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A flexible polymersome system with tunable morphology and release profiles for efficient intracellular delivery

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ABSTRACT

Polymersomes are widely used as drug delivery system however they have shortcomings in drug-eluting properties that are attributable to the high molecular weight of the copolymers forming their membrane. Here we demonstrate for the first time how novel class of polymersomes from very short, liquid to soft star-shaped copolymers can be empowered to form an efficient drug delivery system. The copolymers undergo self-assembly in water into a stable, nano-sized rod or a spherical shape polymersomes. Increasing the Mw of the hydrophobic moieties the CMC value is decreased accompanied with the tendency to form a more spherical structure. The poorly water-soluble anticancer drug camptothecin was loaded into the fabricated polymersomes, resulting in a high drug loading content, and released over a period of over three days. Furthermore, this biocompatible system could deliver a variety of drugs intracellularly in a rapid yet controlled manner. Therefore, this nano system's tailorable properties, biocompatibility and ability to incorporate hydrophobic drugs and release them intracellularly are desirable traits for anti-cancer delivery system and other biomedical applications.

1. Introduction

During the last two decades there has been a significant advance in drug-delivery research that has led to more effective pharmaceutical therapy. Novel controlled release formulations increase the therapeutic efficiency by maintaining the concentration of the drug within the therapeutic window for longer periods, thus allowing optimal effectiveness with minimal toxic effects (Brannon-Peppas and Blanchette, 2012). However, cancer treatment still remains a challenge, in part because many anti cancer drugs are poorly water-soluble which diminish their bioavailability and therapeutic efficacy (Yu et al., 2016). Furthermore, in cancer treatment intracellular delivery is required in order to exert the therapeutic action inside the cytoplasm or other specific organelles (Gupta et al., 2005). Thus, new concepts that offer viable solutions for hydrophobic anti cancer molecules are much needed.

Polymersomes are widely used as drug delivery systems: they are formed easily, vary in size, possess a large drug loading capacity and may allow site-specific targeting (Zhang et al., 2012). Their ability to increase water solubility of hydrophobic molecules enables them to be used to deliver poor water soluble drugs while improving their pharmacokinetics profile (Song et al., 2014). Furthermore, polymersomes have the ability to encapsulate high payloads of both hydrophobic and hydrophilic drugs inside either the hydrophobic core of the membrane or the aqueous cavity (Chen et al., 2010; Christian et al., 2009).

In addition, current polymersome systems can encapsulate hydrophilic molecules within their aqueous interior and hydrophobic molecules within their thick and stable membrane (Meng et al., 2009). This ability, however, also has disadvantages: polymersomes are often impermeable to many small organic molecules, ions, and water (Discher and Eisenberg, 2002), and as a result, they show incomplete (lower than 20%) (Xu et al., 2005) or a very slow release rate (Zhong and Feijen, 2008). This inherent drawback is related in part to the high molecular weight of the copolymers forming the polymersome causing impermeability of their thick membrane (Discher et al., 1999).

Particles from amphiphilic copolymers often suffer from low drug loading, in some cases substantially less than 1% (Cheng et al., 2007). This problem is intensified in cancer therapy where the majority of anti-cancer drugs are poorly water-soluble (Le Garrec et al., 2004). For example, the loading capacity of camptothecin (CPT), a potent anti-cancer drug (Wall et al., 1966), could hardly exceed 1% in nanoparticles prepared from amphiphilic poly (ethylene glycol) (PEG)-polycaprolactone (PCL) based copolymers (Cai et al., 2015b). Attempts to overcome the low CPT capacity were

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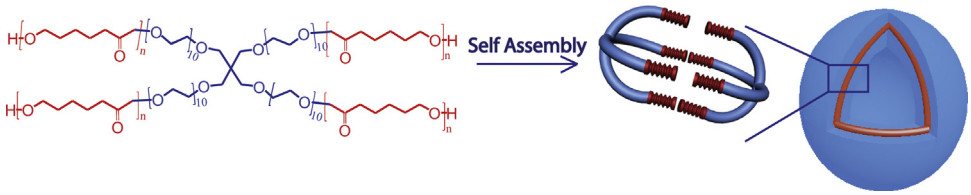

Scheme 1. Schematic illustration of PEG_4-PCL self-assembles into a polymersome structure.

carried out by conjugating the drug to high molecular weight PEG, however, in order to keep the complex water soluble, CPT content was limited to only 0.86%wt (Yu et al., 2005).

In the present research, we hypothesized that amphiphilic copolymers with liquid core, as we termed "soft", can surmount the inherent deficiencies of currently used polymersome systems. Since it has been suggested that multi-armed (star-shaped) copolymers are more stable than linear copolymers of similar composition and molecular weight (Hyung et al., 2013; Mizrahi et al., 2013), applying these copolymers could potentially maintain particle stability without forcing us to compromise on membrane permeability. We synthesized a series of short star-shaped copolymers consisting of a low molecular weight (Mw = 2000 Da) PEG core and PCL segments (PEG_4–PCL) of various lengths. When in water, the copolymers undergo self-assembly into a polymersome structure (Scheme 1). In two samples (PEG_4-PCL 10:2 and 10:4 monomer ratio), the PEG segments weighed more than the PCL segment (1:0.4 and 1:0.8, accordingly). Another sample had a similar weight ratio (10:5 monomer ratio equivalent to 1:1.2 wt ratio), and in two samples (PEG_4-PCL 10:6 and 10:20) the weight ratio was in favor of the PCL segment (1:1.5 and 1:5, accordingly). We chose PEG_4 and PCL because of their low immunogenicity and toxicity (Peppas et al., 1999; Woodruff and Hutmacher, 2010). In this study, we show for the first time, that short, multi-armed copolymers are capable of forming effective vehicles for CPT.

2. Materials and methods

2.1. Chemicals

Four-armed PEG (Mw = 2 KD) was purchased from JenKem Technology Co., Ltd. (Beijing, China). ε-caprolactone monomer (CL), stannous octoate ($Sn(Oct)_2$), chloroform-d, linear PEG standards, 1,6-diphenyl-1,3,5-hexatriene (DPH), Nile red, Dulbecco's phosphate buffered saline (PBS) pH 7.4, Dulbecco's modified Eagle's medium (DMEM) were purchased from Sigma Aldrich (MO, USA). All solvents were purchased from Bio-Lab Ltd. (Israel). Fetal bovine serum (FBS) was purchased from Gibco-Invitrogen Corp (NY, USA). Camtothecin (CPT) 99.58% was purchased from Chem-Impex Int'l inc (IL, USA). Fluoromount G with DAPI was purchased from SouthernBiotech (AL, USA). Penicillin-Streptomycin and L-Glutamine were purchased from Biological industries (Israel). Paraformaldehyde was purchased from Electron Microscopy Sciences (PA, USA). CellTiter 96® Aqueous One Solution Cell Proliferation Assay (MTS) kit was purchased from Promega (WI, USA).

2.2. Syntheses and characterization of PEG_4-PCL

Synthesis of PEG_4-PCL was accomplished according to published procedure (Xie et al., 2008) (Fig. 1A). Briefly, 4.2 mmol PEG_4 were dissolved in 200 mL toluene at 120 °C. 50 mL of the toluene were collected using a Dean Stark apparatus, followed by addition of caprolactone monomer in different ratios (Table 1) and $Sn(Oct)_2$ as catalyst. The mixture was refluxed for 12 h at 110 °C, and when cooled to room temperature, the solvent was removed under reduced pressure by a rotary evaporator. The obtained polymer was dissolved in minimal amount of methylene chloride, precipitated in petroleum ether and dried in a desiccator overnight.

The chemical structures of pure PEG_4 (without PCL) and of the obtained copolymers were evaluated by ^1H NMR using Bruker Avance III 400 MHz NMR spectrometer (MA, USA) in $CDCl_3$. The molecular weight was determined by gel permeation chromatography (GPC) using a Viscotek GPC-mak VE 2001 GPC–solvent/

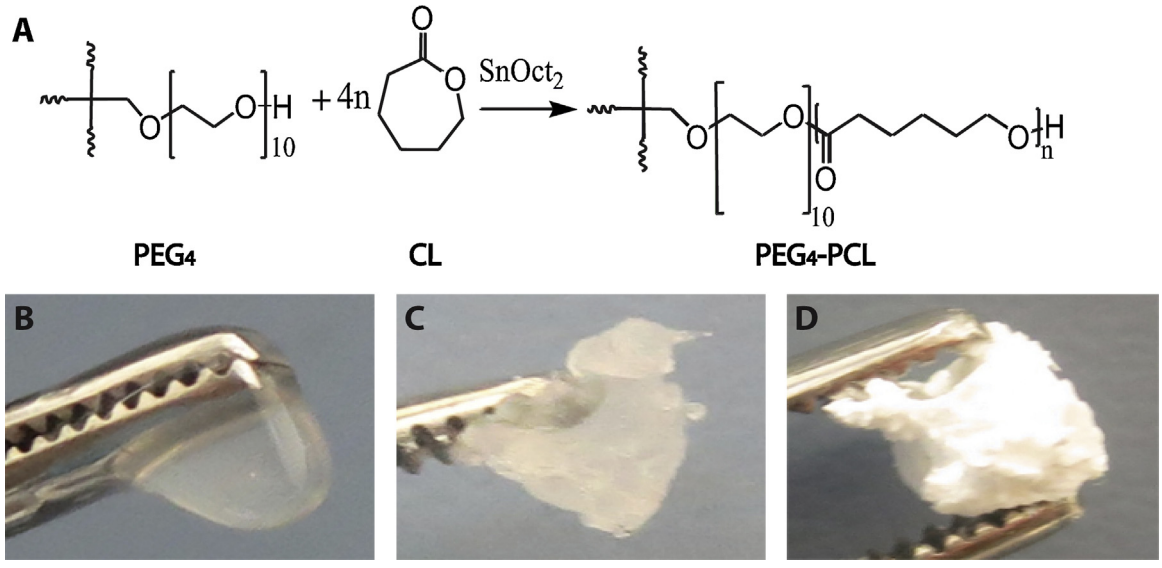

Fig. 1. (A) Synthesis scheme of PEG_4-PCL. Various PEG_4-PCL copolymers at room temperature: (B) 10:2 (C) 10:6 (D) 10:20.