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Original article

Renewable poly(δ-decalactone) based block copolymer micelles as drug delivery vehicle: *in vitro* and *in vivo* evaluation

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ABSTRACT

Polymers from natural resources are attracting much attention in various fields including drug delivery as green alternatives to fossil fuel based polymers. In this quest, novel block copolymers based on renewable poly(δ-decalactone) (PDL) were evaluated for their drug delivery capabilities and compared with a fossil fuel based polymer i.e. methoxy-poly(ethylene glycol)-b-poly(ε-caprolactone) (mPEG-b-PCL). Using curcumin as a hydrophobic drug model, micelles of PDL block copolymers with different orientation i.e. AB (mPEG-b-PDL), ABA (PDL-b-PEG-b-PDL), ABC (mPEG-b-PDL-b-poly(pentadecalactone) and (mPEG-b-PCL) were prepared by nanoprecipitation method. The size, drug loading and curcumin stability studies results indicated that mPEG-b-PDL micelles was comparable to its counterpart mPEG-b-PCL micelles towards improved delivery of curcumin. Therefore, mixed micelles using these two copolymers were also evaluated to see any change in size, loading and drug release. Drug release studies proposed that sustained release can be obtained using poly(pentadecalactone) as crystalline core whereas rapid release can be achieved using amorphous PDL core. Further, mPEG-b-PDL micelles were found to be non-haemolytic, up to the concentration of 40 mg/mL. *In vivo* toxicity studies on rats advised low-toxic behaviour of these micelles up to 400 mg/kg dose, as evident by histopathological and biochemical analysis. In summary, it is anticipated that mPEG-b-PDL block copolymer micelles could serve as a renewable alternative for mPEG-b-PCL copolymers in drug delivery applications.

1. Introduction

In drug delivery applications, amphiphilic block copolymers are used extensively owing their inherent self-assembly behaviour into diverse nanostructures, such as micelles (Gaucher et al., 2005; Lu and Park, 2013). The term "micelles" defines the aggregation of amphiphilic molecule in core-shell structure, above their critical micelle concentrations (CMC) when dispersed in solvent usually water (Azum et al., 2017b; Kumar and Rub, 2016) (Fig. 1). The CMC is defined as the concentration of amphiphilic molecules in solvent, above which they start forming micelles (Azum et al., 2017a). Amphiphilic block copolymers with poly (ethylene glycol) (PEG) as hydrophilic block such as PEG-b-poly (lactic acid) (PEG-b-PLA), PEG-b-poly(caprolactone) (PEG-b-PCL), PEG-b-poly(aspartic acid) (PEG-b-PA) etc., have been extensively studied as drug delivery carriers. The hydrophobic block in such copolymers can be chosen based on the required application; however, those derived from renewable resources have gained utmost interest, because of their environment friendly nature, abundant availability and in most cases biocompatibility, biodegradability and non-toxicity (Zhang et al., 2017). Additionally, polymers from renewable resources fitting in the concept of "acting responsibly to meet the needs of the present without compromising the ability of future generations to meet their own needs" (Vilela et al., 2014). Therefore, several renewable feedstocks from either plant or animal sources have been discovered, to synthesize polymers with tunable properties.

Micellar formulations have already shown their presence in the market. Genexol PM® (micelles of PEG-b-PLA) have been approved

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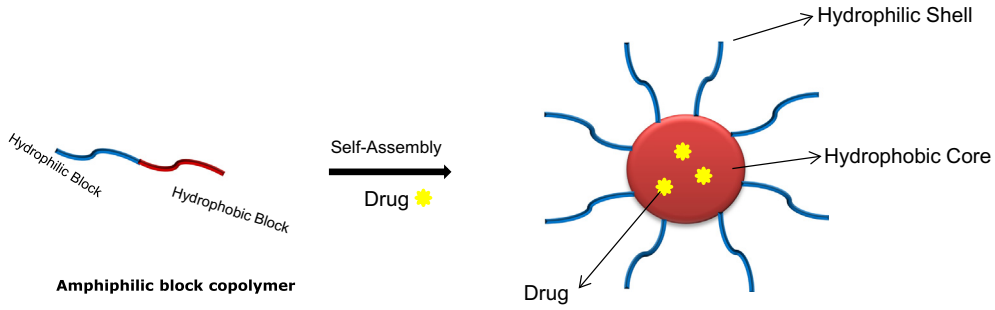

Fig. 1. Pictorial presentation of self-assembly of an amphiphilic block copolymer into micelles when dispersed in water.

in South Korea for the treatment of breast cancer (Weissig et al., 2014) whereas NC 6004 (micelles of PEG-b-PAA) and NK 911, NK10 (micelles of PEG-b-PA) are in clinical trials (Weissig and Guzman-Villanueva, 2015). Genexol PM, though, have been fabricated using renewable polymer (PLA), but is expensive and has certain limitations. A few reports suggested that the drug loading achieved using PLA block polymer or their copolymers are usually low owing to its lower hydrophobicity. Therefore, more hydrophobic derivatives of lactide have been prepared (Trimaille et al., 2004, 2006; Yin and Baker, 1999); however, the synthesis procedure appears to be tedious and expensive. Moreover, in PLA-derived drug delivery materials, the production of excessive acid from PLA degradation can cause deleterious effects on loaded acid-sensitive drugs, thus limits its application (Kang and Schwendeman, 2002; Meyer et al., 2012). Therefore, the quest for new polymers from renewable resources continues, which could serve as a alternative for such existing polymers.

Recently, we have reported the synthesis of amphiphilic block copolymers derived from an economical and renewable monomer i.e. δ-decalactone (Bansal et al., 2015). Studies performed on the poly(δ-decalactone) (PDL) derived copolymers suggested that they have remarkable potential to act as a drug delivery carrier. Therefore, in the present study, we have compared the drug delivery capability of micelles of PDL with non-renewable PCL block copolymer using curcumin as a model drug. Micelles of block copolymers were fabricated using a revised nano-precipitation method (Schubert et al., 2011) as this offers advantages over the other methods. PCL block copolymer have been investigated earlier for the improved delivery of curcumin and therefore, chosen here for a comparative study. It has been demonstrated that mixed micelles prepared from two or more different block copolymers were capable to enhance the formulation stability and drug loading efficiency compared to the micelles prepared from single block copolymer (Attia et al., 2011). Therefore, mixed micelle formulation using mPEG-b-PDL and mPEG-b-PCL copolymer has been also fabricated to study the effect on curcumin loading content and release pattern. Another important parameter, which needs to be addressed for polymers in drug delivery, is toxicity (Ghanghoria et al., 2018). Hence, an *ex vivo* haemolysis study was conducted to measure haemocompatibility of mPEG-b-PDL polymers. Further, micelles were also tested on rats for *in vivo* sub-chronic toxicity.

2. Materials and Methods

2.1. Materials

Curcumin (≥99.5%), triton X-100 (BioXtra), haematoxylin and eosin solution have been purchased from Sigma-Aldrich and used as received. Previously synthesised block copolymers of PDL and PCL have been used in all studies (Bansal et al., 2015) (Scheme 1). All the solvents used were purchased from Fischer Scientific UK.

2.2. Methods

2.2.1. Micelles preparation from PDL and PCL block copolymers

Curcumin loaded micelles of block copolymers were prepared by a single-step nano-precipitation method with minor modifications (Gou et al., 2011). Briefly, curcumin (2 mg) was dissolved along with the polymer (50 mg) in acetone (5 mL) and added drop wise into Milli-Q water (10 mL) under stirring (1000 rpm). The solution was then stirred for 3 h at room temperature and left overnight (open vial) to ensure the complete removal of acetone. Curcumin is light sensitive and hence the whole process was performed in the dark . Empty micelles were prepared using same procedure without curcumin. Mixed micelles of mPEG-b-PDL and mPEG-b-PCL were fabricated by physical mixing (Bae et al., 2007) of both copolymer (25 mg each) in acetone (5 mL) and the method described above was employed to obtain curcumin-loaded mixed micelles.

Curcumin loaded micelles were purified by passing through PD10 Desalting Column (Sephadex G-25 Medium, GE Healthcare Life Sciences). In this procedure, materials of <5 K molecular weight were retained giving the sample (eluent) free from any unencapsulated drug. Separately, the crude micellar solution was also purified by filtration using a membrane syringe filter (pore size: 220 nm) (Millex-LG, Millipore Co., USA) to determine the efficiency of individual purification method. A part of the micellar solution was freeze dried for the determination of drug content.

2.2.2. Characterisation of micelles for size, zeta potential and surface morphology

For the size and polydispersity index measurements, micelle samples (50 μg/mL) in Milli-Q water were analysed on a Malvern NanoZS instrument. Surface zeta potential was measured from same instrument in HEPES 10 mM buffer (pH-7.4). TEM images were taken to confirm the size and to determine the surface morphology. Samples were imaged on TEM grids without staining. All the measurements were performed on three different batches and the mean values were reported.

2.2.3. Curcumin content, stability and in vitro release behaviour from micelles

Drug Content (DC) and Encapsulation Efficiency (EE) of curcumin in micelles were determined by dissolving the known amount of freeze dried samples of micelles in acetone followed by quantification of the drug concentration using fluorescence spectroscopy (Varian) after appropriate dilutions. For analysis, samples were excited at a fixed wavelength (λ_{ex} = 420 nm) and spectra were recorded in a range of 450–600 nm (Fig. 2) (Leung and Kee, 2009). The excitation and emission slit widths were selected at 5 nm and selected emission intensity was 524 nm. Amount of curcumin present in sample was than calculated using curcumin standard calibration curve prepared in acetone. All stud-