# Synthesis and Characterization of Acetalated Dextran Polymer and Microparticles with Ethanol as a Degradation Product

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ABSTRACT: In the field of drug delivery, pH-sensitive polymeric microparticles can be used to release therapeutic payloads slowly in extracellular conditions (pH 7.4) and faster in more acidic areas in vivo, such as sites of inflammation, tumors, or intracellular conditions. Our group currently uses and is further developing the pH-sensitive polymer acetalated dextran (Ac-DEX), which is a biodegradable polymer with highly tunable degradation kinetics. Ac-DEX has displayed enhanced delivery of vaccine and drug components to immune and other cells, making it an extremely desirable polymer for immune applications. Currently, one of the degradation products of Ac-DEX is methanol, which may cause toxicity issues if applied at high concentrations with repeated doses. Therefore, in this manuscript we report the first synthesis and characterization of an Ac-DEX analog which, instead of a methanol degradation product, has a much safer ethanol degradation product. We abbreviate this ethoxy acetal derivatized acetalated dextran polymer as Ace-DEX, with the 'e' to indicate an ethanol degradation product. Like Ac-DEX, Ace-DEX microparticles have tunable degradation rates at pH 5 (intracellular). These rates range from hours to several days and are controlled simply by reaction time. Ace-DEX microparticles also show minimal cytotoxicity compared to commonly used poly(lactic-co-glycolic acid) (PLGA) microparticles when incubated with macrophages. This study aims to enhance the biocompatibility of acetalated dextran-type polymers to allow their use in high volume clinical applications such as multiple dosing and tissue engineering.

KEYWORDS: acid-sensitive polymer, microparticles, tunable degradation, tissue engineering, scaffolds, vaccines
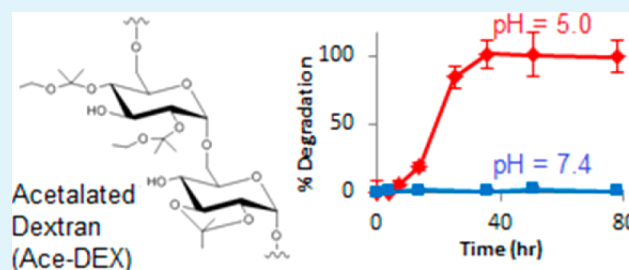
1. INTRODUCTION

Polymers that undergo dramatic property changes in response to environmental stimuli such as pH, temperature, magnetic fields, and electromagnetic radiation have recently been developed.[1] Because these polymers can change shape and solubility in response to a stimulus, they have many biomedical applications in which controlled release of a therapeutic is desired, including drug delivery and tissue engineering.[2] One of the most important avenues for controlled release is the exploitation of natural pH differences in vivo by using pH-sensitive polymers to fashion micro- and nanoparticle drug carriers.[3] The potential for these drug-loaded, pH-sensitive polymeric nanoparticle carriers has been explored by various groups, including delivering therapeutics intracellularly via the pH-gradient in the endocytic pathway,[4,5] delivering immuno-suppressants to acidic sites of inflammation in the gastro-intestinal tract,[6,7] and delivering chemotherapeutic agents to the slightly acidic extracellular pH of tumors.[8,9] It is therefore necessary to engineer a polymer that is relatively stable at the extracellular pH of 7.4 yet quickly degrades and thus releases its drug payload faster in the acidic environment in which the drug delivery is desired. Localized and controlled release of drug through pH-sensitive polymeric devices such as nanoparticles has the potential to increase efficacy of the encapsulated drug,

reduce the possibility of its toxic side effects, and perhaps even lead to dose-sparing (i.e., requiring less drug).[10,11]

Various acid-sensitive polymers have been reported in the literature and fashioned into nanoparticles, such as poly(ortho esters),[12,13] and poly(β-amino) esters,[14,15] and polyketals.[16] One of the most promising examples of a pH-sensitive polymer is acetalated dextran (Ac-DEX). Ac-DEX, which is simply the polysaccharide dextran modified with cyclic and methoxy acyclic acetal moieties (which may be further subclassified as ketal moieties), is a recently described biocompatible, biodegradable, and pH-sensitive polymer.[17] In aqueous acidic conditions, Ac-DEX undergoes degradation when these attached acetal groups hydrolyze, converting the organic-soluble Ac-DEX polymer back into water-soluble dextran. The main advantage of Ac-DEX over other pH-sensitive biopolymers is that its degradation rate is easily tunable through the reaction time used to synthesize the polymer.[18] Longer reaction times create the thermodynamic reaction product, Ac-DEX with high degrees of cyclic acetals which hydrolyze slowly; shorter reaction times create the kinetic reaction product, Ac-

DEX with high degrees of acyclic methoxy acetals which hydrolyze quickly. By simply varying the reaction time, the acetal coverage and thus the degradation rate of Ac-DEX can be carefully controlled, ranging from minutes to months depending on the desired application.[18] In contrast, commonly used polymers in drug delivery such as poly(lactic-co-glycolic acid) (PLGA) or other polyesters are not pH sensitive and have fixed degradation rates depending on the molecular weight, often on the order of months.[19] Ac-DEX microparticles have been explored by our group in various applications requiring pH-sensitive release, including adjuvant-based vaccines,[20] immuno-suppressant delivery to phagocytic cells,[21] and chemotherapeutic delivery to pulmonary cancer cells.[22]

A limitation of Ac-DEX, however, is that one of its degradation products is methanol, along with the more innocuous products of dextran (a sugar) and acetone (a common metabolic byproduct).[17] On one hand, these degradation products are pH-neutral, unlike acidic PLGA degradation byproducts, which can alter the local pH.[23] Unfortunately, methanol is well-known to be toxic to humans because it is metabolized to formic acid and leads to subsequent metabolic acidosis, which can cause blindness, and in serious cases, death.[24] It is important to note that there is little threat of methanol poisoning for a single dosage of Ac-DEX microparticles, since the amount of methanol released would likely be well under the maximum acceptable safe methanol dosage for humans of approximately 28 mg/kg as reported by Paine and Dayan.[17,25] However, it is unclear if applications needing higher volumes of Ac-DEX such as electrospun tissue scaffolds or multiple, high-frequency dosing would result in unsafe levels of methanol release. Regardless, eliminating methanol as a degradation product is a practical next step to enhance the biocompatibility of the Ac-DEX family of polymers for wider therapeutic microparticle, nanoparticle, and tissue engineering applications in the clinical setting.

Thus, the goal of this study was to develop an Ac-DEX-like biopolymer that would degrade into the pH-neutral byproducts of dextran, acetone, and ethanol instead of methanol. Ethanol is a far less toxic alcohol than methanol and is a more ideal degradation product for in vivo applications. We call this analog polymer ethoxy acetal derivatized acetalated dextran (Ace-DEX), with the extra "e" to indicate ethanol as a degradation product. Because Ace-DEX has a different chemical structure than Ac-DEX, the properties of Ace-DEX needed to be characterized and compared to Ac-DEX. After Ace-DEX was successfully synthesized, ^{1}H NMR was used to confirm the exclusive presence of the ethanol degradation product and to quantify the effects of reaction time on cyclic and acyclic acetal coverage. Then, the degradation properties of Ace-DEX microparticles were investigated, including their pH-sensitive degradation characteristics and the ability to tune degradation rate with reaction time. Finally, the biocompatibility of Ace-DEX microparticles was compared to Ac-DEX and commonly used PLGA microparticles in vitro.

2. EXPERIMENTAL SECTION

Materials. All materials were purchased from Sigma-Aldrich (St. Louis, MO) and used as received unless otherwise noted.

Ace-DEX Synthesis. Lyophilized dextran (1 g, MW = 10 400) and pyridinium p-toluenesulfonate (0.0617 mmol) were dissolved in anhydrous dimethyl sulfoxide (DMSO, 10 mL) and reacted with 2-ethoxypropene (37 mmol, Waterstone, Carmel, IN) under nitrogen gas at room temperature. At various predetermined time points (5, 10,

50, 110, 480 min), aliquots were withdrawn and quenched with triethylamine, precipitated with basic water (0.02% v/v triethylamine in water, pH 9), vacuum filtered, and lyophilized for two days. The products were purified by dissolving them in ethanol (200 proof) and centrifuging (5 min, 10,000 × g, Thermo Legend Micro 21). The supernatants were precipitated again in basic water and lyophilized for two days to yield Ace-DEX polymer (1.5 g), a powdery white solid.

Ace-DEX NMR Analysis. To prepare for NMR analysis, Ace-DEX polymer was suspended in deuterium oxide and hydrolyzed with deuterium chloride. After 2 h, a 300 MHz ^{1}H NMR (Bruker 300 Ultrashield) was used to read the samples. The analysis provided a ^{1}H NMR spectrum of Ace-DEX and allowed for the cyclic/acyclic acetal content to be measured. The hydrolysis of one cyclic acetal produces one acetone molecule, whereas the hydrolysis of one acyclic ethoxy acetal produces one acetone and one ethanol molecule. Using these peak integrations and those of the hydrogens on the glucose ring for standardization, the cyclic and acyclic acetal coverages per 100 glucose units were determined. A more detailed description of NMR analysis for Ac-DEX (which follows the same procedure) may be found in the Supporting Information of Bachelder et al.[17]

Microparticle Synthesis. Empty Ace-DEX microparticles were prepared with the single-emulsion technique (water/oil) as previously described with Ac-DEX.[21] Briefly, 100 mg of Ace-DEX was dissolved in dichloromethane (DCM, 1 mL) and added to a 3% poly(vinyl alcohol) (PVA) in PBS (17 mL) solution. This two-phase mixture was homogenized for 30 s (IKA-25 Ultra Turrax Homogenizer, S25N-18G head, 18 000 rpm) and the emulsion was immediately poured into a spinning solution of 0.3% PVA (40 mL) being stirred on a magnetic stirplate. After two hours of spinning to evaporate the solvent and allow for particle hardening, the mixture was centrifuged (5 min, 20 000 × g). The supernatant was discarded, and the resulting microparticle sediment was washed with basic water (pH 9) and centrifuged under the same conditions several times in order to remove excess PVA. Finally, the microparticles were suspended in basic water (pH 9) and lyophilized for 2 days to yield Ace-DEX microparticles, a fluffy white solid.

For comparison in toxicity analysis, Ac-DEX microparticles were prepared with the same procedure as described above, except Ac-DEX was used in place of Ace-DEX. This Ac-DEX polymer (MW = 10k) was synthesized as previously described in Kauffman et al.[21] PLGA microparticles were also prepared with the same procedure, except PLGA (lactide:glycolide 85:15, MW = 50 000−75 000 g/mol) was used in place of Ace-DEX.

Electrospun Fiber Synthesis. A solution of Ace-DEX in ethanol (0.5 g/mL) was prepared. A Gamma High Voltage Power Source (negative: ES30N-5W, positive: ES30P-5W, Ormond Beach, FL) was used to positively charge a single 21G stainless steel needle at 15 kV and negatively charge an aluminum foil collection surface at −15 kV for a total potential drop of 30 kV. The Ace-DEX solution was electrospun at ambient conditions at a flow rate of 2 mL/h using a Harvard Apparatus (PHD 2000 Infusion Pump, Holliston, MA) syringe pump. The distance between the tip of the needle and the collection surface was 20 cm.

Scanning Electron Microscopy. Ace-DEX microparticles were suspended in basic water (pH 9), and a small amount was placed on an SEM pin stub. The samples were allowed to air-dry. Fibers collected on aluminum foil were stuck directly to the SEM pin stub using carbon tape. All of the samples were sputter-coated with a layer of Pd/Au alloy, and imaged with a FEI NOVA NanoSEM 400.

Ace-DEX Microparticle Degradation Analysis. Ace-DEX microparticles were suspended in triplicate in sodium acetate buffer (pH 5.0) or in PBS (pH 7.4) at 1.5 mg/mL. The samples were kept at 37 °C on a shaker plate at 150 rpm. At various time points, aliquots (120 μL) were withdrawn and centrifuged (15,000 × g, 5 min). The supernatants were removed (90 μL) and stored at −20 °C in a 96-well polystyrene plate. After the last time point, the supernatants were analyzed with a microplate reductometric bicinchoninic acid based assay (BCA)[26] according to the manufacturer's protocol (Protein Assay Kit; Pierce, Rockford, IN). The absorbance was measured at 562 nm with a plate reader (FlexStation 3 Benchtop Multi-Mode
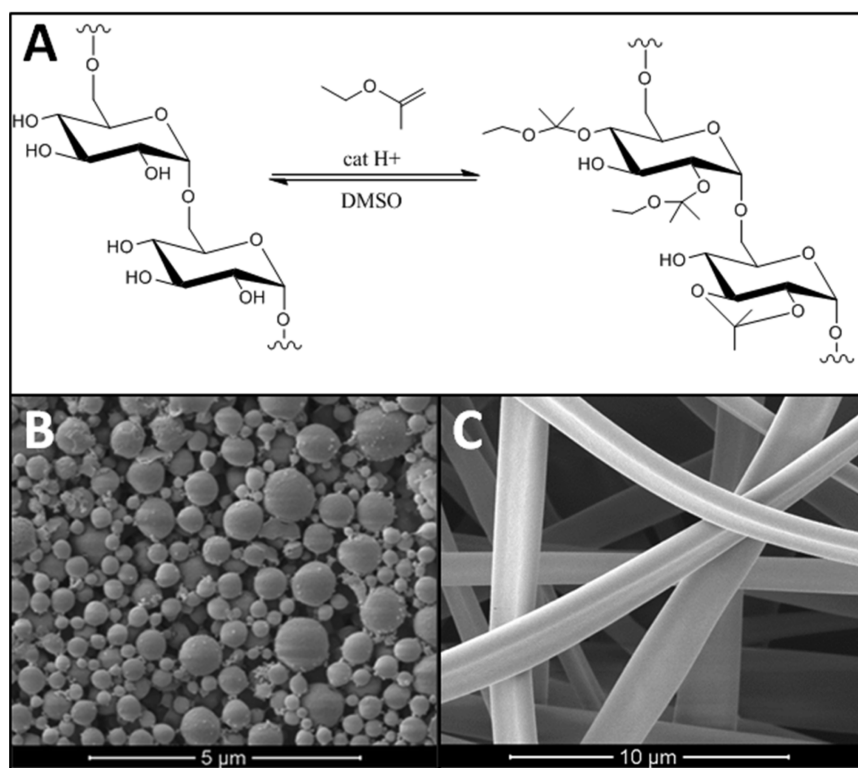
Figure 1. (A) Reaction scheme to synthesize ethoxy-derivatized acetalated dextran (Ace-DEX) polymer. (B) Ace-DEX microparticles formed by the solvent evaporation method. (C) Ace-DEX fibers formed by electrospinning.

Microplate Reader). This assay quantified the amount of the degradation product dextran in the supernatant over time, and a curve was fit to the data. The polymer degradation half-life, $t_{1/2}$, was defined to be the time at which 50% of the Ace-DEX particles had degraded.

Cell Preparation and MTT Assay. Macrophages (RAW 264.7; ATCC, Manassas, VA) were grown and maintained according to the manufacturer's guidelines. Media was made with fetal bovine serum (50 mL; Hyclone, Pittsburgh, PA), penicillin-streptomycin (5 mL; Fischer, Pittsburgh, PA), and Dulbecco's Modified Eagle's Medium (450 mL; Fischer, Pittsburgh, PA). Cells were maintained at 100% relative humidity, 37 °C, and 5% CO_2. Macrophages were plated at a concentration of 1×10^5 cells/mL in a 96-well plate. After 24 h, the media in each well was replaced with media containing Ace-DEX microparticles, Ac-DEX microparticles, or PLGA microparticles ranging from 100 ng/mL to 1 mg/mL.

After another 24 h, the MTT assay was performed to determine cell viability. Briefly, the media in each well was replaced with fresh media (150 μL) and a solution of MTT in media (5 mg/mL, 20 μL). The plate was incubated for 4 h until purple formazan crystals formed. Then, the supernatants were removed and isopropanol (200 μL) was added to dissolve the crystals. The plate was analyzed with a plate reader at an absorbance of 560 nm, and the background absorbance at 670 nm was subtracted. These values were compared to the control wells, which contained only media, to determine the cell viability. Other controls, such as wells with dead cells grown in the absence of media and blank wells, were analyzed as well.

3. RESULTS AND DISCUSSION

Ace-DEX Polymer, Microparticle, and Fiber Synthesis. Ethoxy acetal derivatized acetalated dextran (Ace-DEX) polymer was synthesized via the reaction scheme shown in Figure 1A with reaction times varying from 5 min to 8 h. From this polymer, microparticles and fibers were formed, as shown in images B and C in Figure 1, respectively. Microparticles had spherical morphology and appeared fairly polydisperse by

inspection, with diameters ranging from several hundred nanometers to several micrometers. Meanwhile, the electro-spun fibers had a ribbonlike morphology with a width of several micrometers. In all, there seemed to be little difference between Ace-DEX and its analog Ac-DEX in terms of physical appearance and processing ability. Both polymers are a powdery white solid, and both polymers demonstrate facile fabrication of microparticles and fibers. The Ace-DEX micro-particles in Figure 1B are visually similar to comparably made Ac-DEX microparticles. Our group recently explored the parameters to optimize Ac-DEX microparticles in terms of yield and encapsulation efficiency, including molecular weight, concentration, homogenization speed, and other factors.[21] If desired, it would be straightforward to perform the same study on Ace-DEX microparticles. We have also recently demon-strated that Ac-DEX fibers can be synthesized via electro-spinning (data not shown). However, as discussed in the introduction, Ace-DEX would now be the preferred polymer to use in future tissue engineering applications because it eliminates the methanol degradation product of Ac-DEX.

^{1}H NMR was used to confirm that the Ace-DEX polymer contained acyclic ethoxy acetals as theorized by analyzing its degradation products for the presence of ethanol and acetone. As previously mentioned, cyclic acteals degrade into acetone, and acyclic ethoxy acetals degrade into ethanol and acetone. A representative ^{1}H NMR spectrum of Ace-DEX degradation products is shown in Figure 2. The spectrum has clear acetone (−CH_3, 2.2 ppm, peak A) and ethanol (−CH_3, 1.2 ppm, peak B) peaks, confirming that the original Ace-DEX polymer indeed contained acyclic ethoxy acetals. The analog Ac-DEX has instead been shown to contain acyclic methoxy acetals and to degrade into methanol and acetone.[18] The methanol peak (3.3
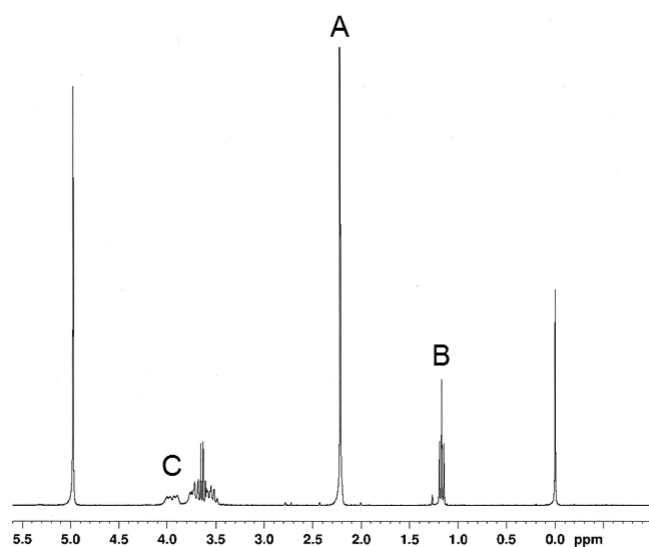
Figure 2. Representative 300 MHz ^{1}H NMR spectrum of Ace-DEX degradation products (dextran, ethanol, and acetone) in DCl/D$_2$O, with labels for the three important peaks: A = acetone $-CH_3$ peaks (6H, 2.2 ppm, integration = 4.80), and B = ethanol $-CH_3$ peak (3H, 1.2 ppm, integration = .45). The integrations were standardized for each glucose unit by C = two glucose ring hydrogens (2H, 4.0 ppm, integration ≡ 1.00).

ppm) is absent from the Ace-DEX degradation products NMR spectrum in Figure 2.

Ace-DEX Acetal Content. As described in the methods section, the integrations of the ethanol and acetone peaks in the ^{1}H NMR spectra may be used to determine the cyclic and acyclic acetal content of the Ace-DEX polymers. Figure 3
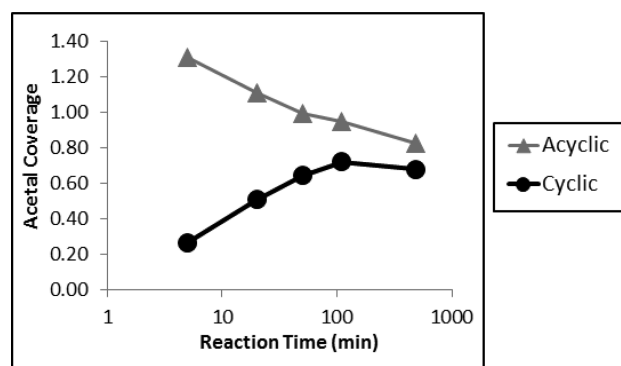

Figure 3. Acyclic and cyclic acetal content per glucose unit in Ace-DEX polymer as measured by ^{1}H NMR.

describes how the reaction time used to synthesize Ace-DEX polymer can control acyclic and cyclic acetal coverage, respectively. Acetal content is measured in acetal groups per glucose unit in the dextran backbone. There is a negative, logarithmic relationship between reaction time and acyclic ethoxy acetal coverage. This result is expected; because acyclic acetals are formed as the kinetic reaction product, they are more favored at shorter reaction times. Conversely, there is a positive, logarithmic relationship between reaction time and cyclic acetal coverage; because cyclic acetals are formed as the thermodynamic reaction product, they are more favored at longer reaction times.

It is evident from Figure 3 that as the reaction progresses, acyclic acetal groups are replaced with cyclic acetal groups. Around 100 min, it appears as if a limit of 0.72 cyclic acetals per glucose unit is reached. The cyclic acetal coverage actually slightly decreases at 480 min, most likely due to small amounts of water contamination in the reaction vessel. For the analog Ac-DEX polymer, Broaders et al. found a similar relationship between cyclic and acyclic methoxy acetal groups and reaction time.[18] In Ac-DEX, however, the natural maximum of approximately 1 cyclic acetal per glucose unit is indeed reached, but this occurs at a much longer reaction time of about 25 h. When comparing comparable reaction times between Ac-DEX (from Broaders' paper[18]) and Ace-DEX, Ac-DEX appears to have a slightly higher degree of substitution for cyclic acetals, yet Ace-DEX has a higher degree of substitution of acyclic acetals. Because cyclic acetals replace acyclic acetals over time, this difference in acetal degree of substitution indicates that the Ace-DEX reaction is slower than the Ac-DEX reaction. It is hypothesized that the extra methyl group on the reactant 2-ethoxypropene causes minor steric hindrance and thus a reduced reaction rate. Understanding the relationship between acetal coverage and reaction time is important because the cyclic acetal content is the key factor in Ac-DEX-type polymer degradation,[18] and this phenomenon is also explored for Ace-DEX in the next section.

Ace-DEX Microparticle Degradation. Although the distribution of the acetal groups along the polymer chain may be highly complex (including 2,3-cyclic acetals, 3,4-cyclic acetals, or acyclic acetals from any of the 2,3, or 4 positions on the glucose subunit) cyclic acetal content alone has been found to control the degradation rate of Ac-DEX micro/nanoparticles. Broaders et al. theorized that because cyclic acetal groups are formed as the thermodynamic product (i.e., longer reaction times), the reverse reaction of acetal hydrolysis also occurs more slowly on Ac-DEX polymers with high degrees of cyclic acetalization; similarly, acyclic acetal groups are formed as the kinetic product (i.e., shorter reaction times), so they undergo faster hydrolysis and lead to faster degrading polymer.[18] Although it was expected that Ace-DEX would behave similarly to Ac-DEX in terms of degradation, it was possible that the extra methyl group on acyclic ethoxy acetal groups of Ace-DEX and its increased hydrophobicity could lead to steric hindrance of hydrolysis or alter the rate of degradation.

To understand the degradation characteristics of Ace-DEX microparticles and how these particles might potentially release encapsulated therapeutics, we degraded microparticles synthesized from varying reaction times using the solvent evaporation technique (shown in Figure 1B) in a pH 5 buffer. This acidic buffer represents an important physiological pH: it models both the phagosomal conditions of macrophages and dendritic cells for immunotherapy applications as well as sites of inflammation. It is advantageous to fashion micro/nanoparticles from an acid-sensitive polymer for these applications in order to localize the release of the encapsulated drug to the desired acidic pH region rather than physiological pH-neutral conditions, thereby minimizing side effects and maximizing efficiency.

Figure 4A shows the degradation profiles of three sets of Ace-DEX microparticles synthesized with varying reaction times in a pH 5 buffer. In general, increasing the reaction times lengthens the time required for the microparticles to fully degrade. This trend is consistent with reports for Ac-DEX; the degradation profile for Ace-DEX from Figure 4A and that of Ac-DEX reported by Broaders et al. were quantitatively similar,[18] which
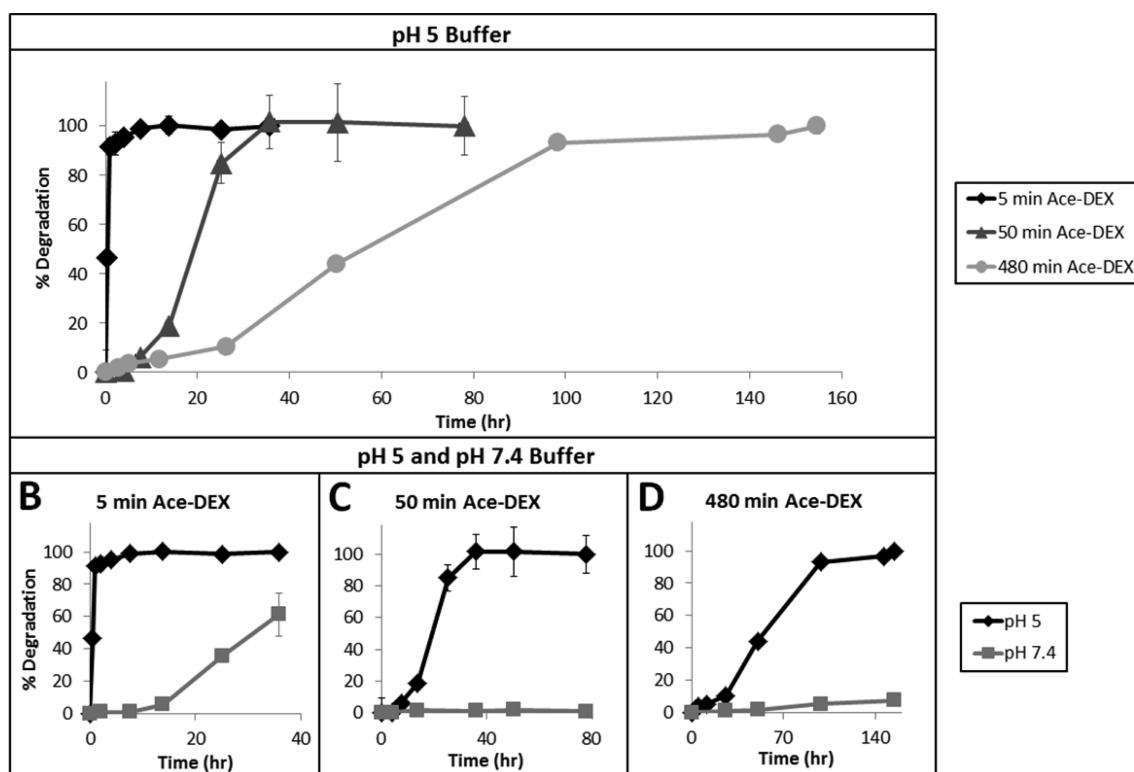
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Figure 4. A. Degradation profiles of microparticles fashioned from 5 min, 50 min, and 480 min Ace-DEX in a pH 5 buffer. **B.** Comparison of 5 min Ace-DEX microparticle degradation in pH 5 and pH 7.4. **C.** Comparison of 50 min Ace-DEX microparticle degradation in pH 5 and pH 7.4. **D.** Comparison of Ace-DEX microparticle degradation in pH 5 and pH 7.4. Data is presented as mean ± standard deviation, n = 3.
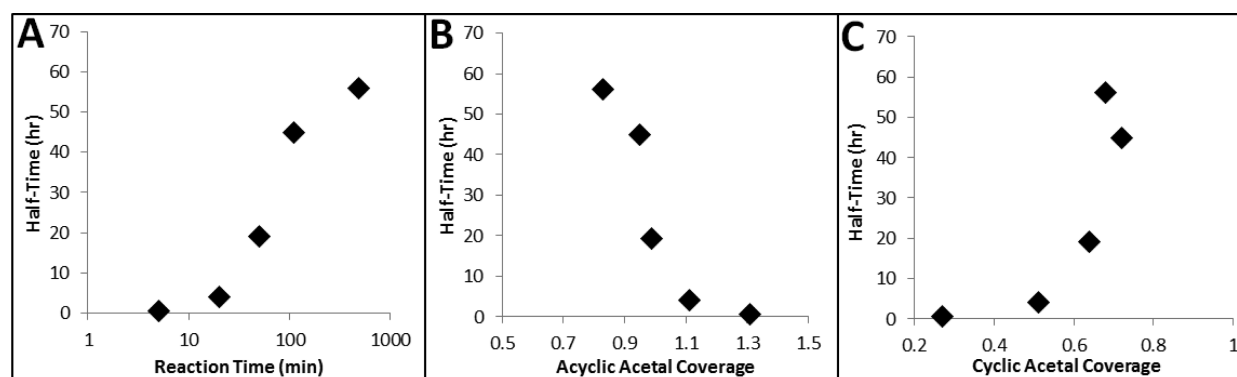

Figure 5. Degradation half-lives ($t_{1/2}$) of Ace-DEX microparticles in pH 5 buffer plotted against (A) reaction time, (B) acyclic acetal coverage, and (C) cyclic acetal coverage.

will be discussed in detail later. It is clear from Figure 4A that the degradation of Ace-DEX microparticles is quite tunable and can be controlled simply by varying the reaction time. The fastest degrading particle set (fashioned from 5 min Ace-DEX, cyclic coverage = 0.27) fully degraded in approximately one hour in a burst fashion, whereas the slowest degrading particle set (made from 480 min Ace-DEX, cyclic coverage = 0.68) required approximately a week to fully degrade and offered a more linear degradation profile. This wide range of degradation rates in acidic conditions means that the degradation of Ace-DEX particles and thus release of the encapsulated drug could be tailored to specific applications. Controlling the polymer reaction time allows for the cyclic and acyclic acetal content of the dextran backbone and thereby the polymer degradation rate to be precisely controlled. It is theorized that even longer degradation times could be achieved by increasing the

molecular weight of the polymer; it has been shown that increasing the molecular weight for Ac-DEX from 10k to 71k lengthened the overall degradation rate approximately 3-fold in pH 5 buffer. Higher cyclic acetal coverages and thus longer-degrading particles might also be obtained by using a more sophisticated reaction vessel which guarantees anhydrous conditions; as was discussed in the previous section, it is unclear if the limit of approximately 0.72 cyclic acetals per glucose unit on Ace-DEX was due to kinetics or slight water contamination and partial hydrolysis.

In Figure 4B−D, the Ace-DEX microparticles were found to have a much slower, more linear degradation in pH 7.4 buffer (physiological conditions) than pH 5. With the exception of 5 min Ace-DEX particles, all other Ace-DEX particles were less than 10% degraded in pH 7.4 at the time of complete degradation in pH 5 buffer. Visually, the difference in
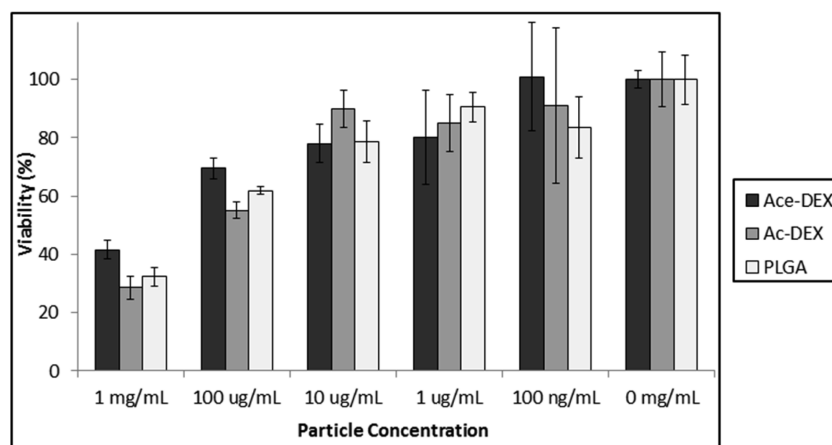
Figure 6. Cell viabilities of RAW macrophages at varying Ace-DEX, PLGA, and Ac-DEX microparticle concentrations after 24 h of incubation by MTT assay. Data are presented as mean $\pm$ standard deviation, $n = 3$.

degradation in acidic and pH-neutral conditions was easy to see; as time goes on, the suspension of microparticles in pH 5 buffer went from cloudy to completely colorless, whereas the suspension of microparticles in pH 7.4 buffer stayed cloudy during the duration of the experiment. Ace-DEX microparticles are therefore well-suited to be tailored for use in a variety of drug delivery applications requiring acid-sensitive degradation, ranging from those which require a quick burst of the encapsulated drug to those which require a controlled, sustained release of drug. Furthermore, Ace-DEX particles may be advantageous to use in place of Ac-DEX particles for frequent and high-concentration dosing applications because Ace-DEX degrades into ethanol instead of methanol.

A second way to quantify Ace-DEX microparticle degradation is by investigating their degradation half-lives ($t_{1/2}$, the time required for 50% of the microparticle to degrade in pH 5 buffer). Half lives are plotted against reaction time, acyclic acetal coverage, and cyclic acetal coverage in Figure 5. Panels A and B in Figure 5 show that half times are directly correlated to reaction time and inversely correlated to acyclic acetal coverage. Figure 5C, on the other hand, suggests that as Ace-DEX cyclic acetal coverage increases, the half-time also increases.

Comparison of Ace-DEX and Ac-DEX Microparticle Degradation. The degradation of Ac-DEX[18] is quantitatively and qualitatively similar to the degradation of Ace-DEX (Figure 4) even though equivalent polymer reaction times lead to higher cyclic acetal coverage with Ace-DEX (Figure 3). Generally, the time required to fully degrade the microparticles is relatively similar for both Ac-DEX and Ace-DEX microparticles formulated from polymer with the same reaction time. On the basis of the kinetics of degradation presented in Figure 5, the rate of degradation differs somewhat between the two polymers. Polymers with low $t_{1/2}$ but longer overall degradation times have an initial burst degradation. Because acyclic acetals hydrolyze faster than cyclic acetals, they are primarily responsible for burst degradation. Thus, if the extra methyl group significantly slowed degradation on acyclic ethoxy acetal groups of Ace-DEX, we would observe a decreased burst (i.e., longer $t_{1/2}$) with Ace-DEX compared to Ac-DEX synthesized under the same reaction time. When comparing our Ace-DEX half-life data to the Ac-DEX half-life data published by Broaders et al.,[18] there is indeed a longer $t_{1/2}$ and thus less pronounced burst with Ace-DEX than Ac-DEX. On the basis of these degradation kinetics, Ace-DEX microparticles may offer a more

sustained release of the encapsulated therapeutic entity as compared to similar Ac-DEX microparticles. It should be noted that, because cyclic acetals are the same between Ac-DEX and Ace-DEX, the two polymers would be expected to have more similar chemical structures and behavior at longer reaction times (for which cyclic acetals are favored). The residual difference in behavior may be attributed to the difference in the methoxy and ethoxy acyclic acetals on Ac-DEX and Ace-DEX, respectively.

In vitro Toxicity Analysis of Ace-DEX Microparticles. Finally, the Ace-DEX microparticles were incubated with RAW macrophages and the cell viabilities after 24 h were determined, as shown in Figure 6. Poly(lactic-co-glycolic acid) (PLGA) was used as a control in this study, since it is one of the most commonly used polymers in drug delivery applications and is FDA-approved.[27] Ac-DEX polymer has also previously been shown to be as biocompatible as PLGA with HeLa cells at concentrations ranging from 1 μg/mL to 10 mg/mL.[17] As expected, there was more cell death at higher concentrations of particles (Figure 6). At all microparticle concentrations, there was no significant difference between cell viabilities when incubated with Ace-DEX, PLGA, or Ac-DEX microparticles. This study validates that microparticles fashioned from Ace-DEX polymer exhibit comparable cytotoxicity with macrophages as other drug delivery polymers; further toxicity studies are needed to conclude if high-volume applications of Ace-DEX indeed have improved biocompatibility compared to Ac-DEX due to the ethanol degradation product as expected.

4. CONCLUSIONS

In conclusion, we have shown the first synthesis and characterization of ethoxy acetal derivatized acetalated dextran (Ace-DEX) polymer and microparticles which degrade into ethanol, acetone, and dextran. Like its Ac-DEX analog, Ace-DEX is a pH-sensitive polymer, degrading more quickly in pH 5 conditions than pH 7.4 conditions. Unlike commonly used polyesters such as PLGA, Ace-DEX exhibits remarkable degradation rate tunability in acidic conditions. We have shown that Ace-DEX microparticles can fully degrade during time scales ranging from approximately one hour to one week simply by varying the reaction time to synthesize the polymer. Finally, Ace-DEX microparticles exhibit comparable cytotoxicity to macrophage cells as FDA-approved PLGA, paving the way for future in vitro and in vivo experiments. On the whole,

Ace-DEX shares many properties (such as powder appearance, processing ability, degradation rates) as the more fully studied analog Ac-DEX; it appears that Ace-DEX microparticles have less of a burst degradation than Ac-DEX microparticles but approximately the same overall degradation time. It should therefore be straightforward to substitute Ace-DEX for Ac-DEX polymer, the latter of which has been investigated for use in wide-ranging therapeutic applications requiring acid-sensitive microparticle degradation, including vaccines, chemotherapy, and immunotherapy. Furthermore, because we have replaced the methanol degradation product with a far safer ethanol degradation product, Ace-DEX polymer has tremendous potential for use in high volume therapeutic applications, such as multiple dosing and tissue engineering, which we intend to explore in future work.

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Notes

The authors declare no competing financial interest.

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