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Pegylated polyelectrolyte nanoparticles containing paclitaxel as a promising candidate for drug carriers for passive targeting

Krzysztof Szczepanowicz[a,*], Monika Bzowska[b], Tomasz Kruk[a], Alicja Karabasz[b], Joanna Bereta[b], Piotr Warszynski[a]

[a] Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences, Niezapominajek 8, 30-239 Kraków, Poland
[b] Department of Cell Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, 30-348 Kraków, Poland

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ABSTRACT

Targeted drug delivery systems are of special importance in cancer therapies, since serious side effects resulting from unspecific accumulation of highly toxic chemotherapeutics in healthy tissues can restrict effectiveness of the therapy. In this work we present the method of preparing biocompatible, polyelectrolyte nanoparticles containing the anticancer drug that may serve as a vehicle for passive tumor targeting. The nanoparticles were prepared via direct encapsulation of emulsion droplets in a polyelectrolyte multilayer shell. The oil cores that contained paclitaxel were stabilized by docusate sodium salt/poly-L-lysine surface complex (AOT/PLL) and were encapsulated in shells formed by the LbL adsorption of biocompatible polyelectrolytes, poly-L-glutamic acid (PGA) and PLL up to 5 or 6 layers. The surface of the nanoparticles was pegylated through the adsorption of the pegylated polyelectrolyte (PGA-g-PEG) as the outer layer to prolong the persistence of the nanocarriers in the circulation. The synthesized nanoparticles were stable in cell culture medium containing serum and their average size was 100 nm, which makes them promising candidates for passive targeted drug delivery. This notion was further confirmed by the results of studying the biological effects of nanoformulations on two tumor cell lines: mouse colon carcinoma cell line CT26-CEA and the mouse mammary carcinoma cell line 4T1. The empty polyelectrolyte nanoparticles did not affect the viability of the tested cells, whereas encapsulated paclitaxel retained its strong cytotoxic/cytostatic activity.

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1. Introduction

Cancer remains one of the leading causes of death worldwide [1,2]. The major obstacle that limits the effectiveness of many anticancer drugs is their inability to selectively target tumor cells; instead, they often strongly affect healthy tissues [2]. To overcome this problem, various drug targeting strategies are being developed. Generally, they are classified into two categories: passive and active targeting. The latter is based on the relatively selective interactions between drug-conjugated targeting ligands with tumor cells [3–6]. In contrast, passive targeting most often utilizes drug nanocarriers and is based on their normal biodistribution within the body upon intravenous administration. Unfavorably, after being injected into the bloodstream, nanocarriers are usually rapidly cleared from the circulation, as they are quickly opsonized and engulfed by macrophages. However, when the fast clearance of nanoparticles is minimized, a significant improvement in their bioavailability is achieved [7] and they may accumulate in solid tumors through a phenomenon called the enhanced permeability and retention effect (EPR). EPR was first reported in 1986 by Matsumura and Maeda [8]. The phenomenon can be explained by the tortuous architecture and increased permeability of tumor vasculature, which facilitate the extravasation of various macromolecules and nanoparticles. At the same time, a lack of proper lymphatic drainage enables retention of macromolecules within the tumors. Hence, the strategy based on EPR phenomenon is now exploited for passive delivering of a wide range of nanoparticles into tumors [8,9].

The concept of nanoencapsulation of drugs as the delivery strategy has been extensively explored. Sequential adsorption of polyelectrolytes, called the layer-by-layer (LbL) technique, is one of the most versatile methods of forming nanocarriers [10–12]. The use of nanoemulsion droplets as cores for encapsulation by the LbL adsorption [13–22] gives the possibility to enclose active components in nanosized containers with shells functionalized for targeted therapy. In order to obtain nanocarriers suitable for pas-

* Corresponding author.
E-mail addresses: ncszczep@cyf-kr.edu.pl, szczepanowicz@gmail.com (K. Szczepanowicz).

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sive drug targeting, a specific surface modification that prolongs their circulation is required. An important strategy to minimize the fast clearance is the formation of protein-resistant coatings, e.g. by the immobilization of polymers such as poly(ethylene glycol) (PEG), poly(acrylamide), poly(vinylpyrrolidone), polysaccharides, and dextrans on the surface of nanocarriers [23,24]. The most popular and promising strategy for the preparation of stealth particles, i.e. particles invisible to the immune system, is the immobilization of a PEG corona onto the particles' surface. PEG is an uncharged, hydrophilic polymer, whose extensive hydration, high conformational flexibility, and high chain mobility account for a steric exclusion effect [24]. The protein resistance of PEG-based coatings depends on both the length of chains and their density [25–28].

Paclitaxel, belonging to a class of taxanes, was registered as the recommended chemotherapeutic for the treatment of ovarian cancer, advanced breast cancer and non-small-cell lung cancer. Paclitaxel is insoluble in water and is currently formulated in Cremophor EL, which increases its bioavailability, but at the same time may have adverse effects, such as hypersensitivity reactions often observed during paclitaxel administration. Hence, the development of new paclitaxel formulations is actually an important approach for maximizing its antitumor activity and minimizing the side effects.

In this paper we describe the preparation of PEG-functionalized, multilayer, polyelectrolyte nanocarriers containing paclitaxel, and present their effects on cultures of two tumor cell lines: the mouse colon adenocarcinoma cell line CT26-CEA and the mouse mammary carcinoma cell line 4T1. The main concept of the nanoparticles preparation is illustrated in Fig. 1.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals for nanoparticle synthesis

Paclitaxel was obtained from Selleck Chemicals USA. The polycations: poly-L-lysine hydrobromide, PLL (MW ~ 15,000–30,000) and poly(fluorescein isothiocyanate allylamine hydrochloride), FITC-PAH (MW ~ 70000), polyanion, poly-L-glutamic acid sodium salt, PGA (MW ~ 15,000–50,000), docusate sodium salt (AOT), methoxypolyethylene glycol amine (MW ~ 5000), Human Serum Albumin (HSA), *N*-hydroxysulfosuccinimide sodium salt (NHS), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), chloroform, and sodium chloride were obtained from Sigma-Aldrich. All materials were used as received without further purification. The ultrapure water was obtained using the Millipore Direct-Q5UV purification system. PGA-g-PEG was synthesized according to the procedure described in Supplementary material.

2.1.2. Materials for cell culture and cytotoxicity tests

Mouse colon adenocarcinoma cell line, CT26 (ATCC CRL-2638) stably expressing human carcinoembrionic antigen (CT26-CEA), was a gift from Dr. Michał Bereta (Jagiellonian University, Kraków). Mouse mammary carcinoma cell line 4T1 (ATCC CRL-2539), stably expressing luciferase, was purchased from Dr. Gary Sahagian's lab (Tufts University, Boston). Reagents for the cell culture: Dulbecco's modified Eagle's medium (DMEM), heat-inactivated fetal calf serum (FCS), trypsin-EDTA and antibiotics (penicillin and streptomycin) were from Lonza. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) was purchased from Sigma-Aldrich and Lactate dehydrogenase (LDH) Cytotoxicity Detection Kit from Clontech Laboratories. Reagents for DNA laddering assay: proteinase K and RNase A were provided respectively by A&A Biotechnology and Syngen Biotech. Dimethylsulfoxide (DMSO), agarose, and EDTA were from BioShop.

2.2. Nanoparticle preparation

2.2.1. Nanocore/nanoemulsion preparation

Nanoemulsion droplets containing paclitaxel were formed by the addition of 0.1 ml of the oil phase containing negatively charged FDA-approved surfactant AOT and paclitaxel dissolved in chloroform to 200 ml of the PLL solution under gentle mixing with a magnetic stirrer (300 RPM). For optimization of nanocore/nanoemulsion preparation conditions, the concentrations of PLL varied from 50 to 300 ppm while the volume of the oil phase was fixed. The optimal oil phase/polycation ratio was determined by measuring the zeta potential and size distribution of nanoemulsion droplets and examining their stability. Due to the toxicity issue, chloroform was evaporated from prepared suspensions by a rotary evaporator under reduced pressure. As a reference sample empty nanoemulsion droplets were prepared.

2.2.2. Polyelectrolyte shell formation

After the formation of stable emulsion nanodroplets containing paclitaxel, the sequential adsorption of polyelectrolytes by the saturation method was performed to make the polyelectrolyte multilayer shell. A fixed volume of nanoemulsion/nanoparticles suspension was mixed with the oppositely charged polyelectrolyte solution using a magnetic stirrer and the shell formation was followed by the measurement of the suspension's zeta potential. The procedure of the sequential deposition of PLL and PGA layers was repeated until an appropriate number of polyelectrolyte layers in the shell was formed. We denote as NCn-PX the nanoemulsion containing paclitaxel encapsulated by n polyelectrolyte layers (NC1 corresponds to the drops with AOT/PLL surface complex). For the preparation of fluorescently labeled nanoparticles FITC-PAH was used instead of the third PLL layer (NCn-FITC). As the reference sample empty nanoparticles (NCn) were prepared using the same procedure.

2.2.3. Formation of the pegylated external layer

To generate the pegylated nanoparticles, PLL-terminated nanoparticles with five polyelectrolyte layers were coated with the single layer of PGA-g-PEG using the same procedure as described above for the multilayer shell formation, i.e., by adding PLL terminated nanoparticles (positively charged) into the filtered PGA-g-PEG polymer solution (NC6-PEG). The whole procedure of the capsule preparation was performed in sterile conditions.

2.3. Nanoparticles characterization

2.3.1. Nanoparticles size distribution measurements and determination of zeta potential

The size distribution and the zeta potential of the nanoparticles was determined by the Dynamic Light Scattering (DLS) and Laser Doppler Electrophoresis (LDA) methods, respectively, both from the Zetasizer Nano Series from Malvern Instruments. The obtained values were an average from at least three runs with a 20 measurements. Additionally, the size distribution of nanoparticles was determined by the Nanoparticle Tracking Analysis (NTA) technique with the NS500 instrument (NanoSight). All measurements were performed at 25 °C in 0.015 M NaCl.

2.3.2. Nanoparticles concentration measurement

The concentration of nanoparticles in suspension were determined by the Nanoparticle Tracking Analysis (NTA) technique with the NS500 instrument (NanoSight). All measurements were performed at 25 °C in 0.015 M NaCl.

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