organic-

soluble material can be obtained.[19] In contrast, SpAc-Dex materials are processible at much lower hydroxyl coverage. SpAc5-Dex and SpAc7-Dex polymers are processible with 50% hydroxyl coverage and reach a maximum of 70% coverage. The fastest degrading SpAc6-Dex tested possesses only 40% of hydroxyls modified. Lower DS has two potential advantages: First, less byproduct is generated upon hydrolysis, which could reduce undesired effects and toxicity. Second, it leaves a larger proportion of hydroxyls available for further modification chemistry. Ac-Dex has been used as the responsive component in multiblock-copolymer architectures and the ability to further modify this block could enable further development in this area.[43–45]

This work demonstrates that new and sometimes improved material properties can be accessed by adjusting the identity of acetals on acetal-modified dextrans. The rate of pH-triggered degradation appears to be especially sensitive to the structure of the acetal group. In addition, the solubility at a given coverage can be impacted by the hydrophobicity of the modifying group. SpAc-Dex materials maintain the biocompatibility of dextran-modified materials such as Ac-Dex, and have nearly identical material processing properties. This suggests broad applicability for the strategy of modifying polysaccharides with chemically diverse acetal groups, and highlights the large unexplored variety of modifications available to materials in this class.

ASSOCIATED CONTENT

Supporting Information.

The following files are available free of charge.

Schematic of acetal formation, NMR and IR spectra of synthesized compounds, NMR characterization acetal composition, DOSY spectra of SpAc5- and SpAc6-Dex, solubility tables, DLS data, and curve fitting of bulk degradation. (PDF)

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