Entrapment of DNA plasmids into modified poly(N-vinylpyrrolidone) nanoparticles for development of DNA-vaccines

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INTRODUCTION

Main impediments in DNA vaccine delivery are a limited DNA transport through mucosal membra-nes and low bioavailability of delivered DNA arising from degradation and instability. Non-viral delivery systems are promising because of their safety, minimal immunological reactions, and ease of production for DNA vaccine. Recent studies have shown that DNA can be delivered efficiently into cells using biocompatible polymers (Sunshine, 2012). Nanoparticles based on modified poly-N-vinylpyrrolidone (PVP) could be proposed for entrapment of DNA plasmids due to their biocompatibility and capability to self-assembly with simultaneous incorporation of biomolecules. The aim of this research was to develop a simple technique for entrapment of DNA plasmids into modified PVP nanoparticles (NPs) and to estimate immune response to these NPs in a mouse model.

MATERIALS AND METHODS

Reagents N-vinylpyrrolidone, β-alanine, glycine, dioxan, di-nitryl-azo-bis-isobutyric acid, mercapto-acetic acid, N,N'-dicyclohexyl-carbodiimide, n-octa-decyl amine, ethyl oxide (all from Sigma-Aldrich) were used. Oligonucleotide used as an adjuvant, CpG-motif 5'-3' (TC*GTC*GTTTTGTC*GTTTTGTC*GTT) was from Beagle (Russia). Recombinant DNA plasmids pTZ57R/G1/4 (1521 b.p.) and pTZ57R/G2/1 (1583 b. p.) encoded Gc and Gn glycoproteins of Rift Valley Fever virus (RVF virus), respectively, were prepared as described earlier (Imatdinov, 2012).

Synthesis of amphiphilic polymers A set of amphiphilic PVPs samples which contain both carboxylic and alkyl groups were synthesized as described earlier (Kuskov, 2010). Then these polymer samples (MM 3500) were modified, in order to get their derivatives containing amino-acid groups.

The obtained amphiphilic PVPs samples were additionally dialyzed for 5-7 days and then lyophilized. Thus, Two PVP polymer samples.

($PVP/_{Ala}$ and $PVP/_{Gly}$) which contained β-alanine fragments (7 mol. %) or glycine (7.5 mol.%) in sidechain and octadecyle hydrophobic fragments were obtained and used for NPs preparation.

Preparation of nanoparticles with entrapped DNA plasmids and CpG oligonucleotide For preparation of nanoparticles with entrapped DNA plasmids and/or CpG-motif water $PVP/_{Ala}$ or PVP/Gly solutions (0.16 wt %) were mixed with DNA solutions (4.5 μg/ml and 5 μg/ml in the case of pTZ57R/G1/4 and pTZ57R/G2/1, relatively), and/or CpG solution (100 pmol/μl). These mixtures were incubated at stirring (1000 r/m) using IKA-Vibrax-VXR for 1-2 hs until NPs were formed.

Determination of nanoparticle size The size of the obtained nanoparticles was determined by means of Coulter 4DM at 20ºC.

Microscopy study of nanoparticles was carried out by scanning nanohardness tester NanoScan-3D (Kelegen, Russia) based on scanning probe microscope (SPM) and electron transmission microscope (ETM) JEM-1400 (Jeol, Japan).

Immunization of mice with nanoparticles The BALB mice (5 animals in each group) were immunized intramuscular with nanoparticles (50 μg of plasmid/mouse). Then on day 20 the blood samples were taken off, and sera samples were tested by ELISA.

Determination of antibodies Concentration of antibodies in sera were tested by ELISA in 95-well plates (Linbro). Briefly, for sensibilisation the antigen for RVF virus diagnostics (diluted to 1:400) from the collection of National Research Institute for Veterinary Virology and Microbiology was used. We also used a horseradish peroxidase-linked goat anti-mouse IgG conjugate (diluted to 1:1500) in this assay. 2,2'–azino-bis(3-ethylbenzthiazoline-6- sulphonic acid) was used for the detection. In 15 min after its addition, the reaction was stopped with 2M H_2SO_4 solution, and the adsorbance was measured using microplate reader (Multiscan MCC/340) at 450 nm.

RESULTS AND DISCUSSION

Nanoparticles were prepared according to the scheme below (Fig.1).

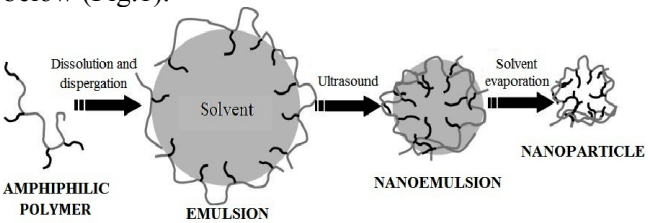

Fig. 1: Preparation of modified PVP nanoparticles.

The mean size of nanoparticles determined by Coulter was around 100 nm.

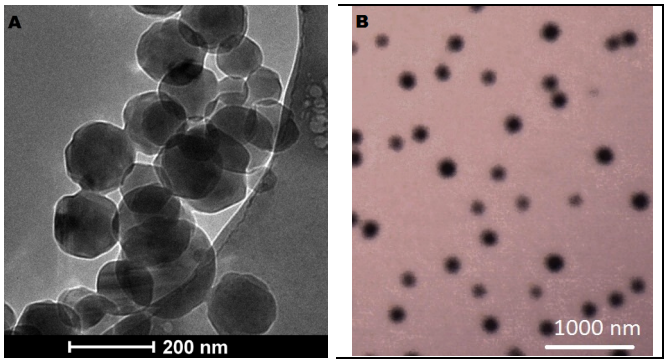

Fig. 2. Micrographs of PVP nanoparticles loaded with DNA plasmids.

The observations of nanoparticles were also carried out by 2 methods of microscopy, namely
we used electron transmission microscope (Fig. 2 A) and a scanning nanohardness tester NanoScan-3D equipped with SPM (Fig 2B).

Table1. Titres of antibodies in sera samples after immunization of mice with PVP nanoparticles.

N	Samples for immuni-zation	Plasmid dose/mouse (μg)	Number of mice in group	Titre on day 20
1	native plasmids mixture G1/4 + G2/1	50	5	1:160
2	plasmids in NPs	50	5	1:320
3	Plasmids in NPs + CpG	50	5	1:640
4	Blank NP	-	3	0

The immune response to the NPs with entrapped DNA plasmids was studied in a mouse model. As seen in Table 1, titres of antibodies in the case of immunization with NPs were higher compared to those when the mice were administered with native plasmids. The addition of CpG adjuvant enhanced this effect.

CONCLUSIONS

Thus, we proposed a new approach to obtain nanoparticles based on PVP modified with amino acids (Gly, Ala) which can be used for entrapment of DNA plasmids and adjuvants. The prepared NPs were used to study the immune response in a mouse model.

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