Microencapsulated DNA as a DNA-vaccine delivery system

V.I. Balysheva[1], N.N. Vlasova[1], E. A. Markvicheva[2], S.Yu. Belov[1], O.Ye. Selina[2] and G. B. Sukhorukov[3]

[1]National Research Institute for Veterinary Virology and Microbiology of Russia, 601120, Pokrov, Vladimir region, Russia.

[2]Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho-Maklaya str.16/10, 1178997 Moscow, Russia.
[3]Queen Mary University, London, UK

Introduction

To develop gene engineered vaccine (DNA-vaccine or the vaccine of the 3rd generation) a plasmid DNA (vector) with a built-in protective protein gene is introduced directly into an animal body. The immune response can be induced through an injection of a "naked" DNA in a salt solution or in via complex formation of DNA with lipids. However, only a small amount of DNA plasmid (0.1-0.2 %) is absorbed by animal cells, while its remaining part is quickly disrupted. To obtain an effective immune response, it is necessary to introduce rather significant DNA amounts, i.e. about 100 µg of plasmids per a mouse [1, 2, 3]. Therefore, to develop a new promising DNA vaccine, an effective polymer delivery system providing a targeted DNA delivery into an animal cell cytoplasm should be evaluated.

Presently a series of methods for a genetic material delivery into a cell cytoplasm has been developed. R. Jason and B.Clark John [4] proposed to use bacteriophages as a promising approach to gene delivery. Another way to improve an efficiency of a biomaterial targeted delivery is based on the use of microcapsules with bioencapsulated DNA [5]. DNA delivery system which we have developed is based on biocompatible biodegradable polyelectrolyte microcapsules. The system can provide DNA preservation in a super colloidal form and a degradation of the microcapsules after their delivery into cells with a subsequent DNA release. In the further research a high efficiency of the system based on DNA and an adjuvant co- microencapsulation for mice immunization will be demonstrated.

The aim of the current research was to develop a simple technique for DNA microencapsulation which could provide rather high DNA microencapsulation efficacy, a good protection of DNA from digestion by nucleases in a microorganism, and DNA effective delivery into cells.

Materials and methods

In the current research sodium alginate (AlgNa), medium viscosity (Sigma), calcium chloride ($CaCl_2$ x $2H_2O$), sodium chloride, poly-L-lysine (PLL), MM 15000-30000, ethylenediaminetetraacetic acid (EDTA) were used. All mentioned above reagents were from Sigma. $CaCO_3$ microparticles were obtained by the method described earlier [6];

Adenovirus (ADV) strain MK-25 (passage 8) in the Siberian mountain deer kidney cell culture (SMDK-60) was used.

ADV DNA was obtained by the phenol-detergent method. Neutralization test was conducted using a routine method with 100 $TCID_{50}$ ADV.

The multilayer polyelectrolyte microcapsules were prepared by the method of layer-by-layer (L-b-L) adsorption of oppositely charged polyelectrolytes on macroporous $CaCO_3$ microparticles (a mean diameter 2 μm).

To adsorb DNA within $CaCO_3$ microparticles, 20-125 μg of ADV DNA were incubated with microparticles at 4 °C for 22 hrs. After the incubation, the DNA concentration in the microparticles was found to be 80-90 % of its initial concentration. The microparticles with adsorbed DNA were twice washed with water. To form the 1-st layer of the microcapsule membrane, the microparticles were centrifuged and transferred to a poly-L-lysine solution (2 mg/ml), then incubated at stirring for 5 min, and then again twice washed with water. To get the 2-nd polymer layer, the microparticles were incubated in an alginate solution (1- 2 mg/ml in

0.2 M NaCl) on a shaker for 15 min, washed twice with a distilled water, and then transferred to 0.1 M EDTA solution for 20-30 min. After dissolving $CaCO_3$ internal core the obtained hollow microcapsules were again washed with water (1000 rpm, 1-5 min). This procedure was repeated several times to get 7 layers polyelectrolyte membrane (PLL-Alg-PLL-Alg-etc).

Results and Discussion

The L-b-L technique to produce polyelectrolyte microcapsules has been developed earlier (7,8). However, we have to optimize it, in order to find a pair of oppositely charged biodegradable polymers, while in the papers mentioned above non-biocompatible and non-biodegradable polymers have been used. As a result, alginate and poly-L-lysine have been selected, since they provided formation of mechanically stable microcapsules, and *in vitro* DNA release from them did not exceed 1% w/v (from DNA initial concentration) for 15 days.

To develop a system for delivery of genetic material (i.e., DNA) into animal cells, ADV DNA was used as a model. ADV is pathogenic virus for rabbits and causes a disease with specific clinical signs, turning into a chronic form and/or resulting in animal death. Since the DNA of this virus is infectious, it has to induce an infectious process when being introduced into susceptible cells.

To obtain DNA sorption in microparticle $CaCO_3$, 20-125 μg of ADV DNA were incubated with 50-100 mg of the microparticles for 12 h at 40°C. The obtained microparticles with the adsorbed DNA were twice washed with distilled water. The maximal ADV DNA sorption from water solution was found to be 85 t- 90 % of its initial concentration. After DNA sorption in $CaCO_3$ microparticles, they were coated with the multilayer polymer (Alg-PLL) membrane. Then the microparticles were transferred to a solution of 0.1 M EDTA, vigorously mixed for the $CaCO_3$ core dissolution and again washed with water. The operation was repeated several times, in order to dissolve $CaCO_3$ core and to get hollow transparent microcapsules.

Four rabbits were intramuscularly administered with 6.0-6.1 μg of the microcapsulated DNA. Two rabbits were injected with native DNA at the same dose (a control). As seen from Table 1, two rabbits which got the encapsulated DNA died on day 4 and day 5, while two other animals got a respiratory form of the disease which was revealed in sneezing, cough and mucous nasal discharges in 6-7 days. On day 21 one rabbit died, and the other one was subjected to euthanasia. The control animals (i.e., those inoculated with native DNA) did not show any signs of the disease for 20 days (an observation time) and survived.

Animal organ samples, namely liver, lung, brain and blood were taken from dead rabbits and treated, in order to get 10% cell suspensions in salt solutions. Into the After that 1 ml of a 10 % suspension of each organ was added to ADV-susceptible cell culture SMDK-60, and the cells were

cultured in vitro at 37 °C. On day 4 and day 5 a specific cytopathic effect was observed in the cell culture where liver and brain suspensions from dead animals have been added (see Table 1).

Animal species	Number	Injected DNA quantity, μg	Disease manifestations	PCR/tested samples	CPE in MSDK-60
Rabbits	2	6.1	Death on day 4 to 5	+ / liver, brain	+
	2	6.0	A subacute respiratory form, a death on day 21	+ / liver, brain, spleen	+
Rabbits, control	2	6.0 (native DNA)	Not revealed	-	-
Mice	2	0.6	Death on day 4	+ / liver, brain, spleen	+
Mice, control	2	6.0 (native DNA)	Not revealed	-	-

Remark : "+" – the reaction runs, "-" – no reaction

Table 1. The effect of microencapsulated ADV DNA on mice and rabbits.

The table demonstrates that microencapsulated DNA was protected from the internal environment of organism (e.g., from nucleases and other enzyme systems), and was successfully delivered into the sensitive cells what resulted in inducing the infectious process in the animals. The PCR analysis of the suspensions of organs taken from the dead rabbits which died on day 4 to day 5, conducted with specific primers, confirmed the presence of ADV DNA in the liver and in the brain of animals (Fig. 1).

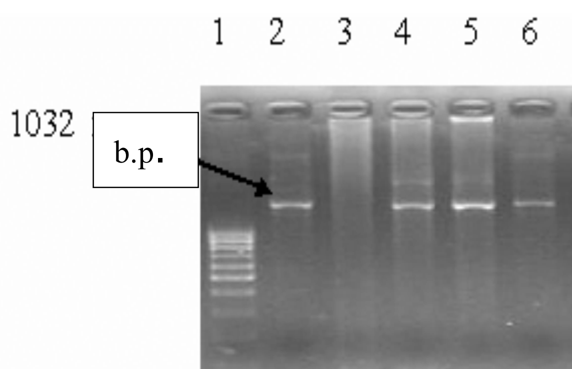

Fig. 1. PCR analysis of various organ samples for ADV DNA detection:

Tracks: lane 1: marker 100 b.p.; lane 2: 10 % brain suspension sample (the rabbit N° 2), lane 3: 10% brain suspension sample (the rabbit N° 1), lane 4: 10 % liver suspension sample (the rabbit N° 2), lane 5: ADV DNA (the strain MK-25); lane 6: 10 % liver suspension sample (the rabbit N° 1).

Conclusion

Thus, a novel technique for DNA delivery into macroorganism based on biodegradable microcapsules has been developed. It provided a high DNA encapsulation efficacy (up to 80-90 %), and DNA protection against nucleases in animal body. The proposed approach can be useful for elaboration of novel DNA vaccines.

Acknowledgments

The research was supported by the Russian Foundation for Basic Research (grant N° 06-04-08277).

References

1. Gendon U.Z. Progress in the development of polynucleotide (DNA) viral vaccines. // Voprosi Virusologii, 1999, No 4, pp.148-154 (in Russ.).
2. Supotnitsky M.V. DNA immunization in livestock infections prophylaxis // Veterinarija,- 1998, No 5, pp 18-24 (in Russ).
3. Chokri Bahloul et al. DNA-based immunization for exploring the enlargement of immunological cross-reactivity against the lyssaviruses // Vaccine, 1998, v. 16, p.417-425.
4. Clark J. R., March J. B.// Expert Rev. Vaccines, 1994, v 3 (4), pp.463-476.
5. Johnson et al. US Patent No 2002/0032165 A1 Mar.14, 2002.
6. Volodkin D.V. et al. // Langmuir, 2004, v.20 (8), p.3398.
7. Sukhorukov,G.B. , 2002, MML Series Vol. 5"Dendrimers, Assemblies, Nanocomposites" (Ed. by R.Arshady and A.Guyot0. Citus Books., pp. 111-147.
8. Möhwald, H, Donath, E, Sukhorukov, G.B., (2002) In: Multilayer Thin Films. Sequential Assembly of Nanocomposite Materials (Ed. by G.Decher and J.B.Schlenoff). Wiley-VCH, pp. 363-392.