High-density doxorubicin-conjugated polymeric nanoparticles *via* ring-opening metathesis polymerization†

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High-density doxorubicin-conjugated polymeric nanoparticles are prepared *via* ring-opening metathesis polymerization and sustained release of nearly 50% of the anticancer agent is observed after 24 h in mildly acidic aqueous solution.

In recent years significant progress has been made towards the design and synthesis of polymer-based nanostructures suitable for biomedical applications.[1] Promising oncology clinical results arising from trials with polymer–drug conjugates provide considerable hope that this class of polymer therapeutics, designed with a defined biological rationale, will lead to improvements in cancer treatment.[2] Specifically, core–shell polymeric nanoparticles, generated through the directed-assembly of drug-containing amphiphilic macromolecules, have emerged as a promising new class of polymer therapeutics[2,3] due to the "enhanced permeability and retention" (EPR effect)[4] of solid tumor tissue. The ability to assemble such nanostructured materials with precise control over size and morphology is largely dependent on the availability of well-defined, macromolecular building blocks. Typically, these amphiphilic macromolecules are constructed using living polymerization methodologies that are compatible with a limited range of organic functional groups. Therefore, utilization of these polymers in nanoparticle therapeutic systems requires additional post-polymerization synthetic steps for drug conjugation,[5] limiting the degree of synthetic control one has over the system. Although such strategies have led to the development of functional drug delivery devices,[6] the assembly of polymeric nanoparticles from highly functionalized copolymer precursors with a pre-programmed degree of drug-conjugation remains a significant challenge. Indeed, the rational design of more sophisticated polymer architectures is an emerging trend in the development of the next generation of polymer therapeutics.[2]

Ring-opening metathesis polymerization (ROMP) offers an attractive route to the preparation of synthetic polymers displaying a diverse array of biologically active moieties with precise control over molecular weight and composition.[7] We have previously described a model polynorbornene-based system which employs ROMP methodology for the synthesis of drug-containing amphiphilic block copolymers capable of assembling into core–shell nanoparticles in aqueous solution with sizes that are suitable for chemotherapeutic applications ($\leqslant$400 nm).[7a] Although the cytotoxicity of our copolymer backbone has not yet been determined, addition polymers derived from norbornene have been reported to be non-toxic in gene-transfer applications.[8] In addition, living ROMP methodology allows for the precise control of polymer molecular weight within the therapeutically relevant regime of <45 kDa where renal filtration is effective for non-degradable polymer clearance.[9]

Herein, we describe the preparation of ROMP-based nanoparticles with core structures composed of chemically-linked doxorubicin, one of the most potent and extensively used chemotherapeutic agents in modern cancer treatment.[10] Significantly, sustained release of doxorubicin from the nanoparticle cores under mild acidic conditions is observed. The present study demonstrates the generality of our ROMP-based approach towards the rational construction of nanoscale polymeric architectures from amphiphilic copolymers containing a pre-programmed degree of chemical functionality.

Our strategy is based upon the preparation of monodisperse amphiphilic block copolymers from an acid-cleavable, doxorubicin-conjugated norbornene monomer (**3**) and a water-soluble hexa(ethylene oxide)-substituted norbornene monomer (**4**). The well-defined, functional-group-tolerant Grubbs' olefin metathesis initiator $Cl_2(PCy_3)_2Ru{=}CHPh$ (**5**),[11] employed herein, allows for the synthesis of highly functional drug-containing polymeric architectures inaccessible by other methods. Monomer **3** was prepared in two high-yielding steps using *exo*-5-norbornene-2-ol (**1**) as a starting material (Scheme 1). A carbamate-linking strategy was employed to allow for hydrolysis and subsequent drug release under acidic conditions.[12] Briefly, compound **1** was treated with 4-nitrophenyl chloroformate and pyridine in CH_2Cl_2 resulting in the activated 4-nitrophenyl carbonate derivative **2**. Subsequent reaction of **2** with doxorubicin·HCl and triethylamine in DMF afforded the carbamate-linked doxorubicin monomer **3**. The water-soluble monomer **4** was prepared as described previously.[7a]

Preliminary ^{1}H NMR studies indicated that monomer **3** could not be polymerized to completion by initiator **5** in a range of organic solvents due to insolubility of the propagating polymer chains. Therefore, to prepare amphiphilic block copolymers under homogeneous reaction conditions, polymerization reactions were initiated with the hexa(ethylene oxide)-substituted monomer **4** in solutions of CH_2Cl_2–MeOH (9 : 1 v/v) (Scheme 2). Polymerization experiments were monitored by ^{1}H NMR spectroscopy and gel-permeation chromatography (GPC). The composition of the block copolymer prepared in this study, denoted $\mathbf{4}_{15}$-*b*-$\mathbf{3}_{15}$, was controlled by the monomer-to-catalyst ratio for each block. Analysis of the block containing monomer **4** after initiation with **5** revealed a M_n = 6300 with a monodisperse size distribution

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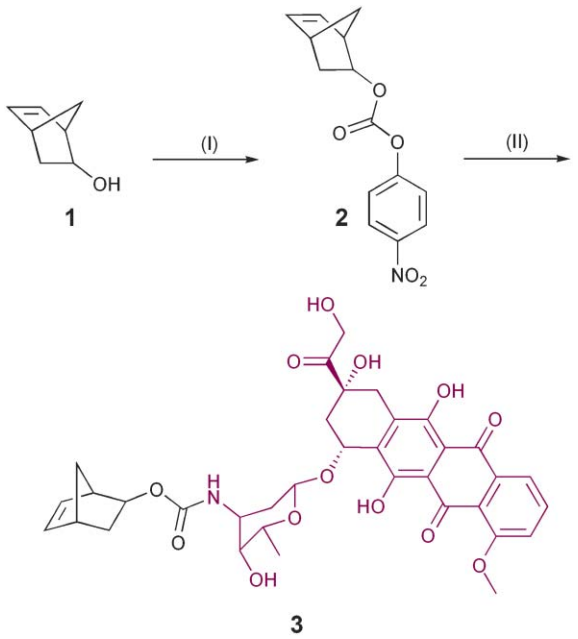


Scheme 1 Synthesis of doxorubicin monomer (**3**). (I) 4-nitrophenyl chloroformate, pyridine, CH_2Cl_2, 99%; (II) doxorubicin·HCl, TEA, DMF, 98%.

(M_w/M_n = 1.19). Upon block copolymerization with monomer **3**, the M_n increases to 12550 while maintaining a narrow size distribution (M_w/M_n = 1.31). The composition of the resulting block copolymer was confirmed by ^{1}H NMR spectroscopy and UV/Vis spectroscopy (see ESI†).

Nanoparticles composed of $\mathbf{4}_{15}$-*b*-$\mathbf{3}_{15}$ were formed by the addition of water to solutions of copolymer (0.01 wt%) in DMSO, and an aqueous solution of the nanoparticles was obtained by dialyzing against deionized water (Scheme 2).[7a] Dynamic light scattering (DLS) was used to measure the effective hydrodynamic diameter (D_H)—calculated using the method of cumulants—of the nanoparticles and a CONTIN analysis was employed to determine the population distribution (see ESI†). A D_H of 230 ± 10 nm was observed with a corresponding polydispersity factor of 0.04 ± 0.03, suggesting a unimodal size

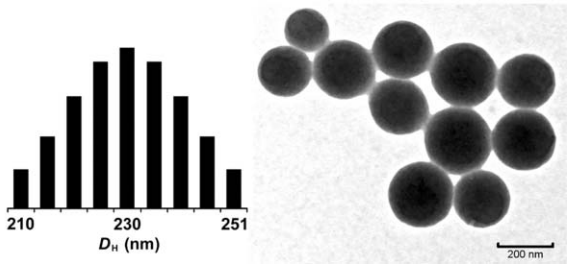
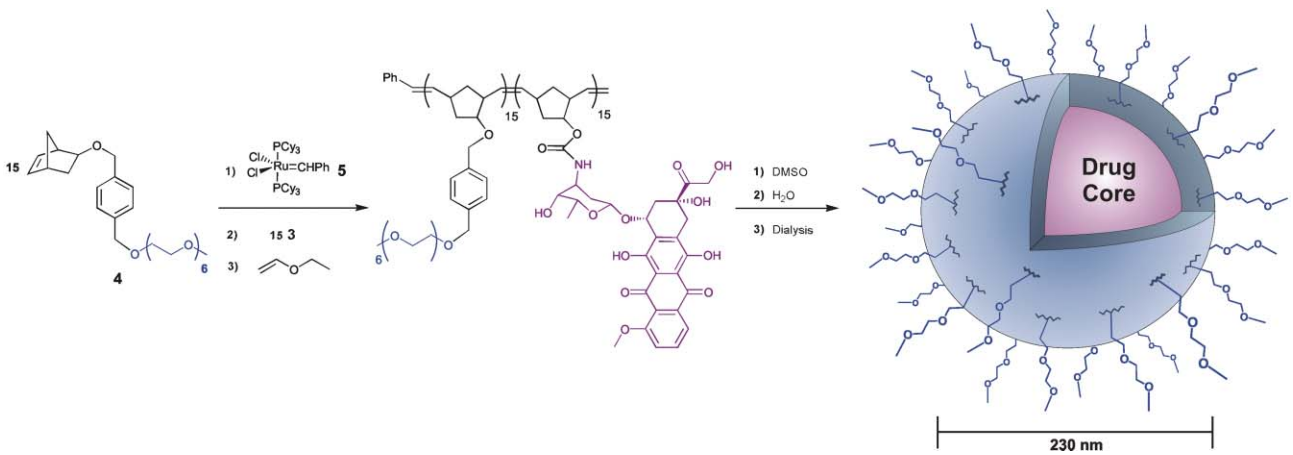


Fig. 1 (Left) CONTIN analysis showing the intensity-weighted hydrodynamic diameter distribution of nanoparticles from $\mathbf{4}_{15}$-*b*-$\mathbf{3}_{15}$ as measured by dynamic light scattering. (Right) TEM image of nanoparticles from $\mathbf{4}_{15}$-*b*-$\mathbf{3}_{15}$ stained with uranyl acetate (2% w/w).

distribution. Fig. 1 (left) shows the CONTIN analysis of the autocorrelation function obtained by DLS and provides further confirmation of the narrow size distribution of particles centered at 230 nm. In the solid state, the nanoparticles were characterized by transmission electron microscopy (TEM). Fig. 1 (right) shows a typical TEM image of the nanoparticles obtained from copolymer $\mathbf{4}_{15}$-*b*-$\mathbf{3}_{15}$. A uniform spherical aggregation morphology is observed with diameters consistent with the DLS data.

In contrast to normal tissue, low interstitial extracellular pH is a well-established pathophysiological characteristic of solid tumors.[13] Additionally, the intracellular phagosomal compartments of human metastatic breast cancer cells have been reported to reach pH values of ⩽4.0.[14] Because polymer-based nanostructures are known to passively internalize in cells *via* endocytosis,[15] the incorporation of acid-sensitive bonds between drug molecules and polymer carrier systems has proven to be an effective approach for releasing drug molecules at tumor sites.[6,16]

The release of doxorubicin from the $\mathbf{4}_{15}$-*b*-$\mathbf{3}_{15}$ nanoparticles was monitored *in vitro* upon incubation in a HCl-adjusted aqueous solution (pH = 4.0) at room temperature. Upon centrifugation, nanoparticles were concentrated into a solid pellet that could be resuspended in solution through mild vortexing without affecting particle size or size distribution. Thus, the amount of drug released was monitored by recording the absorbance of doxorubicin (at λ = 480 nm) in the supernatant after centrifugation. In control samples of nanoparticles suspended in neutral deionized water



Scheme 2 Synthesis of amphiphilic block copolymer $\mathbf{4}_{15}$-*b*-$\mathbf{3}_{15}$ in CH_2Cl_2–MeOH (9 : 1 v/v) and preparation of high-density doxorubicin-conjugated polymeric nanoparticles by water addition to copolymer solutions in DMSO (0.01 wt%) followed by dialysis against deionized water.

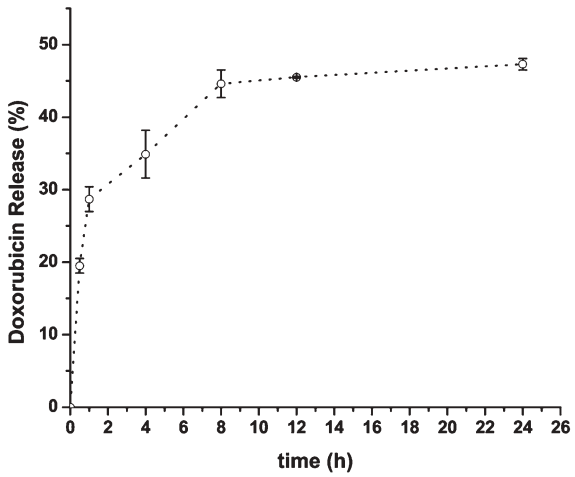


Fig. 2 *In vitro* release of doxorubicin from nanoparticles incubated in pH 4.0 aqueous solution at room temperature.

(pH = 7.0), no absorbance was observed in the supernatant after centrifugation. Additionally, centrifugation did not affect the absorbance of free doxorubicin·HCl dissolved in aqueous solution (pH = 4.0). The total weight of the copolymer, the percentage of monomer **3**, and the extinction coefficient (ε = 10767 cm^{-1} M^{-1}) of doxorubicin in water were used to calculate the concentration of doxorubicin at 100% release from the copolymer. The profile of doxorubicin release from the $\mathbf{4}_{15}$-*b*-$\mathbf{3}_{15}$ nanoparticles monitored over a 24 h period presented three distinct domains: an initial burst phase in which 30% of the drug was released over the first hour, a slower release of the next 15% over the following 7 h, and a plateau region where approximately 2% was released after each 8 h period to give a total release approaching 50% (Fig. 2). These drug release experiments indicated that the doxorubicin-conjugated nanoparticles possessed sustained release characteristics, similar to those observed previously for the release of physically entrapped doxorubicin from polymeric micelles.[17]

In summary, we have shown that ROMP-based amphiphilic block copolymers ($\mathbf{4}_{15}$-*b*-$\mathbf{3}_{15}$) composed of doxorubicin and hexa(ethylene oxide)-substituted norbornene monomers assemble into polymeric nanoparticles in aqueous media. Significantly, this work demonstrates the generality of our ROMP-based approach for the rational construction of a new class of potentially useful polymer therapeutics containing clinically relevant chemotherapeutic agents. Extensions of this work towards more sophisticated, biomolecule-functionalized nanoparticles are in progress.

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