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Volume 2 • Issue 3 • 2018

Review Article

Chronicles of Pharmaceutical Science

ISSN: 2572-7761

Block Copolymer Micelles in Drug Delivery and Cancer Therapy

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Received: April 16, 2018; **Published:** April 24, 2018

Abstract

Block copolymer micelles has been emerged as a better and safer alternatives to small surfactant molecules for drug delivery applications. This review is focused on the design of block copolymers along with the possible mechanism of drug loading in the hydrophobic core of the micelles. Further, the fabrication methods of micelles is being discussed along with shortcomings of individual procedure. In addition, the advantages of polymeric micelles in targeted cancer therapy has been stated giving emphasis on active and passive targeting. Finally, the prospective and improvement possibility in selection of block copolymers has been argued.

Keywords: *Micelles; Targeted delivery; Amphiphilic block copolymer; Drug delivery; Active targeting; Passive targeting*

Volume 2 Issue 3 April 2018

Introduction

A surfactant is a molecule, which comprises both a water soluble and insoluble portion. Surfactants can form micelles after being dispersed in aqueous solutions by self-assembly above their critical micelle concentrations (CMC). The CMC is defined as the concentration of surfactant molecules above which they start forming micelles. However, small surfactant molecules such as sodium lauryl sulphate, polysorbates etc. usually have a very high CMC value thus can dissociate upon dilution in the bloodstream or other biological fluids in vivo. Due to this limitation, the use of these surfactants as drug delivery vehicles is limited and therefore alternative amphiphilic block copolymers surfactants have been developed to address this problem [1]. Polymeric micelles prepared from amphiphilic block copolymers have recently attracted more attention due to their unique structure with low CMC values.

Amphiphilic block copolymers can form micelles in aqueous solvent with a hydrophobic core sterically stabilized by a hydrophilic shell (figure 1). The hydrophobic core serves as a reservoir for drugs with low aqueous solubility while the hydrophilic shell prevents the adsorption of opsonise on the surface. Additionally the nano-scopic sized polymeric micelles (10–200 nm in diameter) are sufficiently large to avoid renal excretion (>50 kDa) as well as small enough to bypass the filtration of inter-endothelial cells in the spleen. All these factors contribute towards the longer blood circulation time of micelles, which leads to improved accumulation at tissue sites

with vascular abnormalities [2-5]. Poly(ethylene) glycol (PEG) is the polymer of choice to be used as the hydrophilic block whereas the hydrophobic block can be chosen based on the required application including but not limited to poly (lactic acid), poly (caprolactone), poly (aspartic acid), poly(decalactone), poly(carbonate), polyion complex etc. [2,3, 6-8].

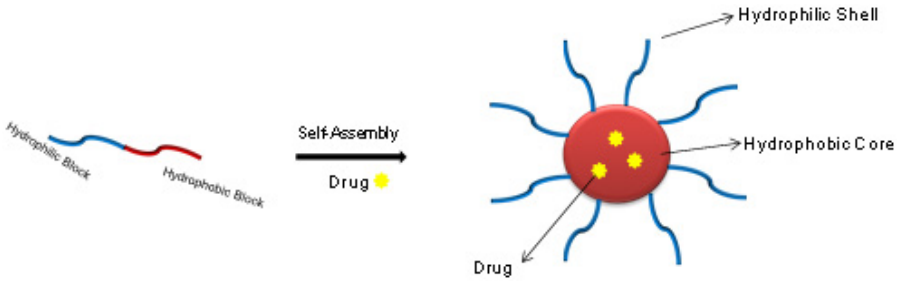

Figure 1: *Pictorial presentation of self-assembly of an amphiphilic block copolymer into micelles when dispersed in water [9].*

Some of the reasons, which makes PEG consistently a polymer of choice for fabricating amphiphilic block copolymers are related to its inexpensive, non-toxic nature, and is a FDA approved polymer for the use in drug products [3]. Additionally, in micelles structure, PEG forms a dense, brush-like shell which imparts steric stability to the formulation [4]. Further, PEG is known to increase the circulatory time of carriers by impeding their uptake by the cells of the Reticuloendothelial System (RES) [10]. Moreover, PEG can be easily functionalised to attach the targeting ligands for targeted drug delivery applications [11-13].

Polymeric micelles have been widely utilised as solubilising tool for hydrophobic drugs [3]. The micelle structures are known to have an anisotropic distribution of water and therefore the core of the micelles is usually water free [14]. During the drug loading procedure, the hydrophobic drugs migrate towards the hydrophobic block (core) due to the hydrophobic interaction. Hydrophobic interaction is defined as the interaction between the non-polar substances in water. This interaction brings the non-polar (hydrophobic) molecules together in order to have minimal contact with water. This is a spontaneous process and is reasonably stronger than other weak intermolecular forces such as hydrogen bonding [15,16]. Therefore, during drug encapsulation procedure, hydrophobic core and drug come together to obtain drug loaded micelles. Furthermore, hydrophilic block provides the steric stability to micelles due to which they remain well dispersed in aqueous solution without aggregation [2,4]. In terms of thermodynamics, the drug solubilisation in micelles core can be considered as a partitioning of the drug between polar and non-polar phases [14]. In addition to the solubilisation tool, micelles have also known to increase the bioavailability, reduce the toxicity and offer the control release of loaded drugs leading to patient compliance [4,17].

As shown in figure 1, drug molecules are generally localised within the hydrophobic core separated from the outside environment by hydrophilic shell. This unique feature prevents the direct interaction of encapsulated drugs with the physiological environment such as cells or body fluids. This in turn, prevents any undesirable pharmacodynamics and pharmacokinetics reactions, which leads in improved bioavailability and reduction in toxicity of a drug. Using polymeric micelles as a drug delivery carrier is certainly beneficial because of various advantages it holds over other carrier systems like easier preparation method with tunable property, good loading capacity and better formulation stability [3,4,23,24]. All these advantages are due to the unique structure (core-shell) of polymeric micelles as discussed above. A number of micelles formulations are already in the clinical trials such as NK012, SP1049C, NC-6004, NK911 etc., (figure 2) while FDA has approved Genexol-PM for the treatment of breast cancer [3,25].

Methods of Fabrication of Drug Loaded Polymeric Micelles

The four frequently used methods for the preparation of micelles and drug encapsulation are described below:

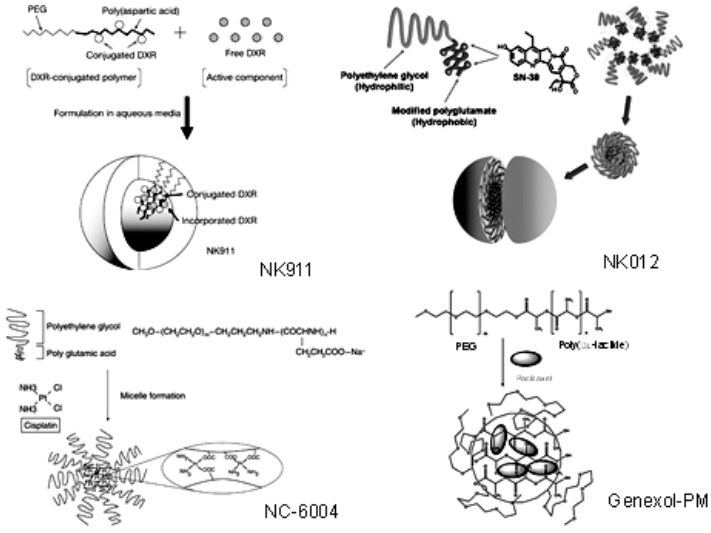

Figure 2: *Schematic presentation of NK911 [18], NK012 [19], NC-6004 [20] and Genexol-PM [21] micelle formulation.*

Dialysis Method

In this method, block copolymer and drug are dissolved in a water miscible non-volatile organic solvent (such as dimethyl sulfoxide and N, N-dimethyl form amide) followed by dialysis of the obtained solution against water. During dialysis, water will gradually replace the organic solvent from the dialysis bag leading to the self-assembly of amphiphilic polymer in micelles with encapsulated hydrophobic drug. It was suggested that during dialysis any unencapsulated drug will be removed from micellar solution leaving behind the drug loaded micelles only [22]. However, it should be noted that the replacement of organic solvent with water is a slow process. Hence, diffusion of some amount of drug into external media (water) might be possible before self-assembly. To avoid this problem Allen., *et al*. prepared the drug loaded micelles by adding the water directly to the drug-polymer solution (in DMSO) followed by dialysis in order to remove the solvent [23].

Oil-in-Water Emulsion Method

In this method, block copolymer and drug are dissolved in a water immiscible volatile organic solvent such as chloroform, ethyl acetate and methylene chloride. The solution is then slowly added to the aqueous phase under stirring to make an oil-in-water emulsion. In some cases, additional surfactants are also used to make a stable emulsion. The organic solvent is then evaporated at room temperature to yield the drug loaded micelles [22,24]. However, the use of chlorinated solvents are not usually recommended in drug delivery applications.

Solvent Evaporation/Film Method

In this method, block copolymer and drug are dissolved in a suitable volatile organic solvent and then the solvent is evaporated to make a thin polymer-drug film on the wall of a flask. The film is then reconstituted with the aid of aqueous solvent by vigorous shaking to produce the drug loaded polymeric micelles [24,25]. Large scale production is possible with the solvent evaporation method. However, the use of this method is not preferred to make micelles from block copolymers with high hydrophobic to hydrophilic ratio. Due to the high hydrophobicity, the complete reconstitution of such polymers by simple mixing is difficult [22].

Co-solvent evaporation/Nanoprecipitation Method

In this method, block copolymer and drug are dissolved in a water miscible volatile organic solvent (such as acetone, tetrahydrofuran) and then added drop wise to water under stirring. The diffusion of solvent in water with simultaneous evaporation triggered the self-assembly of copolymer, yielding the drug loaded polymeric micelles [26,27]. In this method, solubility of copolymer and drug is must in the chosen solvent, which makes this procedure troublesome on few occasion.

Polymeric Micelles in Cancer Therapy

Cancer therapy (chemotherapy) needs targeted delivery of cytotoxic drugs to tumours to avoid unwanted side-effects, which are attributed to the distribution of drugs in normal tissues. Targeted delivery of drugs to the tumors (cancer cells) can be achieved with the aid of suitable drug delivery carriers based on active and passive targeting strategies (figure 3) [28-32].

Indeed, polymeric micelles as a drug delivery carrier for cytotoxic drugs offer numerous advantages in chemotherapy [24, 33-35]. For instance, the incorporation of cytotoxic drugs into micelles has been reported to increase the half-life of drug by circumventing its elimination by the liver and/or kidneys thus increasing the bioavailability [34]. Additionally, small size micelles have been reported to passively target the tumors by the Enhanced Permeability and Retention (EPR) effect [36,37]. Moreover, many anticancer drugs are hydrophobic in nature and hence encapsulating them within polymeric micelles can enhance their aqueous solubility (thus they can be more easily administrable in the body) and consequently bioavailability [24,34, 35]. Furthermore, the controlled release of bio-actives for a longer duration at a tumor site can also increase the effectiveness of treatment [24,34,35]. In addition to that, the shell of polymeric micelles can be modified for active targeting by attaching specific ligands. This modification enhanced the selectivity of polymeric micelles for tumor cells and consequently improved the intracellular drug delivery [34,38]. Thus, the use of micelles for cancer therapy can be beneficial in order to improve the bioavailability and to reduce the side effects of anticancer drugs [36,37].

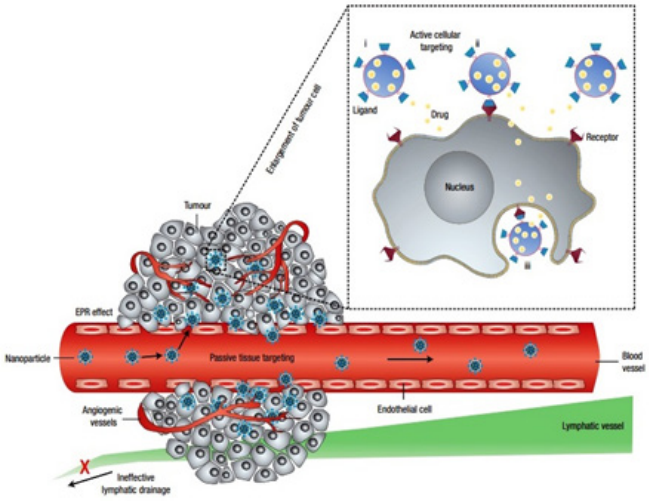

Figure 3: *Schematic presentation of targeted therapy to tumors with the aid of nanoparticles (micelles) by active and passive mechanism [32]. (Reproduced with permission).*

Passively targeted micelles for Cancer Therapy

Targeting solid tumors using long circulatory drug delivery carriers via the Enhanced Permeability and Retention (EPR) effect is considered as passive targeting. The EPR effect was first described by Maeda and co-worker [39]. Physiological and pathological studies of solid tumours suggested that the tumor vasculature possessed some unique characteristics such as incomplete architecture and immature lymphatic capillaries. Tumor vasculature generally has poorly aligned and defective endothelial cells with broad fenestrations (up to 4 μm) and lacking smooth muscle layer (or innervations and functional lymphatics). Additionally, impaired receptor function for vasoactive mediators especially angiotensin II in tumor vascular has been observed (figure 3) [28,30].

The excessive production of vascular mediators, such as vascular endothelial growth factor (VEGF), bradykinin, fibroblast growth factor (bFGF), nitric oxide, peroxynitrite, prostaglandins, and matrix metalloproteinase, are responsible for the hyper-permeability in tumor tissues [40,41]. VEGF, a protein excessively secreted by tumors, plays an important role in the angiogenesis process which includes degradation of vascular basement membrane and surrounding extracellular matrix, as well as vascular endothelial cell division

and migration [5]. This enhanced vascular permeability ensures the adequate supply of oxygen and nutrients for rapid growth of tumor tissues [41,42]. Recently, reduction in vascular permeability in colon carcinomas when treated with anti-VEGF antibody confirmed the role of VEGF in enhanced permeability of tumor vasculature [43]. Furthermore, due to the defective lymphatic function in tumors, continuous draining and renewal of interstitial fluid is minimal [44]. As a result, high retention time of a macromolecule has been observed in tumor tissues compared to normal tissues [32,45]. These two factors (i.e. Enhanced Permeation and Retention) comprise the EPR effect, due to which selective extravasation and accumulation of macromolecules in tumor tissues were observed [37,39,41].

Indeed several polymeric micelle formulations have been reported which accumulate at the tumor sites via the EPR effect [37]. For instance, PEG-poly (g-benzyl L-glutamate) block copolymer micelles loaded with cisplatin, demonstrated high accumulation in solid tumor in Lewis lung carcinoma bearing mice, compared to free drug. The high accumulation at the tumor site was suggested to occur via the EPR effect due to the prolonged blood circulation and small size (approx. 30 nm in diameter) of micelles [46]. This formulation is now in Phase II clinical trials with the trade name "NC-6004"(20). NK105, PEG-poly (aspartic acid) micelles loaded with paclitaxel is another formulation which is in clinical trials. Approximately 50% of carboxylic acid groups of poly (aspartic acid) have been modified with 4-phenyl-1-butanol in the NK105 formulation, which increased the hydrophobicity of polymer and eventually paclitaxel loading (23% w/w approx.). The average size of 85 nm was observed with this formulation after redispersion in aqueous solvent. Approximately, 90-fold increase in the plasma area under curve (AUC), 25-fold increase in tumor AUC in Colon-26 tumors bearing CDF1 mice was observed, when compared with free drug. This high tumor uptake efficiency was attributed to the EPR effect of long circulatory NK105 micelles. Phase II clinical trials of NK105 were conducted in Japan, which was successfully completed in 2010 with positive results. Phase III Studies are on-going on patients with breast cancer and due to end by September 2016 [47,48]. Some more examples of polymeric micelles studied for tumor targeting via EPR effect are listed in table 1.

Polymer	Drug	Size of micelles
PEG2000-PE/Vitamin E (49)	Paclitaxel, Curcumin	15-20 nm
Pluronic® L61 and F127 (SP1049C) [50]	Doxorubicin	30 nm
mPEG-b-poly(D,L-lactide)[51]	Docetexal	16.62 ± 0.31 nm
mPEG-b-poly(D,L-lactide) (Genexol-PM)[52]	Paclitaxel	< 50 nm

Table 1: *Examples of micelles formulations, which demonstrated enhanced tumor uptake by EPR effect. (mPEG- monomethoxyl PEG).*

Actively Targeted Micelles for Cancer Therapy

Tumor targeting potential of polymeric micelles can be further enhanced by attaching the targeting ligands on to the micelle surface (actively targeted micelles). The concept of active targeting is based on the ligand–receptor interactions at the target site i. e. tumor. After reaching the target site, ligand decorated micelles should interact with certain specific receptors present on the tumor cell and then be internalised by receptor-mediated endocytosis (figure 3 and 4) [30,34,38].

Increase in the cellular concentration of anticancer agents via receptor mediated endocytosis leads to superior therapeutic efficacy of the drugs. This in turn reduces the dose size and side effects of cytotoxic drugs [53,54]. The selection of ligands is usually based on any receptor, which is overexpressed by tumor cells or tumor vasculature but have minimal or no expression by normal cells. Commonly used targeting ligands include antibodies, peptides, proteins, carbohydrates, small organic molecules and aptamers. The attachment of a ligand on to the surface of micelles is generally achieved either by the post-modification of a block copolymer with bifunctional spacer molecules or by the direct synthesis of hetero-bifunctional blocks [2]. Several polymeric micellar formulations based on ligand mediated targeting have been reported in literatures and were reviewed recently [24,34,55].

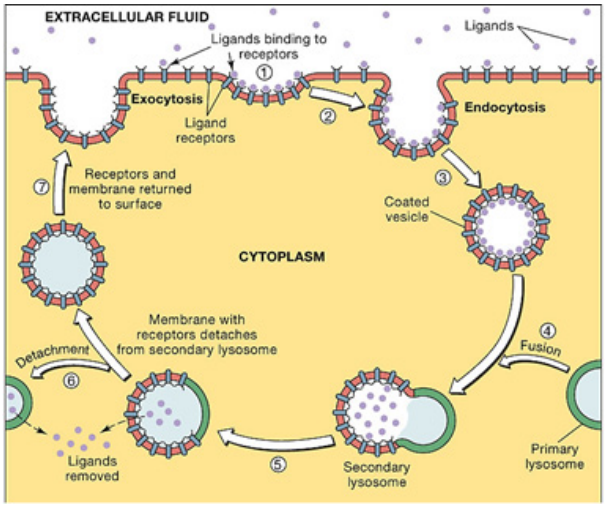

Figure 4: *Receptor mediated endocytosis mechanism of a ligand after being attached to the specific receptor (source - http://droualb.faculty.mjc.edu).*

For instance, monoclonal antinucleosomal antibody (2C5) conjugated poly (ethylene glycol)-block-phosphatidyl ethanolamine (PEG-b-PE) micelles loaded with Doxorubicin (DOX) have been tested in a DOX-resistant ovarian cancer cell spheroid model. The 2C5 conjugated micelles demonstrated higher uptake (two fold) and penetration with greater cell death in spheroids compared to free DOX and non-targeted DOX micelles. The mean size observed for PEG–PE targeted micelles was 15 nm [56]. In another study Herceptin conjugated to d-α-tocopheryl polyethylene glycol succinate (vitamin E TPGS) micelles have been developed for targeted co-delivery of docetaxel and siRNA [57]. Antibodies are very popular as targeting ligands, but only limited conjugation of these moieties on micelle surface is possible due to their large size (~150 kDa). Furthermore, rapid clearance of antibody conjugated micelles might be observed due to their potential immunogenicity [58,59].

Transferrin (T_f) (protein) conjugation is another widely studied approach to fabricate targeted carriers for the specific delivery of cytotoxic drugs to the cancer cells [60,61]. For instance, Yue., *et al*. developed the transferrin conjugated mPEG-b-PLA polymeric micelles for their enhanced uptake in cancer cells [62]. The size range of the micelles was between 85-110 nm. They were tested on three human cell lines, SGC-7901 (gastric carcinoma), SKOV-3 (ovarian carcinoma), and MCF-7 (breast carcinoma) for uptake studies. Higher uptake of T_f- conjugated micelles (TfM-RhB) was evident by confocal laser scanning microscopy (CLSM) (using Rhodamine as marker) on MCF-7 and SGC-7901 cell lines compared to T_f-free micelles (M-RhB). SKOV-3 cells expressed a low level of transferrin and hence little difference in uptake was observed between TfM-RhB and M-RhB (figure 5). This study suggested that the high uptake was due to transferrin receptor mediated endocytosis [62]. High cellular uptake and effective tumor growth inhibition have been also demonstrated by using arginylglycylaspartic acid (RGD) (peptide) [63,64], lactose [65] and galactose [66] (carbohydrates) and A10-aptamer [67] as targeting ligand.

Due to the higher expression level of folate receptors in tumors (100 to 300 times) compared to normal tissue, folic acid (FA) as targeting ligand has been widely studied for cancer chemotherapy [68-70]. Folic acid is a commercially available small molecule that can be easily conjugated on to micelles surfaces [71]. Qiu., *et al*. reported the fabrication of targeted micelles using folate-modified poly (2-ethyl-2-oxazoline)-b-poly (ε-caprolactone) (FA-PEOz-PCL) block copolymer [72]. DOX loaded FA-PEOz-PCL micelles with the size range of 157-191 nm were tested for cellular uptake using folate receptor positive (FR+) Human HeLa cervical carcinoma cell lines (HeLa), human KB nasopharyngeal epidermal carcinoma cell lines (KB), Multidrug-resistant human breast cancer MCF-7/ADR cell lines and folate receptor negative (FR-) human A549 lung adenocarcinoma cell lines.

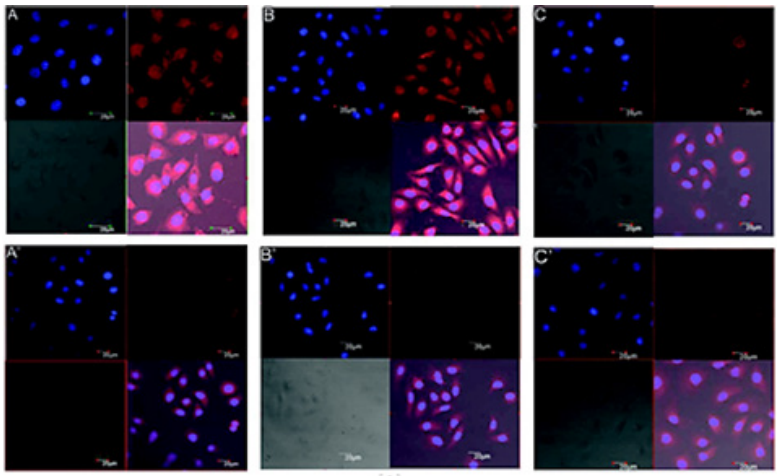

Figure 5: *CLSM images of human MCF-7 (A and A'), SGC-7901 (B and B'), and SKOV3 (C and C') cells incubated with TfM-RhB (A, B, and C) or M-RhB (A', B', and C')[62] (reproduced with permission).*

A higher cellular uptake of folate conjugated micelles (FA4) was observed with FR+ cell lines compared to non-folate micelles (FA0) and DOX. Further, folate receptor mediated endocytosis was confirmed by addition of free folic acid in cell culture media (FA4 + Folate). Addition of free folic acid competes with folate receptors for binding and thus reduced uptake of FA4 as evident by CLSM images. Moreover FA4 demonstrated lower IC_{50} values in FR+ cell lines compared to FA0 [72]. Recently, folic acid conjugate redox-responsive cross-linked block copolymer loaded with doxorubicin has been reported [73]. Microscopy images demonstrate that the conjugation of FA enhanced the cellular uptake efficiency whereas sustained release of drug was observed in environment mimicking tumor.

Conclusion

Polymeric drug-delivery systems have been investigated to address the problems associated with drugs such as poor aqueous solubility, stability and significant side effects. Indeed, polymeric micelles as a drug delivery carrier have demonstrated their potential to address some of the above mentioned problems as discussed earlier. Polymeric micelles can be easily prepared by conjugating a hydrophilic and hydrophobic polymer followed by its dispersion in aqueous solvent. Further, the unique core-corona structure of polymeric micelles provides satisfactory stability to this formulation. Due to these advantages, several polymeric micelles have been studied for the effective treatment of cancer and some of them are in clinical trials.

Apparently, biodegradable polymers because of their low toxicity and biodegradability are the polymers of choice to fabricate micelles for in vivo applications. Undoubtedly, polyesters are the front-runner biodegradable polymers used to generate the micelles. Polyesters derived from renewable feedstocks recently have attracted more attention due to the depletion of fossil fuel reserves and their increased prices. However, new sustainable materials are produced frequently; their applications in drug delivery have been rather less investigated.

Conflict of interest

Author declares no conflict of interest.

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