DOI: 10.1002/zaac.201400030

Biodegradable Block Copolymer Scaffolds for Loading and Delivering Cisplatin Anticancer Drug

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Dedicated to Professor C. N. R. Rao on the Occasion of His 80th Birthday

Keywords: Block copolymers; Self-assembly; Prodrugs; Enzyme catalysis; Drug delivery

Abstract. A carboxylic acid substituted amphiphilic diblock copolymer scaffold with a hydrophilic PEG-chain and hydrophobic biodegradable poly(caprolactone) (PCL), which forms a cisplatin anticancer drug is reported herein. Cisplatin [*cis*-dichloro-diammine platinum(II), CDDP] was anchored on the polymer backbone through Pt–OOC–PCL chemical linkage that enabled self-assembly of the prodrug to produce 110 ± 10 nm nanoparticles in water. These drug loaded nanoparticles were characterized by dynamic light scattering, electron microscopy, and X-ray diffraction. The polymer-drug conjugate burst instantaneously in saline and PBS to release 35 % of the cisplatin drug for immediate administration. The remaining drug that retained in the polymer scaffold underwent slow and controlled release to deliver the drug over a period of 6–7 d. In the presence of esterase enzyme; the biodegradable PCL aliphatic ester backbone broke completely to release 100 % loaded drugs within a few hours. This biodegradable diblock copolymer design strategy opens up new platform for cisplatin-polymer drug delivery approach.

Introduction

Cisplatin is one of the widely employed inorganic anticancer drugs for treating various types of cancers such as colon, lung, ovarian, testicular, and so on.[1] Despite the clinical application of cisplatin; neurotoxicity, non-selective accumulation of drugs in healthy tissues, lack of receptor proteins on the cell membrane and low circulation time are some of the inherent limitations associated with the its administration.[2] Further, more than 90 % of the drugs were found to be rapidly cleared through glomerular filtration which led to therapeutic ineffectiveness in patients.[3] Recently, polymer based drug carriers were explored for loading and delivering cisplatin drug to cancer tissues.[4] These polymer-cisplatin nanoparticles showed enhanced accumulation in the intra-tumoral environment through enhanced permeability and retention (EPR) effect.[5] Poly(aspartic acid),[6] poly(glutamic acid),[7] poly(methacrylic acid),[8] and amphiphilic block copolymers such as poly-(2-hydroxy methacrylate),[9] poly [oligo-(ethyleneglycol)methyl-methacrylate][9] and poly (oxanorborneyl anhydride)-b-poly-[ω-oxanorborneyl-poly(ethyleneglycol)[10] are some of the important examples reported for cisplatin delivery. Most of these polymer scaffolds were found to deliver about 50–60 % of the loaded drugs. This is also partially associated with the non-biodegradability of the C–C bond in these acrylic or norbornene polymers under in vivo (or in vitro) conditions. Thus, new biodegradable polymeric scaffolds that are capable of delivering 100 % cisplatin are very much in demand for cancer treatment.

Poly(caprolactone) (PCL) is one of the widely explored biodegradable aliphatic polyester for various applications in biomedical industry.[11] Micellar assemblies of amphiphilic block copolymers based on polyethylene glycol-block-poly(caprolactone) (PEG-b-PCL) were reported as carriers for hydrophobic drugs.[12] However, these biodegradable diblocks are not suitable for cisplatin conjugation due to the absence of functional groups that are required for cisplatin chelation. Very recently, pH-responsive carboxylic acid functionalized amphiphilic diblocks based on polyethylene glycol-block-poly (γ-carboxylic caprolactone) (PEG-b-CPCL) have been reported.[13] These diblock polymers were successfully demonstrated as oral drug delivery vesicles for camptothecin and Ibuprofen under the gastrointestinal tract.

In the presented investigation, the newly developed carboxylic substituted diblock copolymers are employed as scaffolds for anchoring and delivering cisplatin drugs. The design provides two advantages: (i) the PCL backbone has carboxylic acid functionality for anchoring cisplatin, and (ii) the aliphatic ester linkages in PCL can be completely degraded by esterase enzyme under intracellular environment. Thus, the presented biodegradable diblock copolymer design strategy is one of the first examples for complete delivery of loaded cisplatin (100 %) for efficient cancer treatment. The schematic represen-

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Supporting information for this article is available on the WWW under **http://dx.doi.org/10.1002/zaac.201400030** or from the author.

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tation of the approach is shown in Figure 1. The in-vitro release similar to physiological conditions confirmed the above hypothesis. Thus, the custom designed carboxylic block copolymer architectures opens up new opportunities for controlled delivery of cisplatin.

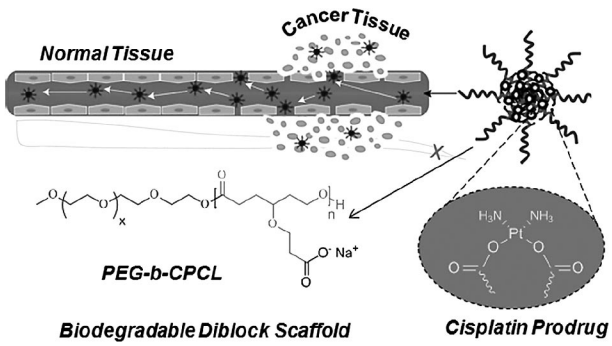
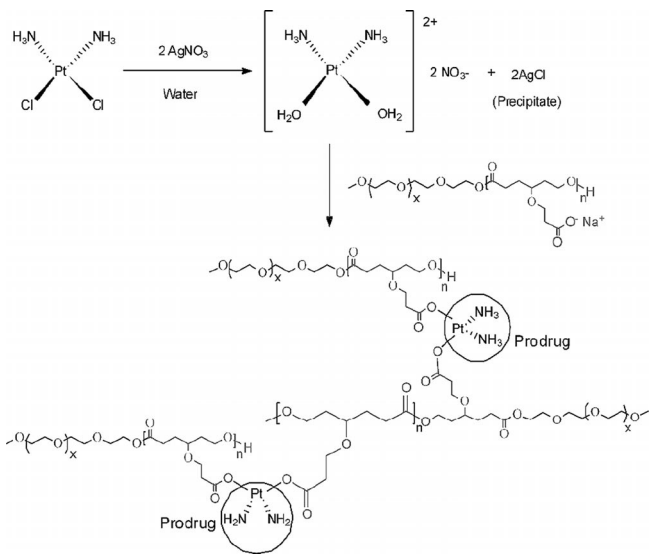


Figure 1. Biodegradable block copolymer approach for cisplatin drug delivery.

Results and Discussion:

Synthesis and Characterization

The monomer was synthesized from commercially available 1,4-cyclohexanediol through multi-step reactions as reported earlier.[13] It was subjected to ring opening polymerization using polyethylene glycol monomethyl ether (MW 2000, about 45 repeating units) and $Sn(Oct)_2$ as catalyst (as shown in Scheme 1). The monomer to initiator ratio was maintained as 100 in the feed. ^{1}H NMR spectroscopic analysis revealed the presence of approx. 103 carboxylic units in the block copolymers (see the Supporting Information for more details, SF-1).[14] The GPC chromatograms showed mono-modal distribution and the molecular weights were determined as M_n = 18,800 and M_w = 25,700 with narrow polydispersity (M_w/M_n = 1.36) (see SF-2, Supporting Information).



Scheme 1. Synthesis of the diblock copolymer and the polymer-cisplatin conjugate.

Cisplatin was treated with $AgNO_3$ yielding the *cis*-diammine-diaqua platinum(II) complex (see Scheme 1) and the AgCl precipitate was removed by filtration.[15] Prior to complexation with cisplatin drug, the block copolymer was converted into its carboxylic sodium salt in double distilled water (pH = 6.8). The aqua complex was stirred with polymer solution for 24 h under dark (see Scheme 1). The resultant polymer-cisplatin complex was filtered through 0.2 μm filters and dialyzed for 48 h to remove any insoluble particle, if present. The dialyzed solution was lyophilized to yield grey colored polymer-cisplatin prodrug. The FT-IR spectra of nascent diblock polymer and cisplatin conjugated polymer are shown in Figure 2. The carbonyl (–C=O) stretching peak appeared as discrete band at 1720 cm^{-1} in nascent polymer, which vanished and a new band appeared at 1558 cm^{-1} with respect to (Pt–O–*C*=*O*) stretching frequency of the metal carboxylate functional group.[16] Additionally, a distinct peak at 545 cm^{-1} corresponding to Pt–O (metal alkoxide) bond stretching was clearly visible in the conjugate. These peaks matched with earlier reports and confirmed the formation of polymer-cisplatin prodrug in the present investigation.[16]

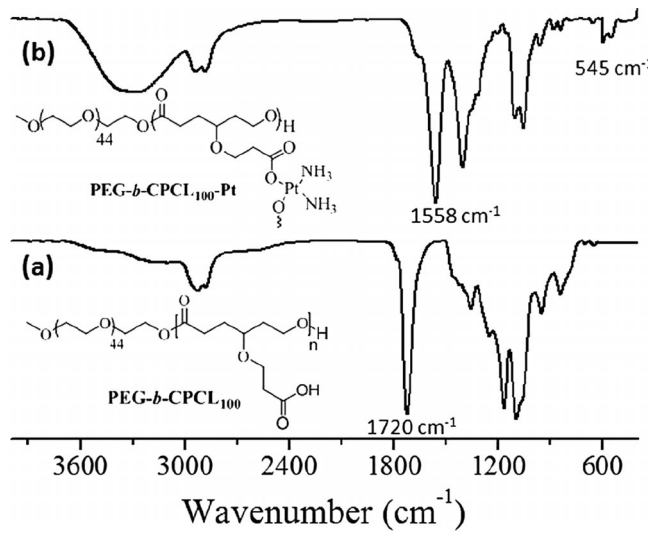


Figure 2. FT-IR spectra of the block copolymer (a) and the cisplatin-polymer conjugate (b).

The drug loading content was estimated by thermo gravimetric analysis (TGA). The TGA plots for polymer, polymer-Pt conjugate, and free cisplatin drug are shown in Figure 3. The decomposition of the polymer started at 310 °C and it completely degraded at 600 °C (< 1% remaining). Cisplatin underwent stepwise decomposition and showed 60% weight loss below 400 °C with respect to the loss of Cl and NH_3 ligands. The platinum content remained unchanged up to 800 °C. In contrast, cisplatin-polymer conjugate showed the combined decomposition profiles: (i) below 380 °C with respect to the ligands (58%), (ii) between 380 to 580 °C with respect to the polymers (24%), and (iii) with residual platinum

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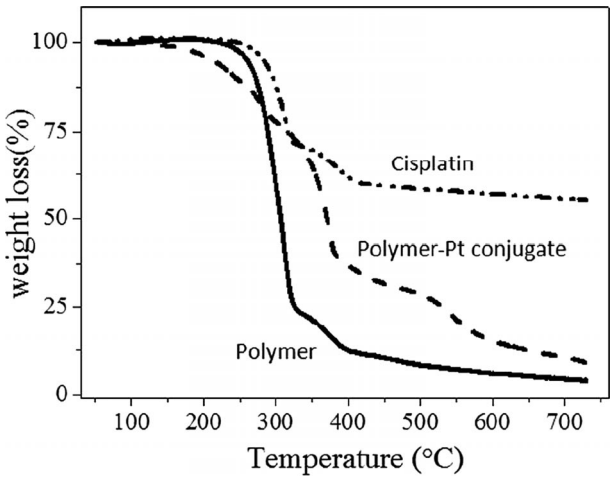

Figure 3. TGA plots of free cisplatin, the polymer, and the polymer-cisplatin conjugate.

above at 600 °C (16 % remaining). Following the procedure reported by *Xu* et al. the drug conjugation efficiency (DCE) was estimated using the equation:[10]

$$DCE = m_{Pt,exp}/m_{Pt,theo} \times 100\,\%$$
$$= (W_{Pt}/M_{Pt})/\,(W_{acid}/2M_{acid}) \times 100\,\%$$

where $m_{Pt,theo}$ is the theoretical molar amount of Pt; $m_{Pt,exp}$ is the experimental molar amount of Pt; W_{Pt} is the weight percent of Pt measured by TGA; M_{Pt} is the molecular weight of Pt; W_{acid} is the weight percent of acid repeating unit calculated by TGA data; M_{acid} is the molecular weight of acid repeating unit.[10] Based on this equation, the DCE was obtained as 50.6 % and the drug loading content (DLC) of polymer sample was calculated as 16 % (see Experimental Section for more details).

Morphology of the Polymer-Cisplatin Conjugate

The amphiphilic diblock copolymer has hydrophilic PEG chains and hydrophobic PCL core for self-organization in water. The hydrophobic core was conjugated through Pt-OOC-polymer linkage, which leads to the nanoparticle assembly having rigid core with flexible PEG tails as corona. The size and shape of the polymer-drug conjugate was studied by dynamic light scattering and electron microscopes. Dynamic light scattering (DLS) profiles of the drug conjugate showed a monomodal distribution with average diameter of 120 ± 10 nm (see Figure 4a). The FE-SEM image of the drug conjugate (see Figure 4b) showed the existence of spherical objects with average diameter of 110 ± 10 nm. HR-TEM image of the drug conjugates also showed the spherical particle morphology with average sizes of 120 ± 10 nm (see Figure 4c). All these three independent techniques showed similar particle sizes, which confirmed the nano-particulate nature. The selective area electron diffraction (SAED) pattern (see Figure 4d) showed highly crystalline states in the drug-polymer particles. Based on the GATAN software data base, these lattices were assigned to 111, 200, 220, and 331 lattices.[17] On the other hand, the SAED pattern of the polymer did not show any diffraction

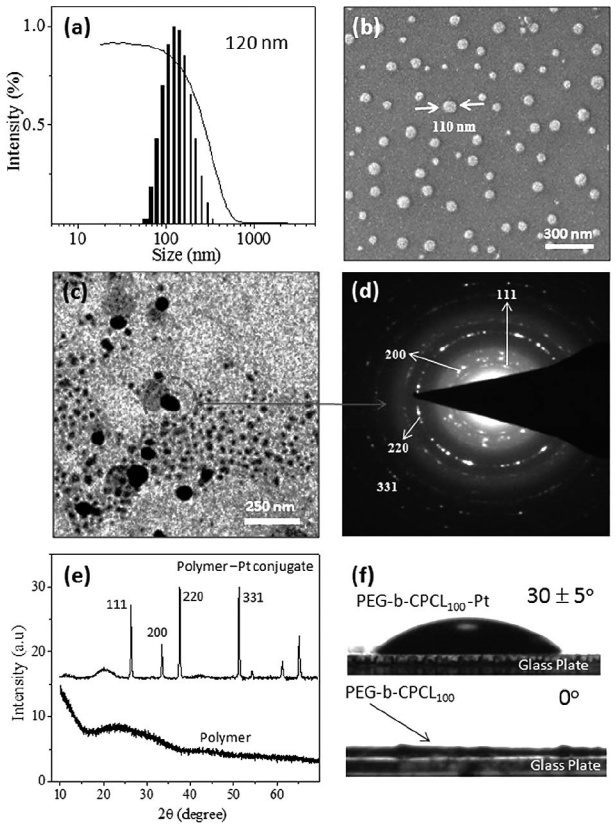

Figure 4. DLS histogram (a); FE-SEM image (b); HR-TEM image (c); selective area electron diffraction (SAED) pattern (d); powder WXRD pattern (e); water contact angle (f) of polymer and polymer drug conjugate.

indicating their amorphous nature (see SF-3, Supporting Information). The bulk sample was subjected to wide angle power X-ray diffraction analysis (see Figure 4e). The polymer did not show any sharp peaks indicating their amorphous nature. On the other hand, sharp crystalline peaks appeared in the polymer-drug conjugate with respect to 111, 200, 220, and 331 lattices (as observed in the SAED images, see Figure 4d). These crystalline lattices were found to match with the cisplatin-data that were reported in the literature.[17] Further, water contact angle (WCA) measurements were also carried out to confirm the hydrophilic or hydrophobic nature of polymer and their drug conjugates. WCA for the block polymers and its cisplatin conjugates were determined by the sessile drop technique. The photographs of water droplets on the polymer film and their corresponding WCA values are shown in Figure 4f. The diblock polymer exhibited WCA value of $<1°$ due to its complete water solubility and highly hydrophilic nature. The WCA of the drug conjugated polymer were escalated to $30 \pm 5°$ (see Figure 4f). This revealed that the formation of Pt-OOC bond (see Scheme 1) enhanced the hydrophobicity of the drug conjugate through cisplatin conjugation. Thus, based on these studies; it may be concluded that the diblock polymer wrapped the cisplatin and stabilized them in aqueous medium as 110 nm size particles for drug administration.

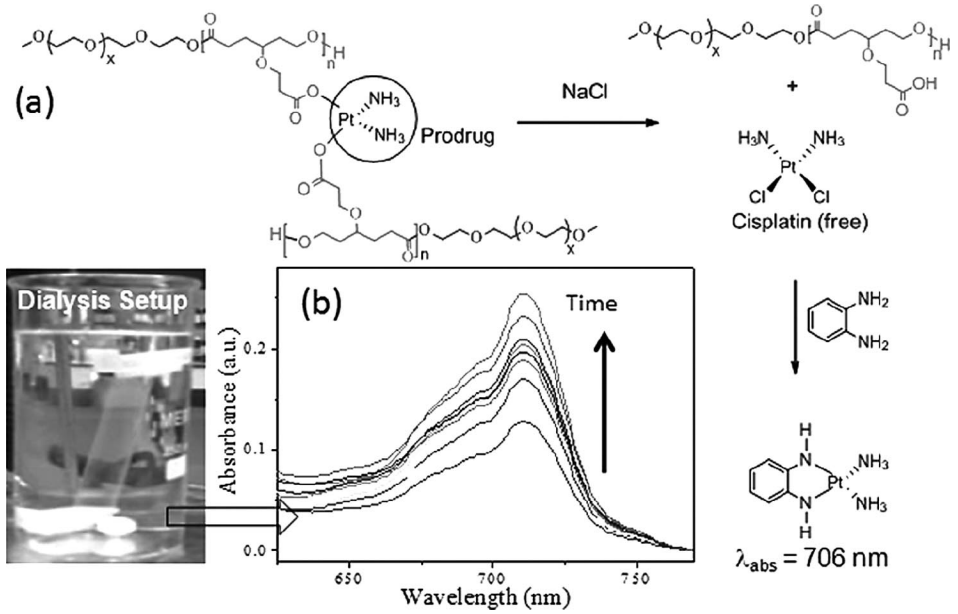

Figure 5. Schematic representation of the cisplatin release from the polymer conjugate in in saline and its complexation reaction with *o*-phenylenediamine (a); absorbance of the cisplatin-OPD conjugate at different time intervals (b) in PBS at 37 °C.

In vitro Drug Release Studies

The in vitro drug release profile of the polymer-Pt drug conjugate was investigated as shown in Figure 5a. The chloride and phosphate anions in saline (aqueous NaCl, pH = 6.8) and phosphate buffer saline (PBS, pH = 7.4), respectively, are highly reactive to cisplatin-carboxylate linkages. The chloride anions attach on the Pt-OOC-polymer linkage to regenerate the cisplatin as drug during the in vitro studies (see Figure 5a). The amount of cisplatin released in the media could be estimated with the help of *o*-phenylenediamine colorimetric assay (see Figure 5b).[9]

In this method, cisplatin released from the polymeric carrier were treated with OPD (*o*-phenylenediamine) for the estimation of the released drug. The amount of cisplatin drug released in this solution could be determined with high accuracy by measuring the absorbance at 706 nm (the absorbance spectra of OPD-Pt complex). Absorbance spectra recorded for the release of the cisplatin in saline over a period of 4 d is shown in Figure 5b. The absorbance spectrum of OPD treated aliquots (see Figure 6c) exhibited an increase in the intensity with release time. The cumulative release was calculated as follows:

Cumulative release (in %) = $C_n \times V_o$ / $m \times 100\%$

where C_n is the amount of loaded cargo in the n^{th} sample, V_o is the total volume and m is total amount loaded in prodrug.

The cumulative release patterns for the conjugate drug in the presence of double distilled water, saline, and PBS are shown in Figure 6. In water, the polymer-drug adduct was completely stable and practically no drug was released even after 6 d. In saline, the chloride ion induced the de-chelation of the polymer-drug conjugate, which typically occurred in two-stages. An initial burst release of the drug-polymer carrier occurred within 10 h with 34% release of cisplatin. Subsequently, the release became very slow and the remaining

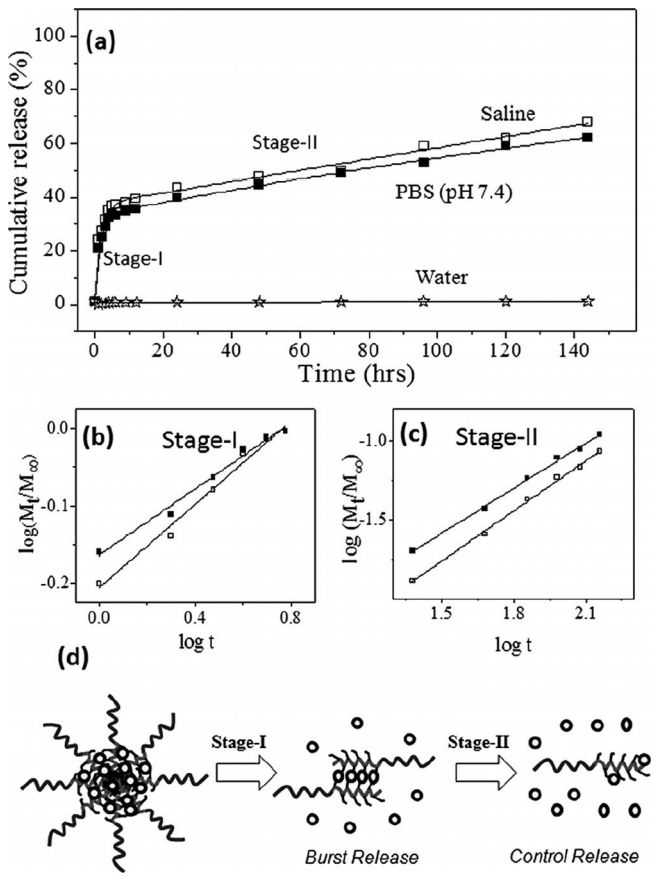

Figure 6. Cumulative drug release of cisplatin in water, saline and PBS (a). Plots of log (M_t/M_∞) vs. log t for stage-I (b) stage-II; (c) of the cisplatin release in saline and PBS; schematic representation of the disassociation of polymer-cisplatin conjugate(d).

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Table 1. Drug release kinetic parameters.

Condition employed	Stage I			Stage II		
	N	*k*	R^2	*n*	*k*	R^2
PBS	0.27	0.625	0.982	0.95	0.1×10^{-2}	0.995
Saline	0.21	0.689	0.981	1.01	0.9×10^{-2}	0.996
Esterase (initial)	0.32	0.125	0.978	–	–	–
Esterase (after 70 h) [a)]	0.39	0.821	0.986	–	–	–

a) The first two phases are similar to that of PBS alone: Stage I with $n = 0.29$ and $k = 0.628$; Stage II with $n = 0.9$ and $k = 0.11 \times 10^{-2}$.

20–30 % drug was released over a period of 5–6 d. PBS also showed a similar profile as noticed in the saline.

The drug release kinetics in saline and PBS in both stages were estimated by the Peppas model (power law).[18] The Peppas power law can be stated as $M_t/M_\infty = kt^n$, where M_t and M_∞ are cumulative releases of drug at time t and infinite time respectively, n is a release exponent, and k is the rate constant. This equation can be written in linear form as

$$\log\left(\frac{M_t}{M_\infty}\right) = n \log t + \log k$$

The diffusion exponent n and the kinetic constant k can be estimated from the slope and the intercept of the plot of log (M_t/M_∞) vs. log t, respectively. According to the Peppas model (if $n < 0.43$), the release of drug happened by both diffusion and erosion processes. The value of n between 0.43 and 1 corresponds to the non-Fickian (anomalous control) in nature. The value of $n = 1$ represents zero order drug release. This equation is normally applicable for the initial 60 % of the fractional drug release or for the values in the interval of $0.1 < M_t/M_\infty < 0.7$.

This model was widely employed for understanding the drug release mechanism from various polymeric carriers such as micelles, vesicles and nanogels and so on. *Zhuang* and coworkers employed this model to study the drug release from the nanogels.[19] *Lecommandoux* and et al. and *Bapurao* et al. used the Peppas model for understanding the drug release from nano-vesicular assemblies.[13,20] In this study, the biphasic release profiles of cisplatin in the presence of saline and PBS were subjected to analysis based on the Peppas model. The plots were fitted to the above equation and their kinetics plots log (M_t/M_∞) against log t are showed in Figure 6b and c. The rate constant k and n values are provided in Table 1. The nanoparticles showed an initial burst release in stage 1 (0–9 h) with low n values ($n < 0.3$) with respect to the 38 % cisplatin release (Figure 6b). This was attributed to the effect of chloride ion, which leads to the disassembly of nanoparticle and subsequent cisplatin release with both diffusion plus erosion pathway. Stage II exhibited the sustained release with n values ($n \approx 1$), which indicates that the remaining 10–15 % of cisplatin release followed zero order kinetics (Figure 6c). Hence, these observations prove that cisplatin-prodrug show sustained release with zero order kinetics, which may help to increase the circulation time in blood plasma.

Based on these kinetic data, the schematic representation of release pattern for cisplatin has been proposed in Figure 6d. However, it was rather surprise that more than 40 % of the Pt-drugs were permanently bonded to the polymer scaffold that was not accessible in saline or PBS. A similar trend was earlier observed by others in acrylic and norbornene based polymer scaffolds.[10] This suggested that under normal conditions (in saline or PBS) only 67 % of the cisplatin could be released from the polymer scaffolds.

Enzymatic Release Studies

The block copolymer scaffold has two parts: (i) biocompatible and non-biodegradable hydrophilic PEG units, and (ii) hydrophobic and biodegradable poly(caprolactone) units having aliphatic ester linkages. The aliphatic ester linkages in the long PCL backbone could be easily chopped under carboxylate esterase enzyme. Since certain cancer cells such as liver and colon have over expression of esterase enzyme;[21] the in vitro analysis of the polymer-cisplatin drug conjugate in the presence of esterase enzyme may provide more insight into their intracellular delivering capability.[22,23]

Cumulative release profiles of the polymer-drug conjugate in the presence of 10 U of esterase are shown in the Figure 7a. It is very clear from the plot that almost all 100 % drug was completely released in less than 10 h by esterase. To further prove the role of esterase enzyme; a control experiment was carried out in which the polymer-drug conjugate was initially exposed to PBS for 72 h (3 d) and subsequently treated with 10 U esterase (see Figure 7a). It is very interesting to notice that only 40 % of the drug is released in PBS up to 72 h and the remaining drug released immediately as soon as it was exposed to esterase enzyme. A further Peppas model was applied to the release profiles of cisplatin from nanoparticles in presence of esterase alone and PBS followed by esterase enzyme. The plots were fitted to above model (see Figure 7), and n and k values are listed in Table 1. In the presence of esterase, the cisplatin delivered in a single step with n value of 0.32. This expresses the release in diffusion plus erosion pathway (see Figure 7b). In the control experiment, it followed three steps kinetics that includes two stages of PBS and a final esterase triggered release. As expected, the drug release kinetics of the initial two phases are similar to that of the PBS alone experiment (see SF 4 for details, Supporting Information). The esterase assisted drug release went along almost identical to that of enzyme initially added experiment. ($n = 0.39$) (see Figure 7c). This difference was attributed to the higher penetrating ability of the enzyme on the nanoparticle, which is already disrupted by the PBS prior to enzyme action.

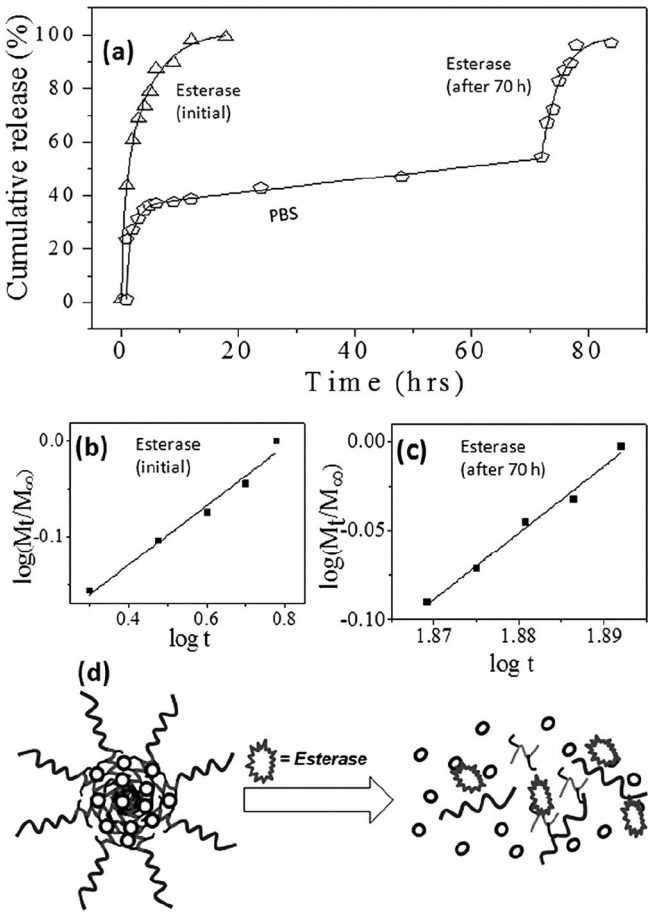

Figure 7. Cumulative drug release (a); plots of log (M_t/M_∞) vs. log t for esterase (initial) (b); esterase after (70 h) (c); schematic representations of the dissociation of polymer-cisplatin conjugate in the presence of esterase (d).

The presented investigation has demonstrated the role of two stimuli for cisplatin release based on PBS buffer (or saline) and esterase enzyme. It was clear from the in vitro studies that the polymer scaffold was biodegradable under esterase enzyme and facilitated the complete drug release, which was not accessible under normal conditions (such as PBS or saline). In general, the release profile of the drugs at intracellular level was assisted by more than one stimuli such as blood plasma (by chloride, phosphate, and other ions), enzymes (like esterase) and so on. Thus, the biodegradable diblock copolymer design, in principle, is capable of delivering 100 % loaded cisplatin drugs through the combination of more than one degradable pathways.

The current investigation has clearly evident for the achievement of biodegradable diblock polymer assemblies for metal based drugs such as cisplatin. The concept was successfully demonstrated based on new diblock PEG-b-PCL design as well delivering the drug by dual stimuli: saline and esterase enzyme. However, the cytotoxicity of the polymer, polymer-drug conjugates and their cellular uptake mechanism are further need to be confirmed. Nevertheless, the current investigation provides the first time insight to the development of biodegradable block copolymer assemblies for loading and delivering cisplatin.

Conclusions

A biodegradable block copolymer structure was developed with enzymatic cleavable polycaprolactone backbone and carboxylic acid functional groups for anchoring cisplatin drugs. The polymer-drug conjugate appeared in the form of 110 nm spherical particles, which could in principle be administered selectively to the cancer tissue via EPR effect. The drug conjugate was very stable and can be stored in water for a long period without any cleavage. The dispersion of the polymer-drug conjugate in saline and PBS leads to the burst release of 35 % cisplatin in the reservoir for immediate drug administration. About 20–30 % of the remaining drugs undergo slow and controlled release over a period of 6–7 d. In the presence of esterase enzyme, the biodegradable PCL aliphatic ester linkage cleaved instantaneously and all the cargoes (100 % of the drugs) released. Thus, the multi-stimuli cleavable biodegradable block copolymer scaffold is a potential vector for cisplatin delivery for cancer treatment.

Experimental Section

Materials: Tin(II) 2-ethylhexanoate [$Sn(Oct)_2$], polyethylene glycol monomethyl ether (MW = 2000, here after referred as PEG), cisplatin and *o*-phenylenediamine(OPD) were purchased from Sigma Aldrich. PEG and catalyst $Sn(Oct)_2$ were dried under vacuum prior to use. All other solvents like tetrahydrofuran (THF) and trifluoroacetic acid (TFA) are purchased locally and distilled and kept in an inert atmosphere prior to use. The monomer *tert*-butyl 3-[(7-oxooxepan-4-yl)oxy] propanoate was synthesized as reported by us earlier.[13]

Measurements: NMR was recorded with a 400-MHz JEOL NMR Spectrophotometer. All NMR spectra were recorded in $CDCl_3$ containing TMS as internal Standard. Gel permeation chromatographic (GPC) analysis was performed with Viscotek VE 1122 pump, Viscotek VE 3580 RI detector, and Viscotek VE 3210 UV/Vis detector in tetrahydrofuran (THF) using polystyrene as standards. Thermal stability of the polymers was determined with a Perkin-Elmer thermal analyzer STA 6000 model at a heating rate of 10 °C·min^{-1} in a nitrogen atmosphere. Water contact angle measurements were performed with a GBX model (DIGIDROP contact angle instrument) using windrop software. Extreme care has been taken in carrying out sessile contact angle measurements to monitor contact angle values within 1 min to avoid the evaporation effects (repetition of the word contact angle). All contact angle measurements were carried out at room temperature (27 °C) under constant humidity (40–50 %). The absorption spectra were recorded with a Perkin-Elmer Lambda 45 UV/Visible spectrophotometer. Dynamic light scattering (DLS) was done with a Nano ZS-90 apparatus utilizing 633 nm red laser (at 90° angle) from Malvern Instruments. FE-SEM images were recorded with a Zeiss Ultra Plus scanning electron microscope. For FE-SEM analysis, the samples were prepared by drop casting on silicon wafers and coated with gold. TEM images were recorded with a Technai-300 instrument by drop casting the sample on Formvar-coated copper grid. The fluorescent micrographs were collected with a Carl Zeiss Axiovert 200 microscope.

Polymer Synthesis: Ring opening polymerization of *tert*-butyl 3-[(7-oxooxepan-4-yl)oxy] propanoate was performed with $[M_0]/[I_0]$ = 100

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and polyethylene glycol monomethyl ether as initiator by following our earlier report. PEG (77.5 mg, 0.0387 mmol) was taken in a flame dried Schlenk tube and dry toluene (2.0 mL) was added in a nitrogen atmosphere. To this mixture, $Sn(Oct)_2$ (7.8 mg, 0.0193 mmol) was added and the content was stirred at 25 °C for 15 min under nitrogen purge. The monomer (1 g, 3.87 mmol) was added to the above mixture and the polymerization mixture was stirred at 25 °C for 15 min under nitrogen purge. The polymerization tube was immersed in preheated oil bath at 110 °C and the polymerization was continued for 48 h with constant stirring. The polymerization mixture was precipitated in MeOH. The polymer was re-dissolved in THF and precipitated again in methanol. The purification was done at least twice to obtain highly pure polymer. Yield: 710 mg (71 %). **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.13 (s, 2 H, OCH_2), 3.64 (m, 3.8 H, PEG and $-OCH_2-$), 3.45 (s, 1 H, $-CH-$), 3.38 (s, 3 H, OCH_3), 2.44 (t, 2 H, $COCH_2$), 2.35 (t, 2 H, $COCH_2$), 1.93–1.81 (m, 2 H, $-CH_2-$), 1.81 –1.67 [m, 4 H, $CH_2(CH_2)$], 1.44 (s, 9 H, *tert*-butyl) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 173.63 (*C*=O), 170.81 (O=*C*–O), 80.72 [O*C*H(CH_2)], 75.58 (OCH_2), 70.68 (OCH_2), 65.13 ($COCH_2$), 61.47 ($COCH_2$), 36.60 (CH_2), 33.04 (CH_2), 29.81(CH_2), 28.86 (*tert*-butyl), 28.22. **FT-IR**: $\tilde{\nu}$ = 2973, 2931, 1726 (C=O ester), 1457, 1364, 1251, 1156, 1156, 1099, 1062, 957, 898, 845, 757 cm^{-1}. GPC molecular weights: M_n = 18,800, M_w = 25,700 and M_w/M_n = 1.36.

Synthesis of Carboxylic Substituted Poly(Caprolactone) (PEG-*b*-$CPCL_{100}$): Trifluoroacetic acid (1 mL) was added slowly into PEG-*b*-$BuCPCL_{100}$ (500 mg) in dry DCM (15 mL) and the polymer solution was stirred at 25 °C for 30 min. The solvents were evaporated and the polymer was re-dissolved in THF and precipitated in cold methanol. The purification was repeated at least twice to get pure polymer. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.13 (t, 2 H, CH_2OH), 3.63 (m, 3.8 H, PEG and OCH_2), 3.55 (m, 1 H, OCH), 2.54 (t, 2 H, CH_2COOH), 2.37 (t, 2 H, $COCH_2$), 1.97–1.65 [m, 4 H, –OCH (CH_2)$_2$]. **FT-IR**: $\tilde{\nu}$ = 3447, 2932, 2450, 1711(C=O acid), 1355, 1257, 1175, 1096, 1059, 955 cm^{-1}.

Preparation of Aquated Cisplatin $[Pt(NH_3)_2(OH_2)_2]^{2+}$: For synthesis of aquated cisplatin, 18 mg (0.059 mmol, 1 equiv.) of cisplatin was partially dissolved in H_2O (18.0 mL). To this mixture, silver nitrate (20.3 mg, 0.119 mmol, 2 equiv.) was added and the resulting reaction mixture was stirred at room temperature for 24 h. Formation of aquated cisplatin confirmed by milky white colored silver chloride precipitation. Silver chloride was removed by centrifuging at 10,000 rpm for 1 h. Finally, the aquated cisplatin was obtained by filtration through 0.2 μm filter.

Synthesis of the Polymer-cisplatin Conjugate: The diblock polymer was dissolved in NaOH (2 mL, 1 mg·mL^{-1}) solution and left for stirring at 37 °C for 30 min. Aquated cisplatin (14 mg, 33 mmol, and lyophilized sample) was added to the prepared activated polymer solution. Reaction mix was left for 12 h at 37 °C. The solution was transferred to a dialysis bag (MWCO = 1000) and dialyzed against large amount of distilled water for 2 d. Fresh distilled water replaced periodically to ensure the removal of un-encapsulated molecules from the dialysis tube.

The drug loading efficiency (DLE) and drug loading content (DLC) were determined by absorption spectroscopy using OPD colorimetric assay from the following equation:

DLE (%) = {weight of drug in vesicles / weight of drug in feed} × 100 %

DLC (%) = { weight of drug in vesicles / weight of drug loaded vesicles} × 100 %

***o*-phenylenediamine (OPD) Colorimetric Assay:** Samples with unknown cisplatin (Pt) content were added to 0.5 mL of OPD solution in *N,N*-dimethylformamide (DMF) (1.2 mg·mL^{-1}) and heated for 2 h at 100 °C. The amount of Pt present in the sample was determined by measuring the absorbance at 706 nm (absorbance maxima of OPD-Pt complex). Molar extinction coefficient was calculated for OPD-Pt calculated as 24,310 L·mol^{-1}·cm^{-1}, alike to literature.[24] The concentration of Pt released from the conjugate was expressed as a ratio of the amount of platinum in the releasing solution from the polymer backbone.

In vitro Drug Release Studies: Cisplatin loaded nanoparticles were taken in a dialysis bag (in 3 mL) and they were immersed in a 100 mL beaker and dialyzed at 37 °C with constant stirring. At specific time intervals, 1.0 mL of the dialysate was withdrawn and replaced with an equal volume of fresh buffer (or) saline. The amount of molecule (or drug) released in each aliquot was measured using OPD colorimetric assay by using absorption spectroscopy to quantify their percentage of cumulative release. Cumulative release (%) = $C_n \times V_o$ / $m \times 100$ where C_n is the amount of loaded cargo in n^{th} sample, V_o is the total volume and m is the total amount loaded in vesicles. For esterase assisted release studies 10 units of enzyme was used, above mentioned procedure was followed for calculation of cumulative release.

Supporting Information (see footnote on the first page of this article): NMR spectra, GPC plots, drug loading content calculations, SAED pattern of polymer are given.

Acknowledgements

The authors thankfully acknowledge research grants from Department of Science and Technology (DST), New Delhi, INDIA, under nano-mission initiative project SR/NM/NS-42/2009 and SERC Scheme project SR/S1/OC-37/2013. Bapurao thanks CSIR, New Delhi, India for research fellowship. Pramod thanks IISER-Pune for research fellowship.

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Received: January 17, 2014
Published Online: April 8, 2014

