Available online at www.sciencedirect.com
SCIENCE DIRECT
Acta Biomaterialia 2 (2006) 207–212
Acta BIOMATERIALIA
www.actamat-journals.com

Brief communication

# Novel block copolymer (PPDO/PLLA-b-PEG): Enhancement of DNA uptake and cell transfection

Shanta Raj Bhattarai [a], Ho-Keun Yi [b], Narayan Bhattarai [c], Pyoung Han Hwang [d], Hak Yong Kim [e,*]

[a] Department of Bionanosystem Engineering, Chonbuk National University, Chonju, South Korea
[b] Department of Biochemistry, School of Dentistry, Chonbuk National University, Chonju, South Korea
[c] Department of Materials Science and Engineering, University of Washington, Seattle, WA 98195, USA
[d] Department of Pediatrics, School of Medicine, Chonbuk National University, Chonju, South Korea
[e] Department of Textile Engineering, Chonbuk National University, 664-14, 1-Ga Dukjin-Dong, Dukjin-Gu, Chonju, Chhalabucdo 56-756, South Korea

Received 24 June 2005; received in revised form 19 September 2005; accepted 6 October 2005

**Abstract**

The cationic lipid mediated uptake of plasmid DNA by cells in monolayer culture was significantly enhanced with an aqueous solution of the block copolymer poly(*p*-dioxanone-co-L-lactide)-b-poly(ethylene glycol) (PPDO/PLLA-b-PEG). Plasmid uptake studies with DNA encoding the β-galactosidase gene and cytotoxicity evaluations were performed on MCF-7, NIH 3T3 and CT-26 cell lines. Transfection yields and time courses for maximum release of FITC labeled DNA in MCF-7 cells were observed and quantified by β-galactosidase assay and spectrofluorometry, respectively. The reported results suggest that the studied block copolymer might be useful for the enhancement of polycation mediated transfection and could find application in gene therapy.


*Keywords:* Poly(*p*-dioxanone-co-L-lactide)-b-poly(ethylene glycol); Biodegradable DNA; Cell transfection; Cell proliferation

## 1. Introduction

Polyelectrolyte complexes formed between DNA and polycations have recently been used for gene transfer in mammalian and bacterial cells [1,2]. Due to electrostatic binding of DNA and polycations, these complexes are spontaneously formed in aqueous media. Polycations like poly(L-lysine) (PLL), poly(*N*-ethyl-4-vinyl pyridinium bromide) (PEVP), and others permit uptake of the complex and can achieve cell transfection in the absence of binding with receptor [3]. Transfection reagents (Lipofectin and calcium phosphate) are commercially available for this purpose, but one problem encountered using these techniques is the relatively low efficacy of DNA (or complex) release from endocytic compartments in the cytoplasm and nucleus of the cell [1,2], resulting in low efficiency of gene expression. Further, due to charge neutralization, these complexes are often unstable in aqueous solutions and precipitate, thereby hindering their application in gene delivery [4].

Recently, nonionic micelles or unimers of surfactant block copolymers have been used to transfer water insoluble drugs and polypeptides [4,5]. Work on these systems suggested that they enhance the transport of charged molecules across cell membranes [6]. To the best of our knowledge, there has been no previous report on applying the block copolymer poly(*p*-dioxanone-co-L-lactide)-b-poly(ethylene glycol) (PPDO/PLLA-b-PEG) as an enhancer to improve polycation mediated cell transfection. Focusing on this application, we prepared an aqueous solution of the triblock copolymer which was synthesized by our research group previously [7], and report here on its ability to enhance DNA uptake and cell transfection.

* Corresponding author. Tel.: +82 063 270 2351; fax: +82 063 270 2348.
*E-mail address:* khy@moak.chonbuk.ac.kr (H.Y. Kim).

1742-7061/$ - see front matter © 2005 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2005.10.007

## 2. Materials and methods

### 2.1. Preparation and characterization of copolymer particles in aqueous solutions

The preparation and characterization of the block copolymer was performed as previously published [7,8]. (The weight average molecular weight of the copolymer was 19,000, copolymer composition was PPDO/PLLA/PEG: 10/20/70, with the molecular weight of PEG fraction at 10,000.) A solution of triblock copolymer with specified concentration in acetonitrile was added dropwise into deionized water at room temperature. Then, the acetonitrile was evaporated from the suspension under reduced pressure and the aqueous suspension was filtered through a 0.45 μm pore disposable filter. The block copolymer concentration in the final suspension was 0.3%.

### 2.2. Size and ζ-potential measurements

The size and ζ-potential measurements were also performed as previously published [8]. The particulate size in the highly dispersed solution was determined by dynamic light scattering using a Malvern System 4700 Instrument. All experiments were performed at $25 \pm 1$ °C. The correlation decay function was analyzed by the cumulate method to determine the average particle diameter. The ζ-potential of the aqueous solution of the block copolymer was determined by a laser zeta potential instrument (ELS-8000/6000, Otsuka Electronics). Measurements were performed at $25 \pm 1$ °C, on samples approximately diluted with 1 mM HEPES buffer.

### 2.3. Cell culture

Cells (NIH 3T3; mouse embryo cell, CT-26; colon cancer cell and MCF-7; breast cancer cell) were purchased from Korea Cell Line Bank (Seoul, Korea). These cell lines were used for the transient transfection and the cytotoxicity experiments, and grown at 37 °C under 5% $CO_2$ atmosphere. The following media were used: (1) Dulbecco's modified Eagle's medium (DMEM) (Gibco) with 10% (v/v) fetal calf serum (Gibco) for CT-26 and MCF-7 cells, and (2) RPMI-1640 medium containing 10% (v/v) fetal bovine serum (FBS) (Gibco) for 3T3 cells. For all media, penicillin (100 U/mL) and streptomycin (100 μg/mL) was used.

### 2.4. Cytotoxicity evaluation

The toxicity of the aqueous solution of the block copolymer was evaluated by quantifying MCF-7, CT-26 and 3T3 cell viability in vitro. Briefly, three different cell suspensions containing $1 \times 10^4$ cells in their respective media (DMEM and REPI-1640) containing 10% FBS were distributed in a 24-well plate and incubated in a humidified atmosphere containing 5% $CO_2$ at 37 °C for 24 h. After removing the medium, different concentrations of the aqueous solution of the block copolymer (1–10 mg/mL) were added to the 24-well plate, and were incubated for 5 h. The cell culture surface was washed with PBS solution, and fresh respective media solution was added to the plates. The number of live cells was counted by the Trypan blue method after 24 h. The cytotoxicity of the aqueous solution of the block copolymer was expressed by the relative viability, which was defined by the number of viable cells relative to the cell control ($= 100\%$).

### 2.5. Preparation of DNA complexes

DNA or DNA/Lipofectin with aqueous solution of the block copolymer was used for preparation of complexes in phosphate buffer (PBS, pH 7.5). The plasmid DNA (5 μg) and Lipofectin (15 μg) were mixed with or without different concentrations (0.2–3 mg/mL) of the block copolymer at a final volume of 1 mL PBS. Before further analysis, the resulting mixture was stored for 30 min at room temperature.

### 2.6. Analysis of DNA complexes

DNA complexes were first analyzed by gel electrophoresis and further verified from sedimentation analysis with spectrophotometer. Samples were prepared as described in preparation of DNA complexes. Resulting samples were stored in room temperature for 6 h and then centrifuged at 1500 rpm (revolution per minute) at 4 °C for 20 min. Ten microliters of the supernatant from each sample was taken out and vortexed for 10 min before loading onto 1% agarose gel for gel electrophoresis. The remaining supernatant portion was discarded, and the sedimented portion of each sample was again diluted with 20 μl autoclaved triple distilled water and re-diluted in 1 mL autoclaved triple distilled water for sedimentation analysis via spectrophotometric measurement of optical density (OD) at 260 nm.

### 2.7. Gene transfection

MCF-7, NIH 3T3 and CT-26 cells were grown at 37 °C under 5% $CO_2$ atmosphere. In the calcium phosphate method, 24 h before transfection, different cell line monolayers in 6 cm plates were supplemented with the serum containing media. Prior to cell transfection, 10 μg of the plasmid, 2 mM $CaCl_2$, HEPES buffer and different concentrations of block copolymer (0.22–11 mg/mL) at a final volume of 1 mL serum and antibiotic-free media was made; and the resulting mixture was allowed to stand for 30 min at room temperature. During the transfection experiment, cells were supplemented with that mixture; and the plates were slowly agitated for 2 min, and incubated for 4 h at 37 °C, 5% $CO_2$ atmosphere. After 4 h, media was replaced by fresh media containing 10% FBS, and again incubated in the same conditions up to 48 h. For the Lipofectin method, complexes (DNA/Lipofectin) at a w/w ratio (1:3) were mixed with different concentrations of block copolymer as

| ID | Title | Pages |
|---|---|---|
| 2587 | Novel block copolymer (PPDO/PLLA-b-PEG): Enhancement of DNA uptake and cell transfection | 6 |