

嘉南藥理科技大學專題研究計畫成果報告

水溶性陽離子型高分子載體合成

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計畫主持人：蕭明達

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1. 摘要

聚陽離子/DNA複合體勢力用來傳遞治療的基因到細胞的一種熱門方法。筆者合成出新型的MDEA-PU利用於基因傳遞的一個非病毒型載體。從所形成之複合體的物理性質、低毒性及高轉染效率的結果顯示出MDEA-PU是個新型生物可分解和生物相容性的基因傳遞載體。

關鍵詞：生物可分解、細胞毒性、轉染

Abstract

Polycation/plasmid DNA polyplexes is popular method by which to transfer therapeutic nucleic acid to cell. In a novel cationic polymer, MDEA-PU, was synthesized and used as a non-viral vector for gene delivery. The formulation characteristics, low cytotoxicity, high transfection efficiency showed that MDEA-PU be the novel biodegradable and biocompatible polycation vector in gene delivery.

Keywords: Biodegradable; Cytotoxicity; Transfection

2. Introduction

A various of polycations such as poly(L-lysine) (PLL) [1], polyamidoamine [2], polyethylenimine (PEI) [3], and poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) [4] have been proposed for the delivery of DNA into mammalian cells. These polycations not only condense DNA into structures small enough to enter cells

through endocytosis. Among them, PEI, a highly branched polycation, is generally considered to be the standard of polymer-based gene delivery due to its superior transfection efficiency against a wide array of cells in vitro [5]. However, these polycations have demonstrated the tendency of polycations to mediate transfection, they are associated with considerable degree of cytotoxicity [3,6-7].

The new biodegradable cationic polyurethane (MDEA-PU) was successfully synthesized. To explore its qualification as a gene vector, its ability of DNA condensation, endonuclease enzyme and albumin protection, buffering capacity, cytotoxicity and transfection efficiency were investigated in order to assess possibilities in gene delivery.

3. Methods

3.1 Polymer/plasmid polyplexes hydrolytic degradation

The polyplexes were incubated in a 37 °C shaker incubator and 20 μ L aliquot samples were removed at various time points and stored at -70 °C for analysis on a 0.7 % agarose gel that was stained with ethidium bromide (0.3 μ g/mL).

3.2 Particle size and zeta-potential measurements

The hydrodynamic sizes of the MDEA-PU/DNA polyplexes were

determined by dynamic light scattering at 25°C using a 5-mW He-Ne laser ($\lambda=633\text{nm}$) as the incident beam at a scattering angle of 90°. The surface charges of the MDEA-PU/DNA polyplexes were conducted by determining the electrophoretic mobility at 25°C with a Zeta potential system.

3.3 Determination of the cytotoxic effect of polymers and polymer/DNA polyplexes

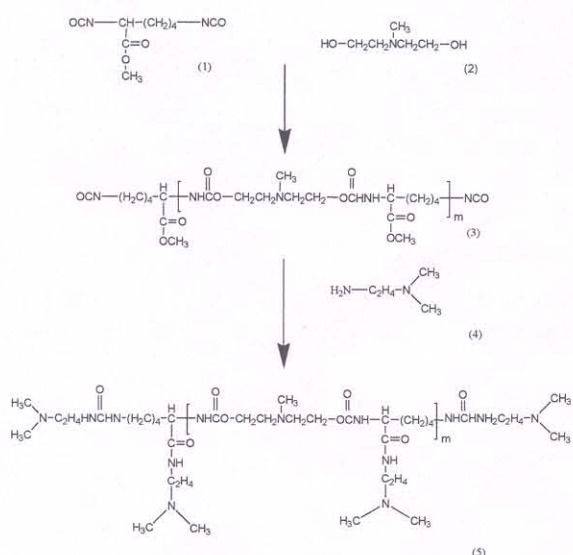
Cell viability of MDEA-PU/DNA and PEI/DNA polyplexes on COS-7 cells were determined by XTT assay [8].

3.4 Transfection studies and β -galactosidase assay

Transfection was performed in COS-7 cells for one hour at a 1 μg DNA per well in DMEM without FBS and then incubated for an additional 48 hours in fresh media with 5% FBS. The results were determined by β -galactosidase assay.

4. Results and Discussion

4.1 Structural Characterizations of MDEA-PU



Scheme 1. Synthesis of MDEA-PU

In scheme 1, the MDEA-PU was synthesized and confirmed by FT-IR, $^1\text{H-NMR}$, and $^{13}\text{C-NMR}$. The GPC data showed that the weight-averaged molecular weight of the MDEA-PU was 37,000 with a polydispersity of 1.8, relative to polystyrene standards in THF.

4.2 In vitro hydrolysis of MDEA-PU/DNA polyplexes

DNA was released from the MDEA-PU/DNA polyplexes in 20Mm HEPES buffer due to the degradation of the MDEA-PU as shown in figure 3. From lane 2 to 8, the results showed that incubation of the polyplexes up to 12 hours resulted in no release of DNA. After initial 15 hours, less DNA was released from the polyplexes. In lanes 11 and 12, DNA was unbounded evidently from polyplexes.

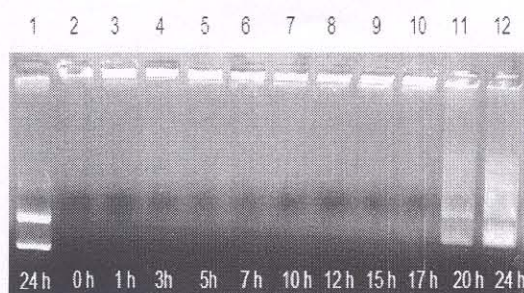


Figure 1. Agarose gel electrophoresis of MDEA-PU/DNA polyplexes at mass ratio of 150/1. Lanes: (1) 625 ng pCMV- β gal; (2)-(8) MDEA-PU/pCMV- β gal (w/w): 150/1 at various time points.

4.3 Size analysis, Zeta-potential and morphology of MDEA-PU/DNA polyplexes

Fig 2 shows the average diameters (100-110 nm) of the polyplexes described above fall within the general size requirements for cellular endocytosis. The zeta-potential of the resulting complex changed from a negative charge to a positive charge when the amount of MDEA-PU was increased. Fig 3 shows the atomic force microscopy (AFM) images of MDEA-PU/DNA polyplexes at mass ratios of 10/1.

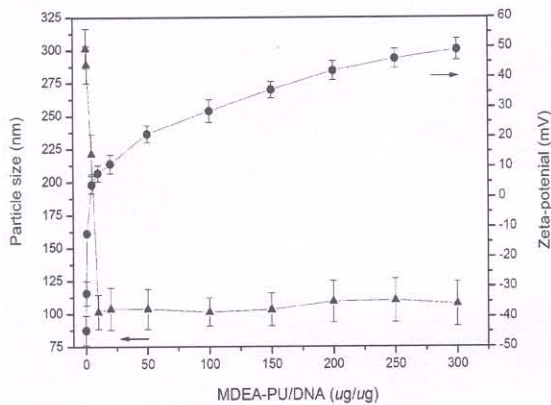


Figure 2. Size and Zeta-potentials of MDEA-PU/DNA polyplexes prepared at different mass ratio. The error bars represent standard deviation as mean \pm SD (n=3).

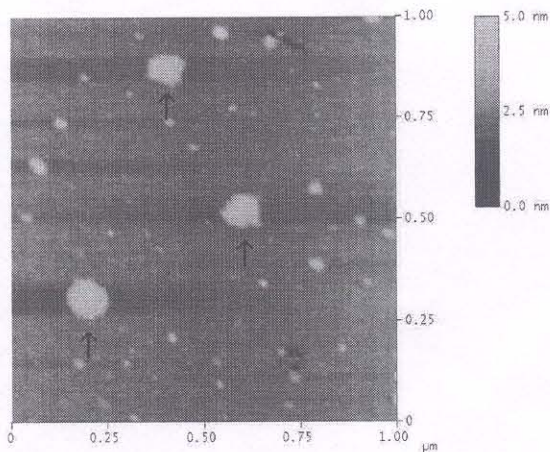


Figure. 3. The AFM images of MDEA-PU/DNA (w/w): 10/1 polyplexes.

4.4 Cytotoxicity and cellular delivery of plasmid DNA via MDEA-PU/DNA polyplexes

The well-studied COS-7 cell line, a normal used model for gene delivery, was transfected in vitro with MDEA-PU/ DNA polyplexes. Relative cell viability of MDEA-PU/ DNA polyplexes and PEI/DNA polyplexes were shown in Fig 4.

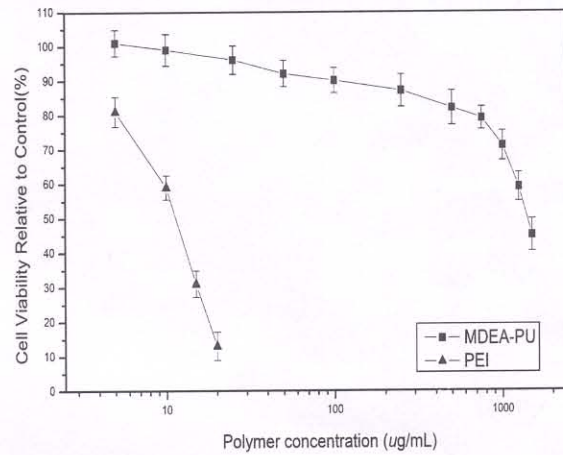


Figure 4. Toxicity of MDEA-PU and PEI in COS-7 cells. Results are presented as mean \pm SD (n=3).

The transfection efficiency of MDEA-PU/DNA complexes was performed by an indication of β -galactosidase expression. A comparison with PEI was made with the optimal transfection efficiency at a mass ratio of 1/1. The transfection efficiency of MDEA-PU/DNA polyplexes was closely to that of PEI in COS-7 cells, and the results were shown in Fig 5.

(a)

