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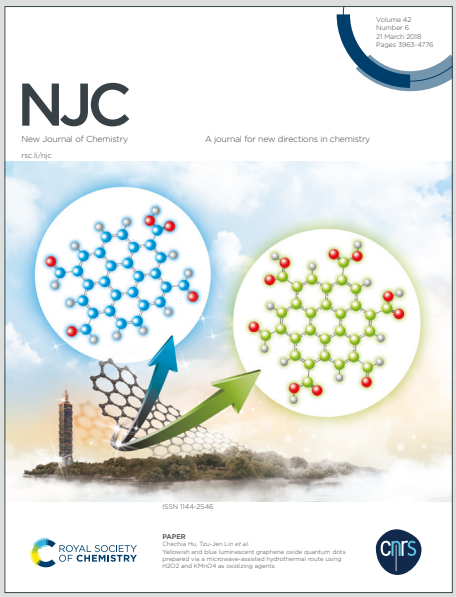

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ARTICLE

Synthetic copolymer conjugates of docetaxel and *in vitro* assessment of anticancer efficacy

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Docetaxel (DTX) is a widely used chemotherapy drug that is associated with numerous side effects and limited bioavailability. Macromolecular conjugates of DTX may improve drug targeting, solubility, reduce off-target toxicity, and overcome mechanisms of multidrug resistance. However, most polymer conjugates of DTX investigated to date make use of biopolymers, which are of fixed structure and are not well suited to optimisation and subsequent reaction to introduce further functionality. Here, we show the preparation of synthetic copolymer conjugates of DTX with drug loading of up to 20% w/w that also has potential for tuning backbone hydrophilicity and the number of reactive sites for conjugation. The intermediates produced are comprehensively characterised, as are the macromolecular conjugates, which are tested in the MCF-7 human breast adenocarcinoma cell line to assess toxicity and anticancer efficacy. The conjugates produced have IC_{50} values within one order of magnitude of DTX, as expected for slow release of DTX by ester hydrolysis. The results suggest that the system is promising for delivery of DTX and future work may examine conjugates of a wider molecular weight range, optimisation of DTX and PEG conjugation efficiency, and *in vivo* biodistribution.

Introduction

Breast cancer constitutes 12% of the global incidence of cancer, with over 2 million new cases and more than 600,000 deaths worldwide in 2018.[1] It is the most commonly diagnosed cancer in women, and the leading cause of cancer-related death. Although a number of treatment strategies exist for breast cancer by targeting particular tumour subtypes based on expression of hormone receptors and Her2, chemotherapeutic cocktails are still widely used alone or as an adjuvant therapy to ensure complete tumour removal, particularly for aggressive subtypes.[2,3] Following the success of the parent taxane paclitaxel, semisynthetic docetaxel (DTX) is now the most widely used chemotherapeutic drug in the treatment of breast cancer.[4–6] Taxanes have limited aqueous solubility, resulting in a moderate bioavailability, and are usually administered in a formulation containing surfactants and/or a non-polar vehicle such as Cremophor EL®, which is associated with some adverse effects.[7] Treatment with DTX itself is associated with severe toxicity and debilitating side effects resulting from a lack of discrimination and selectivity. DTX is an anti-mitotic drug that interferes with cell replication by binding to β-tubulin during mitosis, inhibiting microtubule assembly.[8,9] As a result, DTX induces apoptosis in any dividing cells of the body, especially those which rapidly divide such as hair follicles, finger nails, and cells of the immune system. Sensory nerve damage to the extremities is also a common side effect.[10] Additionally, the development of multidrug resistance as a consequence of taxane-based therapy, particularly in the metastatic context, is strongly associated with a poor clinical prognosis.[11]

Drug conjugation to biocompatible macromolecules has been shown to help ameliorate the adverse effects of chemotherapeutic drugs. For example, paclitaxel is presently available as an albumin conjugate (Abraxane®) which displays improved efficacy and reduced side effects.[12–14] Conjugates of taxanes with biopolymers such as hyaluronic acid have been previously reported.[15–17] However, such natural polymers are of fixed structure and are not readily optimised. In addition to biomacromolecular conjugates, synthetic polymer conjugates have also shown potential as drug delivery agents. Nanocarriers have the potential to improve on 'free' drugs in a number of ways. These include: improving bioavailability,[18,19] reducing the rate of drug degradation and/or metabolism, manipulation of drug pharmacokinetics, on-demand drug release, targeting of specific cell types, combination with a range of imaging modalities, and combination therapies that deliver multiple components simultaneously.[20–28] Further, drug release from

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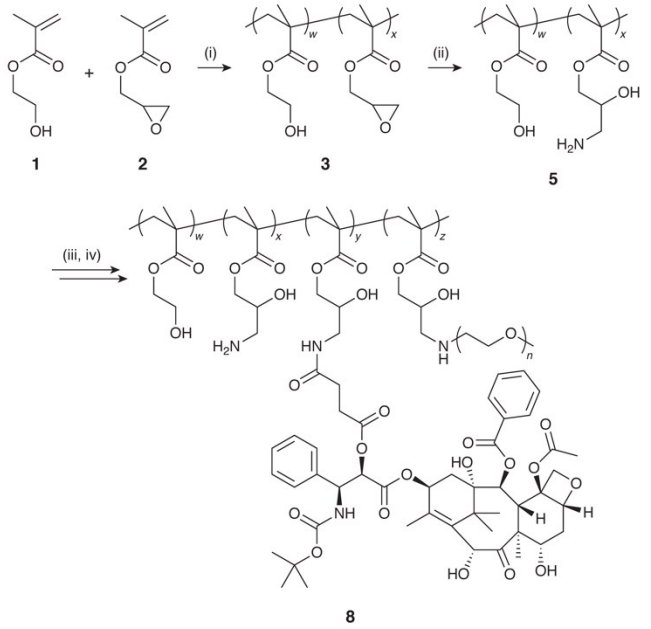
Table 1. Characterisation of copolymer backbones and conjugates prepared in this work.

ID	*w*	*x*	*y*	*z*	DTX wt%	PEG wt%	M_w	M_n	PDI	ΔM_w[1]
3a	0.64	0.36	–	–	–	–	8.65	6.26	1.38	–
3b	0.50	0.50	–	–	–	–	12.8	8.61	1.49	–
3c	0.51	0.49	–	–	–	–	14.8	10.4	1.43	–
3d	0.76	0.24	–	–	–	–	14.2	9.97	1.54	–
5d	0.76	0.24	–	–	–	–	15.5	10.0	1.55	1.3
8a	0.64	0.34	0.02	–	8.3	–	28.6	18.9	1.51	20
8b	0.50	0.48	0.02	–	8.3	–	21.4	18.1	1.18	8.6
8c	0.51	0.47	0.02	–	8.7	–	n.d.[2]	n.d.	n.d.	n.d.
8d	0.76	0.20	0.04	–	20	–	22.5	13.5	1.67	9.6
8e	0.50	0.48	0.01	0.01	5.0	6.9	22.1	17.8	1.24	9.2

[1] Indicative changes in molecular weight ΔM_w are calculated relative to the corresponding unconjugated polymer **3** as this was the most similar to PMMA standards and therefore the most reliably determined value by GPC.
[2] n.d. = not determined.



Scheme 1. Synthesis of DTX–copolymer conjugate. Reaction conditions: (i) CuBr, bpy, ME-Br, MeOH, 80 °C, 2 h; (ii) $NH_{3(aq)}$, MeOH, TEA, 60 °C, 72 h; (iii) mPEG^{2000}-OTs, MeOH, r.t., 72 h; (iv) DTX-2′-Suc-NHS, DMF, TEA, r.t., 2 h.

conjugates can be optimised to be selective for certain biological environments, such as the reduced pH often associated within the tumour microenvironment, resulting in site specific drug release.[29] Conjugation also overcomes mechanisms of multidrug resistance in which ATP-binding cassette transporter proteins are overexpressed in the cell membrane; unlike the free drug, larger conjugates cannot be expelled through such efflux pumps.[2]

The conjugation of highly hydrophobic moieties to a principally hydrophilic backbone would be expected to result in macromolecules that either self-assemble (*e.g.,* into micellar or lamellar structures) or fold to minimise interactions of hydrophobic cargo with the solution. PEGylation may also help to improve hydrophilicity. Off-target toxicity may be addressed by targeting delivery of DTX *via* the leaky vasculature of tumours through a process known as the enhanced permeation and retention (EPR) effect.[30] This could result in better patient outcomes and a reduction in observed side-effects for those undergoing DTX chemotherapy treatment.[22] The principal requirements for EPR targeting are low interaction with blood components, a molecular weight greater than 40 kDa, and particles that can circulate for longer than several hours.[31]

Important considerations for nanoparticle-based delivery of therapeutics are highlighted in other recent work. For example, it is important that the carrier itself does not induce any toxic effects,[32] and that the conjugates are carefully purified to ensure that there is no residual free drug that would result in a skewed IC_{50} value.[33–35] These considerations need to be addressed before animal studies can be attempted.

We reasoned that a synthetic macromolecular conjugate of DTX that has potential for systematically varying the hydrophobic/hydrophilic balance of the polymer, loading of DTX, and also subsequent functionalisation would be a worthwhile pursuit. Evidence of DTX release from the conjugate by ester hydrolysis is provided. Cytotoxicity is assessed in the MCF-7 human mammary adenocarcinoma cell line, demonstrating that the conjugate maintains inhibitory efficacy comparable to equivalent concentrations of free DTX, but could increase the duration over which drug is released.

Results and Discussion

In this work, we describe a novel synthetic polymer conjugate of DTX with high drug loading ability and a size suitable for targeting by the EPR effect. We make use of an amphiphilic random statistical copolymer of 2-hydroxyethyl methacrylate (HEMA, **1**) and glycidyl methacrylate (GMA, **2**) which produces a highly hydrophilic backbone containing reactive groups to allow for drug attachment and functionalisation. We have recently reported the synthesis, characterisation, and biocompatibility of such polymer backbones modified for use in gene delivery applications, both *in vitro* and *in vivo*, where they are well tolerated.[36–38] A number of formulations were prepared to gauge the effect that drug loading and polymer backbone composition had on the efficacy of the conjugates produced.

DTX–polymer conjugates were prepared by first synthesizing a water-soluble, amino-modified poly(HEMA-*ran*-GMA) polymer backbone **5** (Scheme 1). A reactive prodrug derivative of DTX, DTX-2′-hemisuccinate *N*-hydroxysuccinimide ester (DTX-2′-Suc-NHS, **7**), was conjugated to each of the copolymers to give DTX–polymer conjugates **8**. This system allowed for a number of variables to be systematically controlled, including the degree of polymerisation and the composition of the backbone, which in turn governed the molecular weight, number of reactive sites available for drug conjugation, and hydrophilicity. Conjugates of differing molecular weight, drug loading, and functionalisation were synthesised and characterised to determine the effect on anticancer efficacy compared to free DTX (Table 1). A PEGylated conjugate **8e** was also produced by reacting methoxy-PEG tosylate (M_w 2000, **4**) with backbone primary amines.

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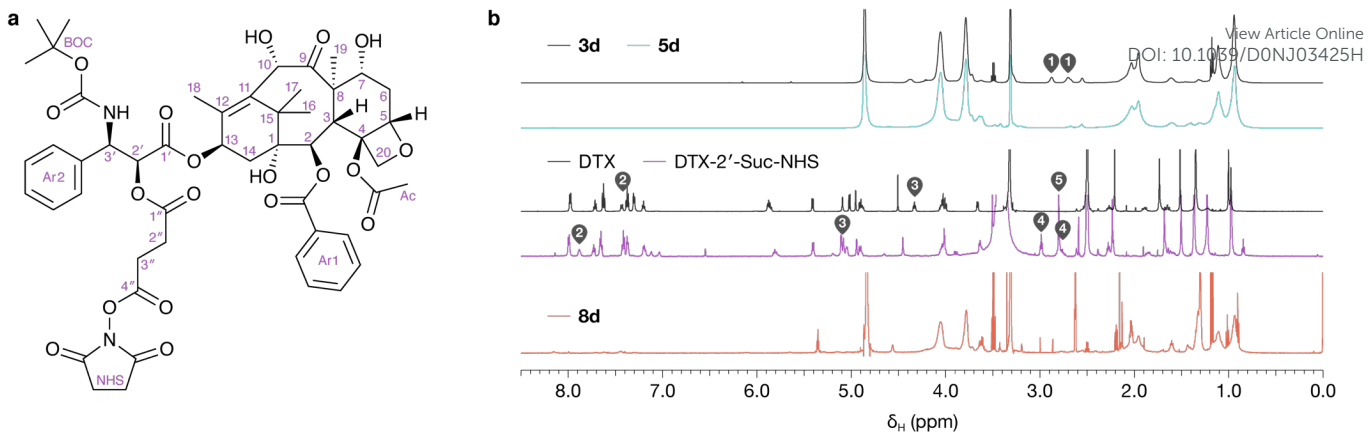

Figure 1. Characterisation of DTX derivatives. (a) Numbering scheme used for DTX. (b) ^{1}H NMR of copolymer **3d**, aminated copolymer **5d**, DTX, DTX-2′-Suc-NHS, and the DTX–polymer conjugate **8d**.

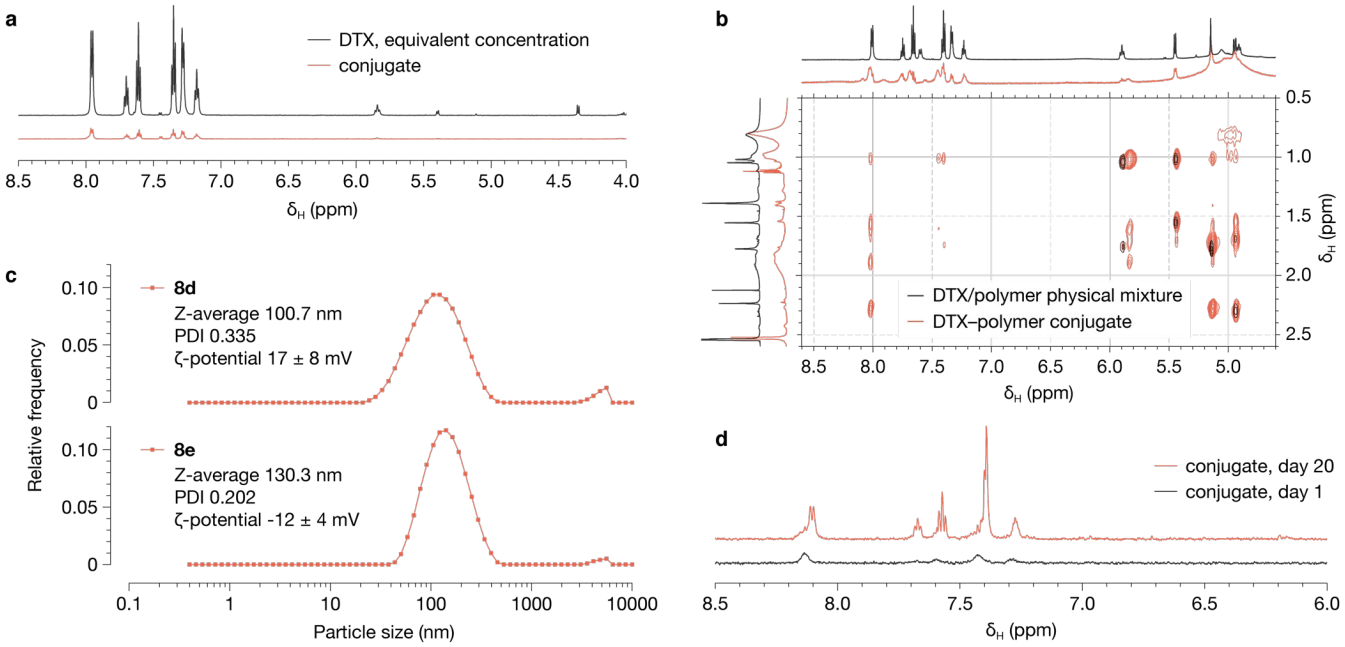

Figure 2. Characterisation of DTX–polymer conjugates. (a) Carr-Purcell-Meiboom-Gill (CPMG) ^{1}H NMR demonstrates successful reaction between DTX-2′-Suc-NHS and aminated copolymer **5**. Strong peak attenuation reveals strong binding (i.e., covalent) of DTX and polymer that is not observed in a simple mixture at the equivalent concentration. Spectra are shown to scale. (b) The NOESY crosspeak at δ_H 1.04 ppm (polymer backbone CH_2 resonances) and DTX aromatic peaks at δ_H 7.40 ppm and 7.45 ppm (Ar2) demonstrate conjugation. These peaks retain the same phase as the diagonal, consistent with a large macromolecule, and are not observed in NOESY NMR spectra of equivalent mixtures of free DTX and polymer. (c) Determination of hydrodynamic size by dynamic light scattering for DTX–polymer conjugate **8d** and PEGylated conjugate **8e**. Both conjugates show a propensity for self-aggregation and/or folding in solution. Zeta potentials agree with expected charge based on free amine (+17 mV) and PEG (-12 mV). (d) Release of highly hydrophobic DTX from polymer can be demonstrated using CPMG ^{1}H NMR. Signals from covalently bound DTX are broadened and suppressed, whereas free DTX resonances are intense, well resolved and often noticeably shifted from their bound counterparts. The release of DTX from the polymer by ester hydrolysis was followed over 20 days. Spectra are shown to scale.

The strategy for conjugating DTX is the addition of a succinate linker at the 2′ position (Figure 1a). In the case of paclitaxel, esterification at this position is known to increase water solubility and successfully release the parent drug more so than modification at the C7 hydroxyl group.[39] In our hands, DTX-2′-hemisuccinate (DTX-2′-Suc) as an intermediate for the synthesis of DTX-2′-hemisuccinate *N*-hydroxysuccinimide ester was unstable owing to hydrolysis during its purification by reverse-phase HPLC. Therefore, once prepared, it was washed, dried and then immediately used to produce the NHS ester. Successful formation of DTX-2′-Suc was indicated *via* ^{1}H NMR spectroscopy by the loss of the DTX 2′ OH peak at δ_H 3.33 ppm. Successful DTX-2′-Suc-NHS synthesis (white crystalline solid, 85% yield) was demonstrated by a suite of NMR spectroscopic techniques including ^{1}H (Figure 1b), ^{13}C, DEPT135, COSY, HSQC, and HMBC and mass spectrometry. Successful reaction was demonstrated by a shift of the 3′ amine (δ_H 7.42 to 7.87 ppm) and 2′ proton (δ_H 4.33 to 5.1 ppm) resonances relative to DTX. The presence of new resonances corresponding to the succinate moiety (δ_H 2.78 and 2.98 ppm) and NHS protons (δ_H 2.80 ppm)

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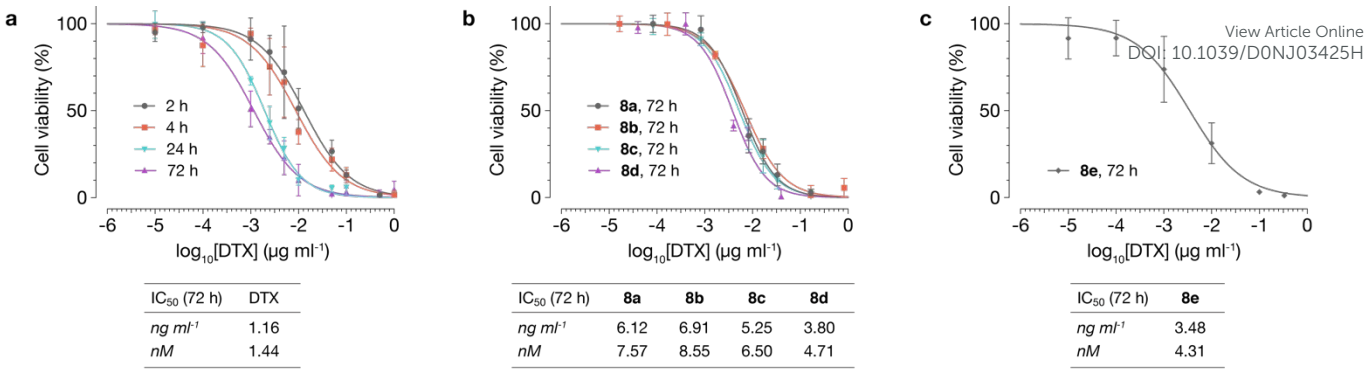

IC_{50} (72 h)	DTX
ng ml⁻¹	1.16
nM	1.44

IC_{50} (72 h)	8a	8b	8c	8d
ng ml⁻¹	6.12	6.91	5.25	3.80
nM	7.57	8.55	6.50	4.71

IC_{50} (72 h)	8e
ng ml⁻¹	3.48
nM	4.31

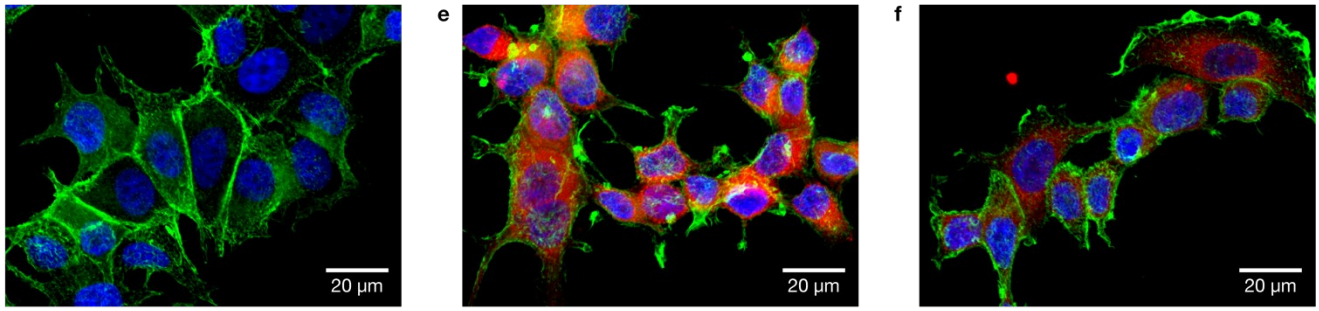

Figure 3. Cytotoxicity assessed by IC_{50} determination in MCF-7 mammary epithelial adenocarcinoma cells using MTS assay. Times in each plot refer to the duration of test compound incubation, after which the compound was removed and cells were incubated in fresh media until the MTS assay was performed at 72 h. For polymer conjugates, [DTX] refers to the equivalent free DTX concentration; that is, conjugate concentrations were normalised based on determined DTX loading. (a) DTX as the free drug exhibits a concentration- and time-dependent toxicity profile; (b) Polymer–DTX conjugates show concentration-dependent cytotoxicity with a higher IC_{50} value than the free drug, presumably due to slow and sustained release of DTX by ester hydrolysis; (c) PEGylated polymer–DTX conjugate displays a comparable efficacy to DTX and DTX–polymer conjugates; (d) representative confocal image of MCF-7 cells receiving no treatment; (e) confocal image of cells treated with DTX–polymer conjugate **8b**; (f) confocal image of cells treated with PEGylated DTX–polymer conjugate **8e**. Confocal images are maximum intensity projections, 40×/1.30 oil immersion, red = RBITC, DTX–polymer conjugate; green = phalloidin-iFluor 488, cytoskeleton; blue = Hoechst 34580, nuclei.

were observed as expected.

Successful conjugation of DTX to copolymers was demonstrated by a range of NMR techniques. ^{1}H NMR spectroscopy of the product showed broadening of the DTX resonances, which was consistent with its successful incorporation into a large macromolecule (Figure 1b).[40] Carr-Purcell-Meiboom-Gill (CPMG) NMR spectra of the conjugates displayed significant attenuation of the DTX resonances compared to simple mixtures of DTX and polymer, consistent with covalent incorporation into a macromolecular structure (Figure 2a).[41] Additionally, conjugation of DTX to the polymer backbone is confirmed by Nuclear Overhauser Effect Spectroscopy (NOESY), revealing a proximal relationship between the polymer backbone and DTX aromatic groups (Figure 2b) that was not observed in simple mixtures of free DTX and the polymer. Furthermore, crosspeaks were observed with the same phase as the diagonal, consistent with a large macromolecule. In Diffusion Ordered Spectroscopy (DOSY) experiments, free DTX diffused at 1.3×10^{-10} m^2 s^{-1}, and polymer **5d** diffused at 5.7×10^{-11} m^2 s^{-1} ([DTX] *ca.* 1.6 mM in DMSO-d_6, 298 K). Under the same conditions, conjugate **8d** diffused at 2.3×10^{-11} m^2 s^{-1}, slower than free DTX or polymer, confirming the formation of a larger macromolecule (Supporting Information Figure S2). While GPC of conjugates **8** demonstrated molecular weight (M_w) increases relative to their parent backbones **3**, the final molecular weights of conjugates may depart from measured values owing to different column interactions compared to GPC PMMA standards. The particle size as measured by dynamic light scattering (DLS, Figure 2c) is consistent with the formation of nanoparticle aggregates which are better suited to passive targeting than is the free drug.[42] The conjugates overcome the poor aqueous solubility of DTX and eliminate the need for addition of polysorbate 80 and ethanol, which are sources of potential hypersensitivity reactions.[43]

DTX release in PBS pH 7.4 + 0.1% v/v Tween 80 at 37 °C (Figure S1) as followed by HPLC gave a first-order release profile with a time constant of 12.2 h. Release of DTX from the conjugates was also detectable by CPMG ^{1}H NMR spectroscopy performed in MeOD. On day 1, only broad, attenuated peaks were present, with no resonances characteristic of free DTX observed, consistent with covalent conjugation. By day 20, the sharp resonances of the drug molecule had returned, demonstrating DTX release from the conjugate (Figure 2d). ^{1}H NMR spectroscopy also provided evidence for cleavage at the 2′ succinimidyl ester position to return the 2′ OH group of DTX and thus the active drug (δ_H 7.44 ppm, br ⟶ 7.39 ppm, t). Here, and also in in the case of paclitaxel, the rate of hydrolysis of the hemisuccinate ester is considerable and therefore alternative linkers with greater stability may be worthy of consideration for sustained release.[39]

In vitro assessment of the DTX–polymer conjugates half maximal inhibitory concentrations (IC_{50}) in the breast cancer cell

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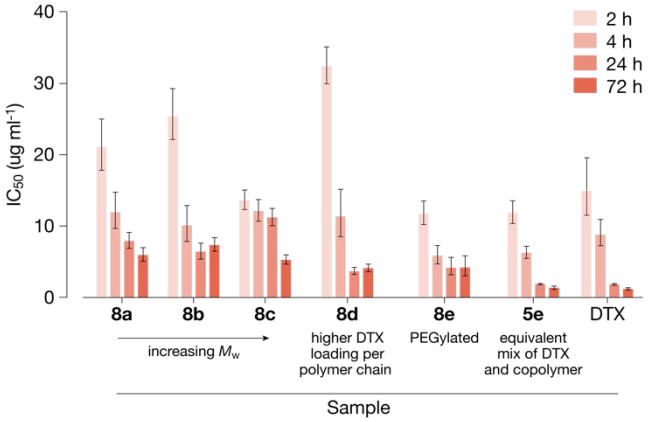

Figure 4. Comparison of IC_{50} values for each of the DTX–polymer conjugates **8a-e**, an equivalent mix of unconjugated DTX and polymer **5e**, and unconjugated DTX following 2, 4, 24, and 72 h treatment. IC_{50} determinations are based on triplicate data. All cells were incubated for a total of 72 h with treatment solutions replaced with media after the treatment time had elapsed, after which MTS assay was used to determine cellular metabolic activity. For statistical comparisons and analysis refer to Supporting Information.

line MCF-7 was performed, to compare the efficacy of the conjugate against free DTX. The aminated polymer backbone **5** prior to drug conjugation was tested for cytotoxicity at greater equivalent concentrations (2, 20, and 200 μg ml^{-1}) than any conjugate treatment (Supporting Information Figure S3). No measurable inhibition of cellular metabolic activity was detected from the backbones alone ($F(4, 10) = 0.6006$, $p = 0.67$), so the polymers were considered non-toxic.

A number of treatment times were investigated *in vitro* to better model the more complex *in vivo* environment which has barriers such as drug half-life, circulation time, and cellular interactions to overcome. This allowed the investigation of cellular interactions and/or time-dependent behaviour of the free DTX and DTX–polymer conjugates to be observed. For cytotoxicity assessments, cells were incubated for a total of 72 h from the addition of treatment-containing media before MTS assays were performed, with shorter treatment times achieved by washing the cells and adding fresh cell media after the desired treatment time had elapsed. As expected, a shorter treatment time correlated with an increased IC_{50} concentration (Figure 3a). Cell cycle arrest is known to occur within 24 h in MCF-7 cells,[43] followed by initiation of apoptosis. The tested conjugate treatments maintained efficacy comparable to DTX against MCF-7 cells, albeit with higher IC_{50} values that are probably attributable to the slow release of DTX from the polymer (72 h treatment times shown in Figure 3b). As expected, varying the treatment time affected the IC_{50} values; all conjugates showed no significant difference in efficacy from DTX for 4 h exposure, while at longer times, conjugates gave IC_{50} values within one order of magnitude of free DTX but significantly higher in all cases. It should be stressed that all conjugates were tested at concentrations normalised to DTX content; therefore, any differences in IC_{50} values can be attributed to polymer conjugation and/or release.

Conjugates **8a-c** were synthesised with similar DTX loading but different polymer backbone molecular weight (M_w); variation of the backbone M_w in this range did not produce any observable differences in anticancer activity, although higher molecular weights could be investigated. Similarly, PEGylated conjugate **8e** also did not alter the cytotoxicity compared to DTX (72 h treatment times shown in Figure 3c) but conjugates with higher PEG content could be assessed. Despite the lack of observable differences in this study, changes in the *in vivo* biodistribution of the conjugates might be expected and this would be worthy of further investigation. A comparison of IC_{50} values for each of the experimental treatment times is shown in Figure 4 (Supporting Information includes a discussion comparing the efficacy of conjugates and statistical analysis). Similarly-sized conjugates with different DTX loading (*e.g.,* **8b** *vs.* **8d**) did not substantially affect conjugate efficacy but as treatments were normalised to DTX content, the delivered total mass of conjugate **8d** was lower. Solubility may determine the maximum possible DTX loading but a higher degree of PEGylation or backbone HEMA content may be further optimised in this system. For comparison, we also mixed DTX and unconjugated polymer (sample **5e** in Fig. 4) in equivalent ratios to the conjugated samples and observed no statistical difference in efficacy versus free DTX.

Our results improve on recent work in several ways. First, the release of DTX from the conjugate followed first-order kinetics with little detectable burst release as a result of careful removal of unbound DTX. Second, we achieved similar IC_{50} values at lower DTX loadings.[34] Third, the drug carrier (polymer backbone) showed no toxicity even when incubated in 1000× excess compared to the IC_{50} of the drug; this is an improvement over a micellar system.[32]

Fluorescently-tagged conjugates were produced by reacting rhodamine B isothiocyanate (RBITC) with backbone primary amines in DMF/water at pH 8–9. Confocal microscopy demonstrated that within 2 h, conjugates had localised within MCF-7 cells (Figure 3d-f). Due to fewer reactive sites on the polymer backbone, the conjugate **8e** has lower RBITC loading and therefore appears dimmer than the non-PEGylated counterpart, conjugate **8b**. The PEGylated conjugate **8e** showed a lower uptake after 2 h that may be the result of reduced membrane adhesion (ζ-potential -12 mV *vs* +17 mV for the unPEGylated conjugate).

Conclusions

In summary, novel synthetic polymer conjugates of DTX of differing molecular weights were produced, characterised, and tested *in vitro*. Conjugates could be prepared with a highly competitive DTX loading up to 20% w/w. Cleavage of the ester bond by hydrolysis and subsequent release of DTX was demonstrated by CPMG ^{1}H NMR spectroscopy and followed by HPLC in physiologically relevant conditions. Conjugates were rapidly internalised in MCF-7 cells. All conjugates showed efficacy within one order of magnitude of free DTX *in vitro*, while the macromolecular carrier was non-toxic at concentrations up to 1000× the IC_{50} of the drug, making this system a potential candidate for *in vivo* study in which its value as a drug delivery platform may be further assessed. The

advantage of this system is the tunability of HEMA to GMA ratios in the copolymer. The reactive groups present allow a great deal of flexibility in attaching imaging probes, solubilisation enhancers, and targeting ligands to the system. Future work may further examine the effects of PEGylation *via* side-grafted chains of varying length in order to optimise solubility, the addition of targeting and therapeutic ligands, biodistribution, and the potential for tumour targeting *in vivo*.

Materials and Methods

Conjugate preparation. Copolymer backbones **3** were synthesised by ATRP as previously described using 2-(Morpholino)ethyl 2-bromoisobutyrate (ME-Br) as the initiator.[36,44] Copolymers were aminated by the action of aqueous NH_3 (30%, 2 ml, 33 mmol) and triethylamine (2 ml, 14 mmol) on **3** (1 g) in dry MeOH (50 ml) at 60 °C for 72 h, after which the product was dialysed and collected by lyophilisation. DTX (25 mg, 30.9 µmol) and succinic anhydride (1.23 eq, 3.81 mg, 38.1 µmol) were combined in dry CH_2Cl_2 (1.5 ml), followed by the addition of dry pyridine (5 µl). The solution was protected from light and stirred at room temperature for 72 h. The crude product was dried under vacuum and purified on silica gel, washed with hexanes and eluted with ethyl acetate. The ethyl acetate fractions were collected and dried under vacuum. DTX-2′-Suc (20 mg, 22 mmol) and SDPP (1.5 eq, 11 mg, 33 mmol) were then combined with MeCN (1.5 ml) and Et_3N (approximately 5 eq, 15 µl). The flask was protected from light and stirred for 12 h. The solvent was removed and the product was dissolved in ethyl acetate and hexanes (2.5:1). The product was precipitated onto a silica gel column with hexanes, then washed twice with hexanes, followed by elution with EtOAc/hexanes (2.5:1). Fractions were dried under vacuum, triturated with Et_2O, and checked by TLC ($CHCl_3$/MeOH 9:1) and HPLC (MeCN/water 1:1). ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm): 0.97 (3H, s, H16/H17), 0.98 (3H, s, H16/H17), 1.37 (9H, s, Boc), 1.50 (3H, s, H19), 1.60 (1H, m, H14), 1.64 (1H, m, H6), 1.68 (3H, s, H18), 1.85 (1H, m, H14), 2.23 (3H, s, OAc), 2.28 (1H, t, H6), 2.78 (m, H2″), 2.80 (s, NHS), 2.98 (2H, t, H3″), 3.63 (m, 3H), 3.89 (1H, m, H20), 4.00 (m, H20), 4.00 (m, H7), 4.45 (1H, s, OH1), 4.9 (1H, d, H5), 4.94 (1H, s, OH10), 5.04 (1H, br, OH7), 5.08 (1H, s, H10), 5.1 (1H, s, H2′), 5.1 (1H, s, H3′), 5.40 (1H, d, H2), 5.80 (1H, t, H13), 7.18 (1H, t, *para* Ar2), 7.36 (2H, d, *ortho* Ar2), 7.41 (2H, t, *meta* Ar2), 7.64 (2H, t, *meta* Ar1), 7.72 (1H, t, *para* Ar1), 7.87 (1H, br, NH), 7.98 (2H, d, *ortho* Ar1). ^{13}C NMR (150 MHz, DMSO-d_6, δ): 9.9 (C19), 13.7 (C18), 22.56 (CH_3, OAc), 27.5 (CH_3, Boc), 55.1 (C3′), 57.0 (C8), 70.8 (C7), 74.8 (C2), 75.0 (C2′), 78.5 (*spiro*, Boc), 83.8 (C5), 127.4 (*ortho*, Ar2), 128.6 (*meta*, Ar2), 128.7 (*meta*, Ar1), 136.8 (*tert*, Ar2), 165.4 (*tert* C=O, Ar1); HRMS (ESI, *m/z*): [DTX-2′-Suc-NHS + Na]$^+$ calcd for $C_{51}H_{60}N_2O_{19}Na$, 1027.37; found, 1027.37. DTX–polymer conjugates **8** were synthesised by combining dry aminated copolymer **5** (29 mg) and DTX-2′-Suc-NHS (30 mg), and adding DMF (dry, 1.5 ml) and triethylamine (20 µl). The solution was protected from light and left stirring at room temperature for 1 h, then washed twice with CH_2Cl_2 and diethyl ether. The product was dissolved in DMF, precipitated in diethyl ether and collected, or dialysed against MeOH/water.

Cell culture. MCF-7 cells were cultured in Minimum Essential Medium α with 10% v/v fetal bovine serum (FBS), 1× GlutaMAX and 0.15% w/v $NaHCO_3$ in a humidified incubator at 37 °C and 5% CO_2. Cells were seeded in 96-well plates at a density of 5000 cells/well in 50 µl of media. Docetaxel and conjugate treatments in media were applied in triplicate at 50 µl per well. After the designated treatment time had elapsed, media was removed, cells were washed with PBS (100 µl per well) for 1 min, then the PBS was removed and prewarmed media was applied (100 µl per well). Plates were incubated for a total of 72 h from the beginning of treatment. Assays were performed using warmed CellTiter 96 AQueous One Solution Cell Proliferation Assay (MTS) solution at 20 µl per well. Plates were read for absorbance at 490 nm 3 h after the reagent was applied. Datasets were normalised and analysed by four-parameter nonlinear regression with an inhibition model. For confocal imaging, cells were seeded on glass coverslips and treated for 2 h with RBITC-labelled DTX–polymer conjugates **8b** and **8e** (25 µg ml^{-1}) diluted in OptiMEM media, and fixed with 4% paraformaldehyde. Cells were stained with CytoPainter Phalloidin-iFluor 488 and Hoechst 34580 in TBST, and mounted using Fluoromount G (Southern BioTech) on SuperFrost glass microscope slides.

Conflicts of interest

There are no conflicts to declare.

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Author Contributions

C.W.E., K.S.I., and M.N. designed experiments and developed the concept. S.E., J.A.K., C.W.E. and M.N. synthesised and characterised the conjugates. S.E. and G.L.N. performed NMR characterisation of DTX and conjugates. S.E., M.N. and C.A.B. performed GPC measurements. C.W.E., J.A.K., S.E., R.S., and T.D.C. performed in vitro toxicity assays and confocal imaging. K.S.I., T.D.C., and C.W.E. supervised the project. All authors discussed the results, assisted with analysis and contributed to writing the manuscript.

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