# Effect of microcapsule composition upon specific antibody induction

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## Introduction

In the course of development of genetically engineered vaccines (i.e. DNA vaccines, or vaccines of the 3rd generation) a plasmid DNA (i.e., a vector) with an incorporated gene of a protective protein is injected directly into animal organism (Gurunathan 2000, Donnelly 1997). The immune response is induced through injection of "naked" DNAs in a salt solution, or together with lipids, the plasmid DNAs being absorbed with animal cells in insignificant amounts (0.1 to 0.2%) and the DNAs major part quickly destructed. In the up-to-date biotechnology various strategies of genetic material delivery to organism are being developed. In this case these are polyelectrolyte capsules that are the transport tool through which the delivery is accomplished.

The work we have carried out was aimed at the development of a procedure to obtain microcapsules having adjustable parameters (namely, their diameter, the capsule thickness, the membrane composition and permeability), methods of nucleic acid encapsulation, and also investigation of gene material targeted delivery using both cell culture and animals.

## Materials and methods

For the investigation some three polymer pairs, namely poly-L-lysine/alginate, DEAE-dextran/carrageenan and chitosan sulfate/carrageenan), were selected. The preliminary selection of the polyelectrolyte pairs was carried out under the following parameters: the polyelectrolytes were to be biodegradable and biocompatible polymers and form a mechanically strong membrane in the process of making the multilayer capsule.

The procedure of making the microcapsules containing DNAs immobilized in there comprised three principal stages that are presented in Fig. 1:

1. Synthesis of macroporous microparticles $CaCO_3$ with DNAs incorporated.
2. Formation of a multilayer membrane on the DNA-containing microparticle surface. To obtain stable microcapsules the PE adsorption on CaCO3 microparticle surface was repeated 6 times, that is, 6 PE-layers were obtained that had the following configuration: (polycation / polyanion)$_3$
3. Obtaining of hollow microcapsules through the carbonate core solution by EDTA treatment of the obtained microparticles.

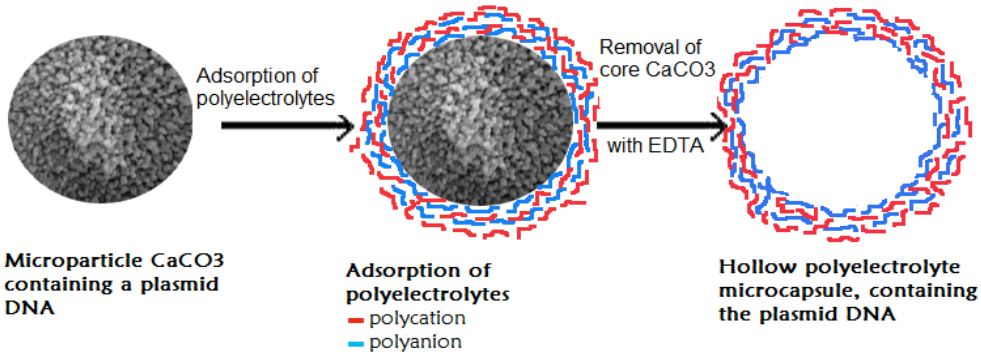


**Figure 1: Scheme of forming a multilayer membrane by sequential adsorption of PE.**

The $CaCO_3$ particles were prepared by method of D.V. Volodkin (Volodkin 2004a & 2004b). The DNAs were incorporated into the microparticles concurrently with making the $CaCO_3$ particles by coprecipitation method. For that the plasmid DNA water solution was mixed with calcium chloride and sodium carbonate single-molar solutions with a magnetic stirrer for 30 seconds. After mixing the microparticle-containing precipitate was separated from the supernatant by centrifugation (1600 g, 1 min). The amount of the sorbed DNAs was determined using spectrophotometry ($\lambda$= 260 nm) from the difference between the adsorption of the primary solution and the solution of the supernatants selected at the precipitation.

The microparticle-sorbed DNAs rates were 94 to 96%. After the sorption was over the DNAs were centrifuged to form the 1st layer of the microparticle capsules and then transferred to a low-molecular (30000) poly-L-lysine solution, incubated for 15 min in a shaker and washed off the non-combined polyelectrolyte. To obtain the 2nd polymeric layer, the procedure was repeated by putting the particles into the alginate solution. After the multilayer capsule formation process was over, the particles were washed with distilled water, transferred to 0.1M EDTA, mixed thoroughly to dissolve the $CaCO_3$ inner core and washed with water once again. The microcapsules from the polyelectrolyte pairs DEAE-dextran/carrageenan and chitosan sulfate/carrageenan were obtained similarly.

The following plasmids were used for encapsulation: a hemagglutinin-expressing plasmid pInf HA (3400 b.p.), a nucleoprotein-expressing pInf NP (4300 b.p.), and also a plasmid pTKShi (6841 b.p.) containing a genome site coding for a classical swine fever virus polypeptide E2.

Animals. Each group contained at least 10 mice for which a universe housing regime was made. Determination of antibody titers in blood sera of mice inoculated with the plasmid preparations was conducted by indirect solid-phase ELISA. The results were evaluated spectrophotometrically using Multiskan MCC 340 (Labsystems, Finland) at $\lambda$= 405 nm.

## Results

Electron microscopic examinations of the microcapsules with the DNAs enclosed in there showed that the microcapsule size was 1 to 2 mcm with the membrane thickness being 0.2 mcm.

To determine the plasmid DNA optimal quantity inducing the maximal antibody induction in mice blood the animals were immunized with the preparations containing various amounts of a nucleoprotein-expressing plasmid Inf NP (see Table 1).

The preparations of the native DNAs incorporated into the microcapsules $(PLL/Alg)_3$, containing the same amounts of the DNAs, were intramuscularly injected to the animals into the thigh area. The blood to be used in these investigations was collected on day 21 after the immunization.

| Quantities of the plasmid injected to mice, mcg | Specific antibody titers | |
|---|---|---|
| | Against avian influenza virus antigen, subtype H7 | Against avian influenza virus antigen, subtype H5 |
| 5 | 1:1600 | 1:400 |
| 10 | 1:2260 | 1:400 |
| 20 | 1:3200 | 1:2600 |
| 30 | 1:3200 | 1:2660 |
| 40 | 1:3200 | 1:3200 |

**Table 1. Antibody titers for blood sera taken from mice immunized with microcapsules $(PLL/Alg)_3$ depending on the quantities of the plasmid Inf NP injected.**
*Note: the titer of white mouse normal serum (0) is 1:50*

As evident from the obtained results, the increase of the injected DNA dose from 5 mcg to 20 mcg per a mouse a raise in antibody titers in the examined blood sera was observed. The further increase in the amounts of the plasmid as injected being both native or taken within the microcapsules did not induce any significant increase in antibody titers without any significant varieties in antibody titers as seen at immunization with either the native plasmid or the microencapsulated one. Perhaps, the microcapsules having the composition $(PLL/Alg)_3$ were degraded too quickly when injected into mouse organism and thus did not provide the desired effect of the titer increase.

Thus, some more microcapsule constructions containing other polymers, like $(CH\text{-}2(mod)/Car)_3$ or $(DEAE\text{-}dextran/Car)_3$ were tested (see Table 2).

Into the microcapsules the plasmids Inf HA and Inf NP expressing avian influenza virus hemagglutinin and nucleoprotein, respectively, were incorporated. The preparations of native plasmids or the ones enclosed into microcapsules were injected to mice intramuscularly. On day 14 in the blood sera of mice immunized with microcapsules $(CH\text{-}2(mod)/Car)_3$ the highest antibody titers were observed. On day 21 the blood serum antibodies raised with immunization of microcapsules $(DEAE\text{-}dextran/Car)_3$ or $(CH\text{-}2(mod)/Car)_3$ containing plasmids equalized and were 3 to 4 times as higher than the ones obtained through immunization with microcapsules $(PLL/Alg)_3$.

| Blood sampling, day | Antibody titers | | | | | |
|---|---|---|---|---|---|---|
| | Inf HA | | | Inf NP | | |
| | $(PLL/Alg)_3$ | $(CH\text{-}2(mod)/Car)_3$ | $(DEAE\text{-}dextran/Car)_3$ | $(PLL/Alg)_3$ | $(CH\text{-}2(mod)/Car)_3$ | $(DEAE\text{-}dextran/Car)_3$ |
| 14 | 1:625 | 1:1460 | 1:1125 | 1:125 | 1:1290 | 1:2290 |
| 21 | 1:125 | 1:125 | 1:100 | 1:1460 | 1:625 | 1:625 |

**Table 2. Antibody titers at immunization of mice with microcapsules of various compositions which contained plasmids Inf HA or Inf NP.**

Similar results were obtained with inoculation of an encapsulated plasmid pTKShi to mice (see Table 3).

| Blood sampling, day | Antibody titers | | |
|---|---|---|---|
| | $(PLL/Alg)_3$ | $(CH\text{-}2(mod)/Car)_3$ | $(DEAE\text{-}dextran/Car)_3$ |
| 14 | 1:800-1:1600 | 1:6400-1:25600 | 1:6400-1:12800 |
| 21 | 1:3200-1:6400 | 1:12800-1:25600 | 1:12800-1:25600 |

**Table 3. Antibody titers at immunization of mice with microcapsules of various compositions which contained the plasmid pTKShi.**

As evident from Table 3, at the injection of encapsulated plasmid DNAs in equal amounts the antibody induction depended on the microcapsule composition. The highest antibody titers were observed on day 14 in the blood sera of mice immunized with microcapsules $(CH\text{-}2(mod)/Car)_3$. On day 21 following the immunization with microcapsules $(DEAE\text{-}dextran/Car)_3$ or $(CH\text{-}2(mod)/Car)_3$ the blood sera antibody titers equalized and were 3 to 4 times as higher as compared to the ones seen after the immunization with microcapsules $(PLL/Alg)_3$.

## Conclusions

Thus, the microcapsule composition effects antibody induction in mouse organism. Here we demonstrated potency of biodegradable microcapsule-incorporated avian influenza virus DNAs delivery into cell, which in turn might serve as a basis for developing effective DNA-vaccines.

## References

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