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Cationic PLA nanoparticles for DNA delivery: Comparison of three surface polycations for DNA binding, protection and transfection properties

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Abstract

Biodegradable cationic nanoparticles (cNP) made of poly(lactide) (PLA) have been shown to be promising carrier systems for in vivo DNA delivery and immunization. In previous work, we have described a versatile approach for the elaboration of cationic PLA cNP based on the use of pre-formed particles and subsequent adsorption of a model polycation, the poly(ethylenimine) (PEI). Here, we evaluated two more polycations, chitosan and poly(2-dimethyl-amino)ethyl methacrylate (pDMAEMA)) to determine the most suitable one for the development of PLA cNP as DNA carriers. Cationic PLA–PEI, PLA–chitosan and PLA–pDMAEMA nanoparticles were compared for interaction with plasmid DNA and, more importantly, with regards to the biological properties of bound DNA. pDMAEMA coating yielded the most positively charged nanoparticles with the highest DNA binding capacity (32 mg/g). Loaded with DNA, all three cNP were in the same size range (∼500 nm) and had a negative zeta potential (−50 mV). PLA–chitosan was the only cNP that released DNA at pH 7; the two others required higher pH. Adsorption and release from cNP did not alter structural and functional integrity of plasmid DNA. Moreover, DNA coated onto cNP was partially protected from nuclease degradation, although this protection was less efficient for PLA–chitosan than others. The highest transfection efficiency in cell culture was obtained with PLA–pDMAEMA carriers. We have shown that at least three different cationic polymers (chitosan, PEI, pDMAEMA) can be used for the production of PLA-based particulate DNA carriers and most probably other cationic polymers can also be used in the same purpose. PLA–pDMAEMA cNP were the most promising system for DNA delivery in this in vitro study. Our future work will focus on the in vivo evaluation of these gene delivery systems.

Keywords: Cationic poly(D,L-lactide) nanoparticles; Poly(ethylenimine); Chitosan; Poly(2-dimethyl-amino)ethylmethacrylate; DNA delivery

1. Introduction

Gene delivery and DNA vaccination are of increasing interest because of the variety of human diseases that can be treated by this strategy, such as cancer and infectious diseases. However, clinical application of this approach requires the development of safe and efficient delivery vectors for in vivo gene transfer and/or immunization. The two main systems for DNA delivery are viral and synthetic vectors. Viral systems are very efficient for in vivo transfection, as well as immunization. Their major drawback is the anti-vector immunity that limits the administration of repeated doses, and some safety issues still remain for human use [1]. Non-viral systems based on biocompatible chemical agents are preferred in terms of safety, stability, the relative ease of large-scale production and characterization, and the lack of intrinsic immunogenicity.

Among synthetic vectors, the use of nanoparticles or microparticles prepared with biocompatible and biodegradable

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poly(D,L-lactide-co-glycolide) (PLGA) or poly(D,L-lactide) (PLA) polymers has become one of the most successful methods for in vivo DNA delivery. Several authors have reported enhanced cellular and humoral immune responses using plasmid DNA encapsulated into PLGA or PLA microspheres [2,3, reviewed in 4,5]. Other particulate DNA carriers promising for in vivo gene delivery include biocompatible polymers such as the polysaccharides chitosan [6, reviewed in 7] and alginate [8], nanoparticles obtained from oil-in-water microemulsions and liposomes. More recently, to limit DNA degradation during the formulation process, Singh et al. adsorbed the plasmid at the surface of cationic particles [9]. To allow DNA binding through electrostatic interactions, PLGA microparticles were modified to display a positively charged surface by inclusion of a cationic surfactant, cetyltrimethylammonium bromide (CTAB). Compared to naked DNA, these cationic microparticles substantially improved the immune responses generated by DNA, both in mouse [10] and macaque models [11].

Since then, several groups have reported other techniques of elaboration of cationic particles either associating CTAB, or other cationic surfactants, within an oil-in-water microemulsion [12,13], within solid lipid nanospheres [14,15], within a physical gel of a non-ionic triblock copolymer [16] or using polymers to modify the interface of particles. In this latter strategy, the polymer was added during the elaboration process as reported by several authors, and particles covered in poly(ethylenimine) (PEI) [17,18] or chitosan [19,20] were obtained. A more versatile approach that we reported recently was based on the use of pre-formed particles onto which a polycation was adsorbed [21]. In the initial study, PEI was used as the model polycation, and subsequently several types of PLA–PEI nanoparticles were shown to efficiently complex DNA [22].

In the work reported here, we evaluated two other polycations in addition to PEI to determine the most suitable one for the development of cationic PLA nanoparticles as DNA carriers. We used PEI, chitosan and poly(2-dimethylamino)ethyl methacrylate (pDMAEMA) polymers to functionalize PLA particles. The polyelectrolyte was adsorbed onto the surface of the particles via electrostatic interactions, according to the general approach described in Trimaille et al. [21]. The three types of cationic nanoparticles (PLA–PEI, PLA–chitosan and PLA–pDMAEMA) were compared with regards to DNA binding and release properties, integrity of the released DNA, protection from nuclease degradation and in vitro transfection efficiency.

2. Materials and methods

2.1. Materials

PLA (PLA50 M_n = 30,000 g/mol, molecular weight distribution M_w/M_n = 1.7) was purchased from Phusis (Grenoble, France). PEI, chitosan, DMAEMA and Pluronic F68 surfactant were purchased from Sigma–Aldrich (Saint Quentin Fallavier, France). The 5.2 kbp pGL3–control plasmid encoding the firefly luciferase under the control of simian virus 40 promoter was from Promega (Charbonnières les bains, France). The plasmid was propagated in the *Escherichia coli* strain DH5α and purified using the endotoxin-free Giga-prep kit from Macherey Nagel (Hoerdt, France). Residual endotoxin content in the DNA preparation was measured using the QCL-1000 Quantitative Chromogenic Limulus Amebocyte Lysate (LAL) kit (BioWhittaker, Walkersville, Verviers, Belgique) and was below 1 EU/mg plasmid. DNAse I enzyme was obtained from Roche Applied Sciences (Meylan, France). The 293T cells were purchased from American Type Culture Collection (ATCC, Manassas, VA). Material for cell culture was purchased from Invitrogen (Cergy Pontoise, France). Cell Culture Lysis Reagent (CCLR, 5×), Luciferase Assay kit and recombinant luciferase protein were from Promega. Micro-BCA assay was from Pierce (Bezons, France). All other chemicals were of analytical grade and obtained from Sigma–Aldrich.

2.2. Nanoparticle preparation and cationic polymer adsorption

PLA nanoparticles were produced by the emulsification–diffusion method [23,24] in the presence of Pluronic F68 surfactant following the procedure that was previously described in Trimaille et al. [21,25]. The pDMAEMA polymer was obtained by free radical polymerization in solution. The adsorption of polycations onto particles was carried out in different buffers depending on colloidal stability of the formed particles. The adsorption procedure was the following: 1 ml of particles was added to 1 ml of polycation solution. The final adsorption medium contained 5 mg/ml of PLA nanoparticles and either 44 μg/ml of PEI in water, or 83 μg/ml of chitosan in 10 mM pH 4.75 acetate buffer, or 75 μg/ml of pDMAEMA in 0.5× Earle's balanced salt solution. Adsorption was let to occur under stirring for 10 min at room temperature. After adsorption, the particles were cleaned from the excess polycation by centrifugation/redispersion in MilliQ water except for chitosan, for which particles were redispersed in 10 mM pH 4.75 acetate buffer.

Particle surface characterization was performed by measuring the electrophoretic mobilities for different pH values at constant ionic strength by the technique of laser Doppler anemometry using a Zeta Sizer 3000HS (Malvern Instruments, UK). The conversion to zeta potentials was performed as described in Messai et al. [22].

2.3. Adsorption of plasmid DNA onto cationic polymer-functionalized nanoparticles

Plasmid DNA was adsorbed on the surface of cationic nanoparticles in different NaCl concentrations (0, 50, 100 and 150 mM), in a final volume of 160 μl. The nanoparticle concentration in the adsorption mixture was 1 mg/ml; to