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Extended Release of Native Drug Conjugated in Polyketal Microparticles

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Supporting Information

ABSTRACT: Polyketals, which can be biodegradable, have good biocompatibility, and are pH-sensitive, could have broad applicability in drug delivery and other biomedical applications. However, facile synthesis of high molecular weight polyketals is challenging, and short durations of drug release from polyketal particulate formulations limit their application in drug delivery. Here we report the synthesis of a di-isopropenyl ether monomer and its use to synthesize high molecular weight estradiol-polyketal conjugates by addition polymerization. Microparticles were prepared from the estradiol-polyketal conjugate, where estradiol was incorporated into the polymer backbone. The particles had high drug loading and significantly prolonged drug release. Release of estradiol from the drug-polyketal conjugate microparticles was acid-responsive, as evidenced by faster drug release at low pH and with co-incorporation of PLGA. Tissue reaction to the microparticles was benign *in vivo*. Polyketal drug conjugates are promising candidates for long-acting drug delivery systems to treat chronic diseases.

The development of biodegradable particulate drug delivery systems, which can provide very prolonged drug release with a minimal initial burst and without undesirable degradation products, is desirable for the treatment of chronic diseases.[1,2] Polyketals are a new class of biomaterials, which are acid responsive and biodegradable.[3,4] In contrast to polyesters (e.g., PLGA), the degradation of polyketals yields pH-neutral products (ketone and alcohol).[3,5] Polyketal microparticles have been used to deliver drugs to treat acute inflammation[6] and cardiac dysfunction in preclinical studies.[7] Unfortunately, the drug release duration from such polyketal microparticles is still relatively short,[6] perhaps because the drugs were encapsulated rather than chemically constrained within the particles.[8] Biodegradable delivery systems able to release drugs in native form for extended periods (e.g., many months) are desirable, especially for treating chronic conditions.[9,10] An approach to achieving that goal would be to incorporate drug molecules within the backbone of high molecular weight polyketals (drug-polyketal conjugate). Carriers made from such drug-polyketal conjugates could have enhanced drug loading,[11] increased duration of release, and less initial burst release; these advances would be significant improvements over existing drug delivery systems.

Facile methods of synthesizing relatively high molecular weight drug-polyketal conjugates have not yet been reported. Polyketals have been synthesized by the ketal exchange reaction between diols and 2, 2-dimethoxypropane.[6,12] The efficiency of these reactions is low, resulting in relatively low molecular weight polymers, perhaps because of experimental limitations (prolonged refluxing and production of methanol, which is a chain-terminating side product and must be removed during polymerization).[13]

Here, we report the synthesis of high molecular weight polyketals by reacting di-isopropenyl ether (DIPP) monomers with diol monomers, catalyzed by a Lewis acid through a simple addition polymerization, without producing side products (Figure 1A). Drugs containing two hydroxyl groups, such as estradiol, corticosteroids, and prostaglandins could be readily copolymerized with DIPP to form drug-polyketal conjugates. As proof-of-principle, a model drug (estradiol) was used to synthesize an estradiol-polyketal conjugate from which microparticles were made (Figure 1B). Since estradiol was

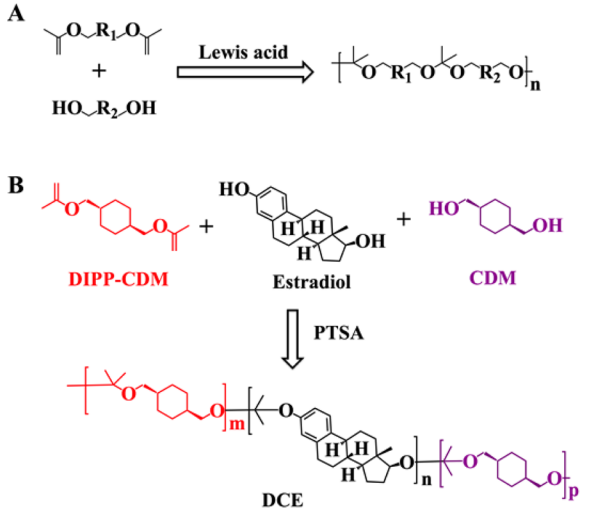

Figure 1. (A) Polyketal synthesis via Lewis acid-catalyzed addition polymerization of di-isopropenyl ether monomers and diol monomers. (B) Synthesis of estradiol-polyketal conjugate (DCE; m = n + p) via Lewis acid PTSA-catalyzed addition polymerization of DIPP-CDM, estradiol, and CDM.

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conjugated into the polymer via ketal bonds, their hydrolysis will lead to the release of estradiol from the estradiol-polyketal conjugate in its native form. We studied the release kinetics from estradiol-polyketal conjugate microparticles and from others composed of a blend of estradiol-polyketal conjugate and PLGA. The hypothesis for the latter experiment was that the degradation of the acid-labile polyketal, and hence the release of estradiol in its native form, would be hastened by the local acidity produced by the concurrent degradation of PLGA.[14]

Di-isopropenyl *trans*-1,4-cyclohexanedimethanol ether (DIPP-CDM) was synthesized from a primary diol (*trans*-1,4-cyclohexanedimethanol, CDM) and isopropenyl acetate through the addition−elimination sequence of alcohol and acetic acid using $[Ir(cod)Cl]_2/Na_2CO_3$ as a catalyst (Figure S1).[15] The progress of the reaction was monitored by NMR, which showed that trans-isopropenylation of isopropenyl acetate with CDM was the dominant reaction and produced a mixture of DIPP-CDM (major product) and minor side products (monoacetate-CDM and diacetate-CDM, Figures S1 and S2). The transacetate reaction produced monoacetate-CDM and diacetate-CDM, but this reaction was minor (only a small peak of acetate at 2.12 ppm in Figure S2).

For step-growth polymerization of high molecular weight polymers, the purity of the monomers is crucial because impurities can interfere with polymerization.[16,17] Equal molarities of the two different monomers are important, which can also be affected adversely by impurities. Initial attempts to purify DIPP-CDM using a silica gel column, which is acidic, did not yield DIPP-CDM due to the acid sensitivity of the isopropenyl group.[18] Addition of the organic base 0.1% triethylamine to the eluent solvent did not prevent the hydrolysis of DIPP-CDM. DIPP-CDM was stable in basic aluminum oxide columns, but due to the minor difference in polarity between DIPP-CDM and acetate side products (both are nonpolar), they could not be separated by this column. DIPP-CDM was successfully purified with a C18 preparative column. To prevent hydrolysis of DIPP-CDM in water/acetonitrile (eluent solvent), 0.1% triethylamine (organic base) was added, and DIPP-CDM was obtained as a white needle-like powder with a yield of 75 wt %. NMR spectra of DIPP-CDM in deuterated benzene (C_6D_6) confirmed the structure of DIPP-CDM (Figures 2A and S3). The peak of the protons of the double bond of isopropenyl ether was observed at 3.88−3.93 ppm, and the peaks of acetate protons from side products (monoacetate-CDM and diacetate-CDM, 2.12 ppm, shown in the ^{1}H NMR spectrum before purification, Figure S2) were not detected after purification (Figure 2A). The purity of DIPP-CDM as determined by HPLC (Figure S4) was over 99%.

Polymerization of DIPP-CDM and diol monomers was subsequently performed at room temperature for 3.0 h, catalyzed by a Lewis acid: *p*-toluenesulfonic acid (PTSA, tetrahydrofuran (THF) as reaction solvent) or pyridinium *p*-toluenesulfonate (chloroform as reaction solvent). Polyketals with varying compositions were synthesized from DIPP-CDM together with CDM, isosorbide, 1,4-butanediol, and/or 1,10-decanediol (polyketals P1−P5 in Table S1). They were purified by precipitation into their antisolvents to remove catalyst and unreacted monomers. High yields (69−89 wt %) and molecular weights in the range of 45−193 kDa were obtained. Addition polymerization between DIPP monomers and diol monomers resulted in a significant increase of polyketal molecular weight compared to the results of a previously reported ketal exchange method (M_n < 3 kDa).[19] All polyketals were water insoluble and could presumably be used to prepare nanoparticles or microparticles for drug delivery.

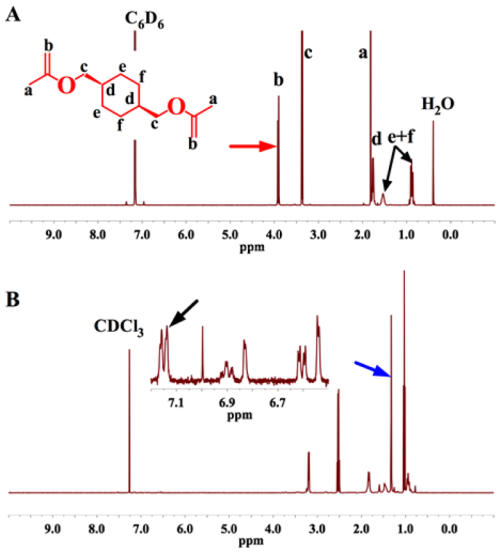

Figure 2. NMR of DIPP-CDM and DCE. (A) Structural analysis of DIPP-CDM using ^{1}H NMR. All peaks were assigned to DIPP-CDM, indicating the high purity of DIPP-CDM. The red arrow indicates the characteristic peak of the double bond of isopropenyl ether (3.88−3.93 ppm, peak b). (B) ^{1}H NMR spectrum of DCE. The blue arrow shows the characteristic peak of the methyl group (1.27 ppm) of the ketal bond in the polyketal. The inset is a magnification of the spectrum from 6.50−7.20 ppm. The black arrow shows the characteristic peak of estradiol at 7.15 ppm.

Using the polymerization procedure above, estradiol, a diol drug widely used in hormonal therapy, was copolymerized (13.8 wt % of reagents) with CDM and DIPP-CDM in THF to produce an estradiol-polyketal conjugate (abbreviated DCE, Figure 1B and Table S1). Free estradiol was removed by repeated precipitation of DCE into methanol. ^{1}H NMR spectra of DCE showed a peak at 7.15 ppm characteristic of estradiol (Figure 2B). In addition, the proton shift of the methyl group of the ketal bond at 1.27 ppm suggested the successful polymerization of estradiol and DIPP-CDM. The M_n of DCE determined by GPC was 23.3 kDa (Table S1). The drug loading of DCE, determined by degradation by acid treatment in methanol, was 11.5 wt %, with a loading efficiency of 83.3 wt %. A maximum drug loading of 37.8% was achieved by copolymerizing DIPP-CDM with estradiol as the only diol (DE; with no CDM), with a loading efficiency of 69.0%. The lower loading efficiency was probably due to the steric hindrance around the hydroxyl group in estradiol. The M_n of DE was 6.5 kDa, lower than that of DCE (Table S1). DCE was used in subsequent studies.

DCE microparticles were prepared by the single emulsion method to be ∼10 μm in diameter (Table S2), for easy injectability through a 27G needle and so that they would persist in tissue.[20] The yield of DCE microparticles was 96 wt %, and their drug loading was 11.1 wt %. In contrast, the estradiol content in PLGA microparticles made by an analogous process (see Methods in SI) was 1.0 wt %, with an encapsulation efficiency of 49.6%.

The release of estradiol from DCE microparticles in mini dialysis devices (see Methods) in PBS (phosphate-buffered saline) was studied at pH 7.4 (physiological pH) and at pH 5.0

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to document pH lability. At predetermined time intervals, dialysis media were completely replaced, and the concentration of estradiol was determined by HPLC. HPLC and LC-MS analyses confirmed that estradiol was released from DCE microparticles in its native form (at pH 7.4, Figure S5). No burst release was observed, and 50% of estradiol was released from DCE microparticles at pH 7.4 in ∼14 weeks (Figure 3A).

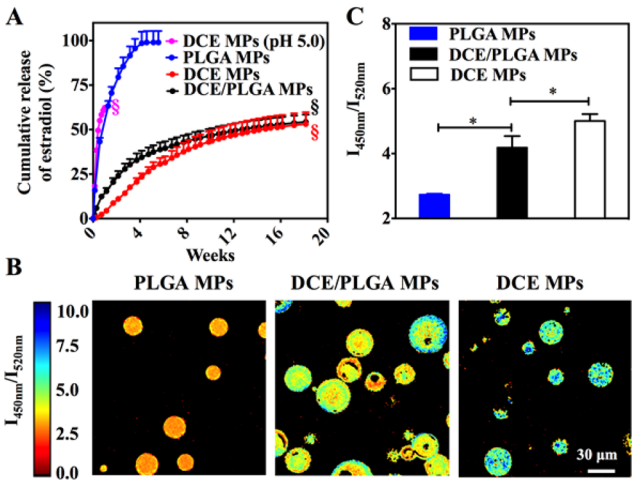

Figure 3. DCE microparticles: drug release and acidic microenvironment. (A) Cumulative release of estradiol (%) from microparticles at 37 °C in PBS (pH = 7.4, 0.1 wt % Tween 80). §: Release experiments were interrupted prior to completion; see text for further discussion. Data are means ± SD; n = 4. (B) Mapping microenvironment pH in microparticles containing lysosensor yellow/blue dextran. A higher $I_{450\ nm}/I_{520\ nm}$ reflects a higher pH. (C) Quantitation of the average I_{450}/I_{520} in each microparticle type (data are means ± SD; n = 8); * indicates $p < 0.05$.

(Note that the release experiment was not taken to completion with the DCE and DCE/PLGA groups, but interrupted at 20 weeks. At that time a large amount of both types of microparticles were still observed.) In comparison, at pH 7.4 PLGA microparticles exhibited burst release, released 50% of estradiol in <1 week, and all the estradiol was released in 4 weeks, before degradation of PLGA microparticles was complete. No oligomers of estradiol conjugates were detected by HPLC in the release media from DCE microparticles. In contrast, conjugates of drugs to polyester polymers (such as PLGA, PLA) release the native forms of drugs over a relatively short period (typically less than a month) and subsequently release soluble carboxyl group-containing oligomer-drug conjugates.[21,22] At pH 5, DCE microparticles released 50% of their estradiol in 3 days, indicating that their degradation was acid sensitive (Figure 3A). For DCE microparticles, release at pH 5 was stopped after a week when the point was clearly made that degradation at pH 5 was much more rapid. Other degradation byproducts of DCE microparticles were acetone and CDM (confirmed by ^{1}H NMR studies of release media at pH 5.0; see Figure S6, which also shows release from DE microparticles). This pH sensitivity may be useful in applications such as intracellular and anticancer drug delivery.

Given the acid-catalyzed degradation of polyketals,[3] we hypothesized that drug release could be modulated by co-incorporating PLGA, the degradation of which is known to create a local acidic microenvironment.[14] Microparticles with 50 wt % DCE and PLGA (50:50, ester terminated, M_w 24,000–38,000) were prepared (abbreviated DCE/PLGA microparticles; ∼14 μm, Table S2) with a yield of 67.2 wt % and drug loading of 6.0 wt %. Scanning electron microscopy (SEM) showed spherical DCE and DCE/PLGA microparticles (Figure S7). We documented an acidic microenvironment in DCE/PLGA microparticles (Figure 3B) by producing maps of the ratio of the fluorescence intensity at 450 nm to that at 520 nm ($I_{450\ nm}/I_{520\ nm}$) of LysoSensor Yellow/Blue dextran,[14,23] encapsulated within the particles; a higher I_{450}/I_{520} indicates a higher pH. (These particles were larger [∼30 μm] than those made of polymer alone [10–14 μm, Table S2], presumably because a double emulsion method was used to load the dye.) The pH in PLGA microparticles was lower than in DCE/PLGA microparticles, which was lower than in DCE microparticles (Figure 3C). This low-pH microenvironment was the likely cause of the much more rapid release of estradiol from DCE/PLGA microparticles in the first 3 weeks (Figures 3A and S8), compared to DCE microparticles.

DCE microparticle biocompatibility was evaluated after injection at the rat sciatic nerve, where nerve, muscle, and connective tissues are in proximity. On dissection 4 and 21 days after injection, microparticles were visible at the injection site (Figure S9). Tissues were sectioned and stained with hematoxylin and eosin. By light microscopy, in all tissues, particle-shaped lucencies (from particles dissolved in staining) and inflammatory cells were seen near muscle and nerve. Macrophages, lymphocytes, and some neutrophils were observed on day 4, while macrophages, lymphocytes, and some foreign-body giant cells were observed on day 21. Inflammation outside of the particle mass and myotoxicity were scored (see Methods in SI). Scores were low at both time points, with minimal myotoxicity and mild inflammation that improved from day 4 to 21 (p = 0.04) (Figure S9 and Table S3). Deeper layers within the muscle had normal morphology without inflammation. Nerve tissue appeared intact. These results were similar to results obtained with polyester microparticles at the same location.[24]

In summary, we have developed a facile method to synthesize di-isopropenyl ether monomers and high molecular weight polyketals by Lewis acid-catalyzed addition polymerization of di-isopropenyl ether and diol monomers. Estradiol was used as a model drug in the synthesis of a hydrophobic estradiol polyketal conjugate. Because estradiol itself is a building block of the polymer, drug loading was high, and estradiol release was slow. *In vitro* release of native estradiol from estradiol-polyketal conjugate microparticles occurred over more than 4 months. Drug release kinetics was altered by adding PLGA. Tissue reaction *in vivo* was benign. These materials may be useful for very prolonged sustained delivery as well as for pH-responsive biomaterials.[25] The approach described here could be applicable to other alcohol- or thiol-containing drugs which do not have interfering reactive functional groups, such as unprotected amines and carboxylates. Polyketals with functional pendant groups could also be synthesized from functional diols, such as protected amine or maleimide group containing diols, to allow drug conjugation to the polymer.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b02435.

Experimental details and data (PDF)

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Notes

The authors declare no competing financial interest.

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