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Highly lipophilic pluronics-conjugated polyamidoamine dendrimer nanocarriers as potential delivery system for hydrophobic drugs

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ABSTRACT

In the study, four kinds of pluronics (P123, F68, F127 and F108) with varying hydrophilic-lipophilic balance (HLB) values were modified and conjugated on 4th generation of polyamidoamine dendrimer (PAMAM). The obtained results from FT-IR, ^{1}H NMR and GPC showed that the pluronics effectively conjugated on the dendrimer. The molecular weight of four PAMAM G4.0-Pluronics and its morphologies are in range of 200.15–377.14 kDa and around 60–180 nm in diameter by TEM, respectively. Loading efficiency and release of hydrophobic fluorouracil (5-FU) anticancer drug were evaluated by HPLC; Interesting that the dendrimer nanocarrier was conjugated with the highly lipophilic pluronic P123 (G4.0-P123) exhibiting a higher drug loading efficiency (up to 76.25%) in comparison with another pluronics. Live/dead fibroblast cell staining assay mentioned that all conjugated nanocarriers are highly biocompatible. The drug-loaded nanocarriers also indicated a highly antiproliferative activity against MCF-7 breast cancer cell. The obtained results demonstrated a great potential of the highly lipophilic pluronics-conjugated nanocarriers in hydrophobic drugs delivery for biomedical applications.

1. Introduction

In recent years, amphiphilic copolymers or thermoresponsive materials have paid much attention in drugs delivery studies and biomedical applications [1,9,10]. These amphiphilic copolymers could self-aggregate forming nano (micro) particles which load hydrophobic drugs in its core. The materials have been expected to solve problems of several kinds of hydrophobic drugs such as water solubility, biological effects, low absorption and the short storage time in blood stream [6,16,30,33–35]. Several kinds of nanocarriers based on thermosensitive amphiphilic (co)polymers such as poly (*N*-isopropylacrylamide) (*p*-NiPAM), polylactide-*co*-glycolide (PLGA), poly(2-(dimethylamino)ethyl methacrylate)-poly (methacrylate isobutyl polyhedral oligomeric silsesquioxane) (PDMAEMA-POSS), poly (ethylene glycol)-*block*-poly (propylene glycol)-*block*-poly (ethylene glycol) (pluronic), etc, have a great drug loading efficiency and sustainable release. In fact, Stevanovic et al introduced some drug delivery systems from PLGA-based nanoparticles into effectively delivering rifampicin, vincristine sulfate and paclitaxel [28,30,34,35]. Also, *p*-NiPAM showed a high curcumin loading capacity, reaching to 86% of total fed drug and a prominent controlled release system [3]. In addition, PDMAEMA-POSS drug-encapsulated copolymers display excellent gene transfection efficiency compared with polyethylenimine or PDMAEMA homopolymers [17]. Pluronic-based nanocarrier encapsulating doxorubicin has been highly expected to clinical trial [7].

Besides, development of amphiphilic copolymer nanocarriers, dendrimer nanocarriers are also one of the most studied materials. The dendrimers contain internal cavities which can be ultilized as a novel nanocarrier for drug, protein and gene delivery. Moreover, dendrimer having externally exposed amine or carboxylic groups could be decorated with target-specific or/and drug molecules [15,31,32]. Several reports indicated that anticancer drugs (camptothecin, 6-mercaptopurin, methotrexate, 5-FU, and paclitaxel) were encapsulated into the PAMAM dendrimer exhibiting a significant enhancement of its water solubility, storage stability, reduction of side-effects, and anti-tumor activity [8,19–21,25]. However, there are a few disadvantages accompanied with the amine-terminated dendrimer-based carriers including hemolytic toxicity and cell lysis due to a strong interaction of its positively charge and a negative charge on cell membrane resulting in disruption of the cellular membrane [2,4,5,13,14]. So, to reduce the toxicity, the amine-terminated dendrimer can modified its external functional groups with less toxic

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agents or biocompatible polymers such as poly (ethylene glycol), Pluronic F127. When conjugated with PAMAM, the functionalized molecules will shield or reduce cationic surface of PAMAM in order avoid interaction with anionic surface of the cell membrane leading to reduce toxicity of PAMAM and enhancing its biocompatibility [2,5,18,22,29].

Pluronics, poly (ethylene glycol)-*b*-poly (propylene glycol)-*b*-poly (ethylene glycol) copolymers are the biocompatible materials. Major difference of hydrophilic domains (PEG) and hydrophobic domain (PPG) in the structure of pluronics give different HLB values causing whether the copolymer solubilize in water or oil. Among of the copolymers, pluronic F127 and its derivatives have been the most widely studied materials for delivering several kinds of drugs and proven its loading efficiency [9–12,22]. Pluronic F127 was also used to functionalize PAMAM dendrimers. With over twenty kinds of commercially available pluronics [9], extensive studies of pluronics-conjugated materials would be an attractive field.

This study introduced pluronic-conjugated PAMAM G4.0 nanocarriers (G4.0-P123, G4.0-F68, G4.0-F127 and G4.0-F108) which utilized the highly inner cavity of dendrimer molecule and hydrophobic interaction within pluronic chains to increase drug loading capacity and control its delivery system. Effect of varying lipophilic pluronics on drug loading efficiency of its conjugated PAMAM dendrimer nanocarrier was also evaluated. Moreover, fibroblast biocompatibility of the conjugated nanocarriers and anti-proliferative activity against MCF-7 breast cancer cell of the drug-loaded nanocarriers were studied (Fig. 1).

2. Materials and methods

2.1. Materials

Pluronic P123 (P123), Pluronic F127 (F127), Pluronic F108 (F108), and Pluronic F68 (F68) were obtained from Sigma-Aldrich; characteristics of the pluronics is presented in Table 1. 5-FU was purchased from Merck Chemicals. 4-Nitrophenyl chloroformate (NPC) and tyramine (TA) were supplied from Acros Organics. Several kinds of Regenerated Cellulose dialysis bags with molecular weight cut-off at 3500 Da, 6000–8000 Da, 12,000–14,000 Da and 25,000 Da were purchased from Spectrum Laboratories Inc. All other chemicals were used without further purification.

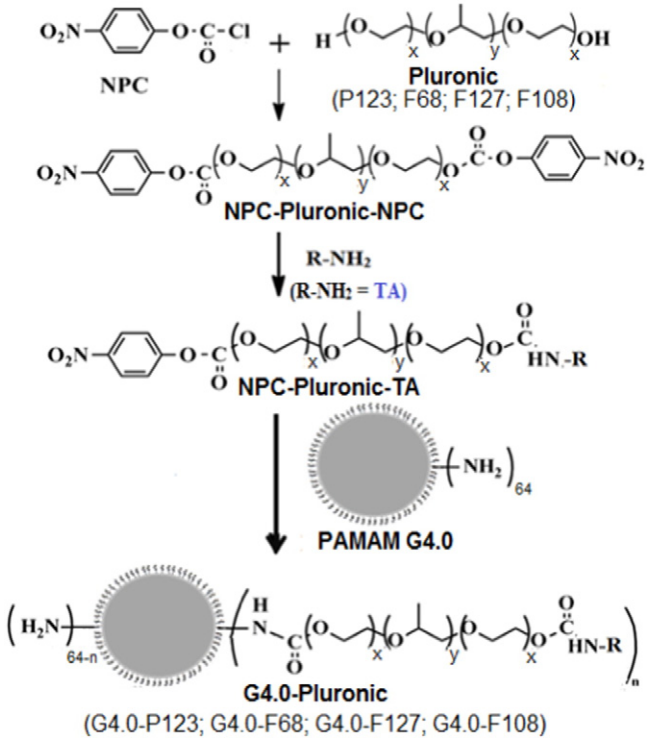

Fig. 1. Synthetic scheme of PAMAM G4.0-pluronic.

Table 1
Physicochemical characteristics of pluronic from Sigma-Aldrich.

Pluronic	Mw (Da)	Average EO units (x)	Average PO units (y)	HLB
P123	5,800	39	69	8
F127	12,600	200	65	22
F108	14,600	265	50	27
F68	8,400	152	29	29

Average molecular weight (Mw) and Hydrophilic-lipophilic balance (HLB) are provided by the Sigma-Aldrich. EO and PO are ethylene oxide and propylene oxide, respectively.

2.2. Synthesis and purification of dendrimer generations

PAMAM dendrimers (from Generation-0.5 to Generation 4.0) were synthesized from the core ethylenediamine (EDA) using step-wise Michael addition reaction, and amidation of multifunctional groups as reported of Donald Tomalia [31]. First, the PAMAM G-0.5 product was synthesized from ethylenediamine core and methyl acrylate via Michael addition for 4 days in the dark at 0–5 °C. The second step was the amidation reaction of the PAMAM G-0.5 external methyl carboxylate moieties with an excess amount of ethylenediamine to obtain PAMAM G0 generation. The synthetic processes of higher generations were conducted in same way. Low Mw dendrimers (from PAMAM G-0.5 to PAMAM G2.0) were purified via removal of small molecules by vacuum evaporator. Higher molecular weight dendrimers were purified through use of Regenerated Cellulose Membrane MWCO 3500 Da (for PAMAM G2.5); 5,000 Da (PAMAM G3.0); 6000–8,000 Da (PAMAM G3.5) and 12,000–14,000 Da (PAMAM G4.0) dialysis bags with MeOH as solvent in 48 h, dried and stored the products in MeOH. Generations of the dendrimers were characterized by ^{1}H NMR Spectrometer, ^{1}H NMR PAMAM G4.0: $\delta_H = 2.550$ ppm (a), $\delta_H = 2.770$ ppm (b), $\delta_H = 2.352$ ppm (c), $\delta_H = 2.746$–2.758 ppm (d) and $\delta_H = 3.225$–3.259 ppm (e). Other dendrimers were also confirmed by and ^{1}H NMR, MS and TEM [26].

2.3. Preparation of mono NPC-pluronics

Firstly, pluronics were activated in a free solvent process. Briefly, copolymer (1.25 mmol) was melted under vacuum at 70 °C, then NPC (3.75 mmol) was added to the molten form and stirred for 4 h to complete NPC activation. The reaction was adjusted to the room temperature and 10 mL of THF was added into the mixture. The polymer solution was dialyzed in THF to obtain NPC-activated pluronic. ^{1}H NMR result indicated typical peaks at δppm = 3.4–3.8 (—OCH_2—CH_2O—, pluronics); 4.44 (—CH_2—O—NPC, pluronics); 7.39–8.27 ($\mathbf{H}_{arom}$, NPC). Degree of activation was over 97% for all pluronics (by ^{1}H NMR).

Secondly, preparation of mono NPC-pluronics, to substitute one of two NPC groups of the activated pluronics, tyramine (TA, 0.055 mmol) was dissolved completely in 1 mL DMF and 5 mL THF, and added drop-wise into 5 mL of THF solution containing the activated pluronic (0.05 mmol) and then was stirred overnight. After this time, the reaction mixture was precipitated in excess diethylether, filtered and dried in vacuum condition to obtain a NPC-pluronic-TA copolymer. TA substituted about 55% of NPC in the activated pluronic. ^{1}H NMR ($CDCl_3$) of NPC-pluronic-TA, δppm = 3.4–3.8 (—OCH_2—CH_2O—, pluronic); 4.44 (—CH_2—O—NPC, pluronic); 4.2 (CH_2-O-TA, pluronic); 6.9–7.1 ($\mathbf{H}_{arom}$, TA); 7.39–8.28 ($\mathbf{H}_{arom}$, NPC).

2.4. Preparation of pluronics-conjugated PAMAM G4.0

To obtain the pluronics-functionalized PAMAM G4.0, 20 mL of THF solution of NPC-Pluronics-TA (0.2256 mmol) was added drop-wise