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Facilitated Self-Assembly of Novel Dendron-Based Copolymers

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Abstract— **Self-assembly of newly synthesized dendron-based amphiphilic copolymers with controlled hydrophilic-lipophilic balances has been investigated to evaluate their potential as a novel nanocarrier. The hydroxyl-terminated polyester dendron (G3) bearing a focal alkyne moiety was used to mediate the combination of poly(ε-caprolactone) (PCL) with multiple polyethylene glycol (PEG) moieties. Four types of PCL-G3-mPEG with different block lengths were prepared and their structures were confirmed by ^{1}H NMR, FT-IR, and GPC. Critical micelle concentration (CMC) values varied from 6.50×10^{-8} to 3.52×10^{-7} M, which were lower than those reported for linear PCL-mPEG. TEM revealed that all PCL-G3-mPEG micelles were spherical with an average diameter of 20 nm. The drug release profile for each PCL-G3-mPEG was investigated by loading indomethacin (IMC), as a model drug, within the micelles. IMC was released in a controlled manner over 72 hours. Synthesized copolymers used in this study were also found to be non-cytotoxic at concentrations up to 100 μM. The low CMC, along with the controlled morphology, release profile and biocompatibility, all demonstrate the potential of the dendron-based micelles as a novel nanocarrier.**

I. INTRODUCTION

Over the past decade, significant advances have been made in the development of polymeric micelles to treat and detect cancer effectively; and various design strategies have been implemented to enhance cancer targeting(Peer, Karp et al. 2007; Sutton, Nasongkla et al. 2007). The hydrophilic-lipophilic balance (HLB) between polymer chains is a crucial factor used to describe the self-assembly behavior of polymers and is strongly associated with the degree of micellar dissociation and blood circulation time augmenting the enhanced permeability and retention (EPR) effect. In addition, by controlling the HLB it has been shown that a variety of morphologies can be induced (e.g. vesicular, spherical, cylindrical micelles) via self assembly as a result of the interplay between thermodynamic forces(Israelachvili, Mitchell et al. 1976). A well-defined density of targeting ligands on the surface and their adopted geometry are also important to produce enhanced selective binding to cancer tissues as supported by recent studies on multivalent cancer targeting(Kostiainen, Hardy et al. 2005; Hong, Leroueil et al. 2007). To this regard, a dendron, a segment of a dendrimer, is a unique material that not only retains the properties of its parent dendrimer (symmetry and monodispersity) but through distinctive chemical modifications of its focal point and periphery can be hybridized with other materials to create amphiphilic structures that self-assemble and exhibit unique biological responses(Rosen, Wilson et al. 2009).

In this study, we have synthesized novel conical dendron-based copolymers with a controlled HLB through a multi-step synthetic procedure and the self-assembly behaviors such as critical micelle concentration, size, and shape were studied along with thermodynamic aspects. Release of indomethacin (IMC) was investigated using indomethacin (IMC) as a model drug and a cell viability assay determined that the synthesized copolymers were non-cytotoxic at the concentrations tested.

II. EXPERIMENTAL

A. Reagents

Hydroxyl-terminated poly(ε-caprolactone) (PCL) polymers (M_n 3500 and M_n 14000) were purchased from Polymer Source (Montreal, Canada). Methoxy polyethylene glycol amines (mPEG-NH_2, M_n 2000 and M_n 5000) were purchased from JenKem Technology (Beijing, China). Polyester-8-hydroxyl-1-acetylene bis-MPA dendron (G3 dendron) and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification.

B. Synthesis and characterization of PCL-G3-MPEG

Each terminal hydroxyl group of PCL3.5K and PCL14K was tosylated (Ts) or brominated (Br), respectively, to introduce an azide group required for *'click'* chemistry with the G3 dendron bearing a focal alkyne moiety. PCL3.5K-Ts and PCL14K-Br were then converted into PCL-N_3 using an excess amount of NaN_3. *'Click'* chemistry between G3 dendron and PCL-N_3 was carried out at 80 °C in the presence of PMDETA and CuBr as a catalyst, followed by conjugation of mPEG-NH_2 after activation of hydroxyl groups at the periphery of the dendron using *p*-NPC and pyridine (Figure 1). The chemical structures and the molecular weights of copolymers were confirmed by ^{1}H-NMR, FT-IR, and gel permeation chromatography (GPC). The critical micelle concentration (CMC) of PCL-G3-mPEG was determined by a fluorescence method using pyrene as a probe. The morphology and size distribution of micelles was analyzed by transmission electron microscopy (TEM) and dynamic light scattering (DLS).

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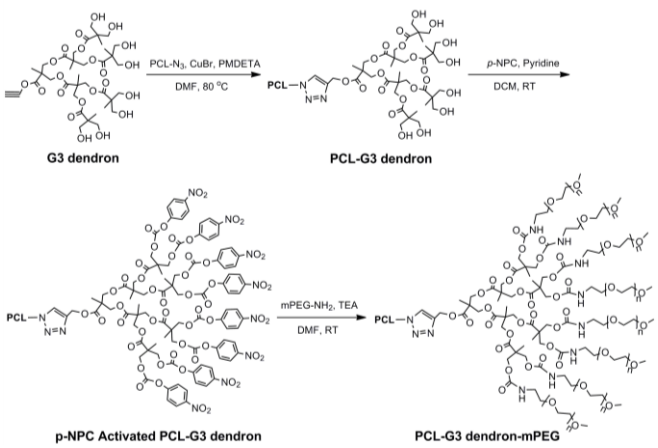

Figure 1. Synthesis of PCL-G3-mPEG. Copper bromide; CuBr, Pentamethyldiethylenetriamine; PMDETA, Triethylamine; TEA, para-nitrophenyl chloroformate; *p*-NPC.

C. IMC loading and release test.

IMC-loaded micelles were prepared using the dialysis method. The drug loading efficiency was measured using UV-Vis after dissolving IMC loaded micelles completely in DMSO. For the IMC release test, a dialysis bag (MWCO 3.5K) containing IMC loaded micelles was immersed in 30 mL PBS (pH 7.4, 0.01M) and placed in a shaking water bath at 37 °C and shaken at a speed of 50 rpm. At predetermined time intervals, 5 mL of solution was withdrawn and fresh PBS was added to replenish the volume. The released samples were lyophilized, dissolved in DMSO and IMC content was analyzed by UV-Vis.

D. Cell Culture Treatment and Cytotoxicity Assay of Dendron-based Copolymers

The KB cell line (ATCC, Manassas, VA, USA) was grown continuously as a monolayer in a humidified incubator at 37 ^{o}C and 5% CO_2 in GIBCO RPMI 1640 medium. The medium was supplemented with penicillin (100 units/mL), streptomycin (100 mg/mL), and 10% heat-inactivated fetal bovine serum (Invitrogen Corporation, Carlsbad, CA, USA) before being used for experiments. 5×10^3 cells were seeded in 96-well plates one day prior to the experiments in RPMI medium. Cells were treated with a range of concentrations from 0.01-100 μM of each copolymer. After each incubation time, the cells were washed with PBS containing calcium and magnesium and incubated for an additional day. Cell viability was assessed by using a CellTiter 96 Aqueous One Solution (MTS) Assay (Promega, Madison, WI, USA) following the manufacturer's protocol. UV absorbance was measured at 490 nm and mean cell viabilities were determined relative to a negative (untreated) and positive control (0.1% Triton-X, Sigma-Aldrich).

III. RESULTS AND DISCUSSION

In order to prepare four types of amphiphilic PCL-G3-mPEG with different hydrophobic PCL and hydrophilic PEG block lengths, the hydroxyl group of PCL was converted to azide and various reaction conditions for *'click'* chemistry were tested. As a result of the dendron hybridization, the molecular weights of PCL-G3-mPEG depending on the conjugated mPEG increased significantly, resulting in copolymers with a large peripheral hydrodynamic volume compared to the core PCL block volume (Table 1).

TABLE I
MOLECULAR WEIGHTS OF THE VARIOUS PCL-G3-MPEGS

Sample	M_n^{Theor}	M_n^{NMR}	M_n^{GPC}	PDI
PCL3.5K-G3-mPEG2K	21990	26280	24290	1.07
PCL3.5K-G3-mPEG5K	44720	48090	38900	1.06
PCL14K-G3-mPEG2K	32490	32000	27710	1.16
PCL14K-G3-mPEG5K	55220	58780	54140	1.38

The conical shape imposed on each PCL-G3-mPEG copolymer allows for the preferential formation of spherically shaped aggregates in aqueous solution with a low entropy penalty due to the pre-organized geometry(Kratzat and Finkelmann 1996; Chen, Zhang et al. 2007). Low CMC values were obtained, ranging from 6.50×10^{-8} M to 3.52×10^{-7} M, which were lower than those of linear PCL-MPEG copolymer counterparts(Kim, Shin et al. 1998; Forrest, Won et al. 2006; Liu, Zeng et al. 2007). PCL14K-G3-MPEG2K had the lowest CMC value among them. We observed a trend that as the hydrophobic proportion of the PCL-G3-mPEG molecular weight increased, the CMC decreased.

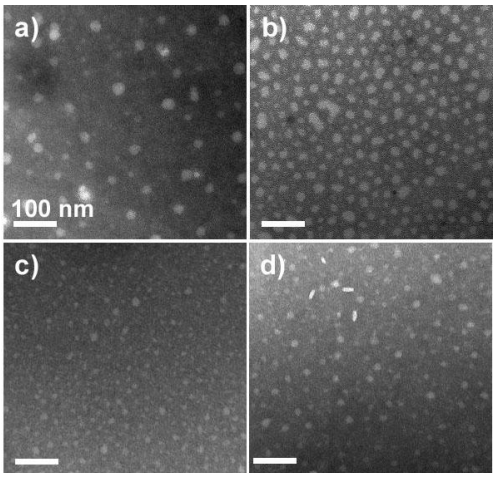

Figure 2. TEM images of self-assembled structures. (a) PCL3.5K-G3-mPEG2K, (b) PCL3.5K-G3-mPEG5K, (c) PCL14K-G3-MPEG2K, (d) PCL14K-G3-MPEG5K. All samples were prepared at 0.2 mg/mL and stained with a single drop of 2% PTA. Scale bars = 100 nm.

TEM images showed that all PCL-G3-mPEG micelles were spherical in shape with an average diameter of 20 nm (Figure 2). The size of PCL-G3-MPEG micelles was smaller

than linear PCL-mPEG micelles (ca. 40 nm) (data not shown). The discrepancy in sizes can be attributed to the dense packing of PCL-G3-mPEG copolymer into micelle structures due to their pre-formed architecture(Suek and Lamm 2008). For the IMC release test (Figure 3a), each micelle was shown to achieve a biphasic controlled release profile. Micelles that were composed of the PCL14K had a decreased rate of drug release which is most likely due to the increased hydrophobic interactions between the IMC and the PCL. For all micelles, release was determined to be complete after approximately 72 hours.

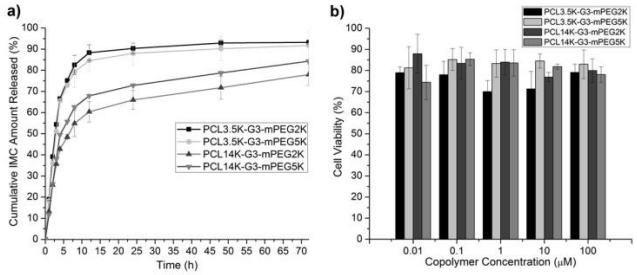

Figure 3. (a) Release profiles of IMC from dendron-based micelles. (b) MTS assay results for cell viability of dendron-based copolymers. n=3 for all measurements.

Cytotoxicity of the various copolymers was assessed using an MTS assay (Figure 3b). All copolymers exhibited non-significant cytotoxicity after 24 h incubation over a concentration range of 0.01 μM to 100 μM. These results provide strong evidence that the dendron-based PCL-G3-mPEG copolymers are well suited for drug delivery application.

In summary, a systematic study of the synthesis and evaluation of PCL-G3-mPEG as a potential drug delivery platform is presented. G3 dendron was able to mediate the combination of PCL with multiple PEG moieties in a controlled manner and the self-assembled structures produced spherical morphologies with high drug loading content. PCL-G3-mPEG micelles exhibited a controlled release of IMC over a period of 72 hours and negligible cytotoxicity at high concentrations up to 100 μM. For these reasons, dendron-based micelles have a great potential to be used as a novel drug delivery platform.

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