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GENE DELIVERY

Dendrimer/DNA complexes encapsulated in a water soluble polymer and supported on fast degrading star poly(DL-lactide) for localized gene delivery

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Abstract

Polyamidoamine (PAMAM) dendrimer/DNA complexes encapsulated in a water soluble polymer, poly-α,β-[*N*-(2-hydroxyethyl)-L-aspartamide], were supported on a cholic acid functionalized star poly(DL-lactide) film with a fast degradation rate to mediate localized gene delivery. The *in vitro* gene transfections of two types of cells, HEK293 and NIH3T3, were investigated. The expressions of pGL3-Luc and pEGFP-C1 plasmids in HEK293 cells indicated that the star poly(DL-lactide) supported PHEA encapsulated PAMAM/DNA complexes could effectively mediate transfection, with transfection efficiencies which were comparable to that of solution-based transfections. Whereas the PAMAM/DNA complexes directly supported on the star poly(DL-lactide) film showed a much lower expression level for HEK293, which indicated the existence of PHEA played an important role in the efficient transfection. The solid support-based transfection for NIH3T3 cells exhibited higher expressions of pGL3-Luc compared with the solution-based transfection. Encapsulating PAMAM/DNA complexes in PHEA could further improve the gene expression in NIH3T3. During the cellular transfection, the degradation of the cholic acid functionalized star poly (DL-lactide) film could be obviously detected and the degradation did not show any unfavorable effects on the gene expression, which implied this solid support-based gene delivery device had great potential for localized transfection.

Keywords: Gene delivery; Tissue engineering; Biodegradation; Dendrimer; Functional polymers

1. Introduction

The aim of *in vivo* gene transfer strategies is to direct gene delivery to a specific target tissue. In this respect, conventional gene delivery suffers from the limitations such as the toxicity to nontarget cells, ectopic gene expression, limited transfection efficiency due to the DNA complexes aggregation, degradation, or clearance from the tissue, and a strong immune response [1–3]. To overcome extracellular barriers in gene therapy, the adaptation of controlled release technology to gene delivery based on substrate-mediated transfection offers the potential to enhance the gene transfer by maintaining elevated local DNA concentrations in the cellular microenvironment, preventing the degradation of DNA, and localizing vector delivery to specific tissues [1,2]. In the substrate-mediated delivery, also termed as "solid-phase transfection", DNA is supported on or entrapped in an extracellular matrix (ECM), which supports cell adhesion as well as delivers DNA to the cellular microenvironment [1,2].

Similarly to protein delivery systems, the naked plasmid DNA could be directly encapsulated in matrices, such as poly(lactide-*co*-glycolide) and collagen, for transfection [4–8]. Although these naked DNA delivery systems could result in transfection *in vivo*, the levels of gene expression are generally low due to the biodegradation and clearance of DNA, and the low level of cellular uptake of naked DNA [8]. To realize high efficient transfection, researchers further used the vector/DNA complexes, which were encapsulated in or supported on matrices, for transfection [8–11]. Since the vectors protect genes from extracelluar nuclease degradation and facilitate the cell internalization, the transfection efficiency can be obviously improved.

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However, during the fabrication, the DNA may undergo irreversible aggregation and oxidation which lead to the loss of full bioactivity [6], it is still a challenge to achieve high transfection efficiency unless under some particular conditions such as incorporation of an anionic lipid on the matrix [11].

Moreover, the extensively investigated matrices for the substrate-mediated delivery are biodegradable polyesters such as polylactide, and poly(lactide-*co*-glycolide). Despite the well-established importance of these polyesters in biomedical fields, this series of polymers still have limitations in particular applications. It is well known that the bulk degradation mechanism of these polyesters causes unfavorable effects for drug and gene deliveries [12,13]. In the initial degradation stage, the hydrolytic degradation rate of these polyesters may not satisfy the requirement of gene delivery since the degradation does not result in obvious weight loss and the DNA or vector/DNA complexes could not be released out quickly for transfection. While in the later degradation stage, the bulk erosion mechanism induces internal autocatalysis and causes the fast release of acidic degradation products, leading to unfavorable effects on gene expression.

Therefore, the major goal of this work is to develop new materials as matrices to support vector/DNA complexes for solid-phase transfection with high efficiency. In this study, we used cholic acid functionalized star poly(DL-lactide) with a fast degradation rate and surface erosion mechanism [14] as the matrix to support PAMAM/DNA complexes. PAMAM dendrimers are a class of spherical, nanoscopic, highly ordered polymers with primary amino groups on the surface, which have been extensively investigated as gene vectors [15]. The positive charges caused by the amino groups at neutral pH enable the dendrimers to associate, condense and transport DNA into various types of cells with relatively low cytotoxicity [16].

In our investigation, we found that the solid support-based transfection efficiency was lower for particular cell lines compared with the solution-based transfection if the PAMAM/DNA complexes were directly supported on the star poly(DL-lactide) surface. Thus we further used poly-α,β-[*N*-(2-hydroxyethyl)-L-aspartamide] (PHEA), a water soluble polymer with degradability and good biocompatibility [17,18], to encapsulate and protect PAMAM/DNA complexes. Our results showed the existence of PHEA played an important role in the efficient transfection and could significantly improve the expression level.

2. Materials and methods

2.1. Materials

Polyamidoamine (PAMAM) dendrimer (Generation 5 with 128 surface amino groups) was purchased from Aldrich. Cholic acid (Acros) was purified by recrystallization from methanol. DL-Lactide (Aldrich) was purified by recrystallization from ethyl acetate. Stannous octoate $Sn(Oct)_2$ (Aldrich) was purified by distillation under reduced pressure and then dissolved in dry toluene prior to use. L-Aspartic acid, phosphoric acid, and PEG2000 (Shanghai Chemical Co. China) were of analytical grade and used as supplied. Ethanolamine (Shanghai Chemical Co. China) was distilled before use. *N*, *N*-Dimethylformamide (DMF) was purified by distillation over P_2O_5 and CaH_2.

The reporter plasmids, pEGFP-C1 and pGL3-Luc, were purchased from Invitrogen and Promega, respectively. Plasmids were amplified in *Escherichia coli* and extracted and purified by E.Z.N.A. fastfilter endo-free plasmid maxi kit (Omega). Plasmids were suspended in water and stored at −20 °C.

HEK293 and NIH3T3 cells were obtained from China Center for Typical Culture Collection (Wuhan, China) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco), supplemented with 10% fetal bovine serum (FBS), 2 mg/mL $NaHCO_3$, and 100 Unit/mL penicillin/streptomycin. Cells were incubated at 37 °C in humidified air/5% CO_2. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was from Amresco. Dimethylsulfoxide (DMSO) was from Sigma.

2.2. Polymer synthesis

Cholic acid functionalized star oligo/poly(DL-lactide), CA-(DLL)n, was synthesized by the ring-opening polymerization of DL-lactide using cholic acid with hydroxyl groups as an initiator. The detailed synthesis procedure and the polymer characterization were reported in our previous article [14]. Poly-α,β-[*N*-(2-hydroxyethyl)-L-aspartamide] (PHEA) was synthesized according to a published procedure [18].

2.3. PAMAM/DNA complex formation

PAMAM was diluted to an appropriate concentration in water and stored at 4 °C until use. The PAMAM/DNA complexes were made up by adding a solution of dendrimer in water to an equal volume of plasmid DNA in water at a particular PAMAM to DNA charge ratio, followed by 30 min incubation at room temperature. The charge ratio was based on the calculation of the electrostatic charge present on each component, the number of terminal NH_2 groups on the dendrimer versus the number of phosphate groups in the DNA.

2.4. CA-(DLL)n film preparation

Flat films of CA-(DLL)n with a thickness of 2.6 μm were formed on glass slides with a diameter of 14 mm by solvent casting. After solvent evaporation, the samples were dried in a vacuum oven for 24 h. The samples were sterilized by UV light irradiation for 1 h prior to use.

2.5. Preparation of CA-(DLL)n film supported PAMAM/DNA complexes

The PAMAM/DNA complex solution (100 μL containing 1 μg plasmid DNA) was overlaid on the surface of the CA-(DLL)n film and air-dried in a laminar flow hood for at least 4 h. After drying, the sample was placed in the well of a 24-well plate and then kept in a desiccator until required.