Immobilization of animal cells in alginate/chitosan microcapsules and their cultivation

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Introduction

Today immobilization of animal cells in microgranules or microcapsules increasingly attracts the attention of experts in biotechnological industries. Cells in immobilized state are noted to keep their vitality for a long time (20 days or more) and at that keep intensive production of biologically active substances [1, 2, 3]. Natural polymers like sodium alginate and chitosan are most spread compounds suitable to form microgranules or microcapsules; being introduced into organism, they do not induce immune reaction and are able to stay long inside it.
The purpose of our work is to estimate mechanical strength of alginate-chitosan microcapsules and immobilization of animal cells into microspheres with their functional characteristics being preserved.

Materials and methods

Reagents. Sodium alginate (Sigma), calcium chloride (Carpov Chemical Plant, Ltd.), sodium chloride, (REACHEM, Russia), sodium salt EDTA, (Angar Plant of Chemical Reagents) were used. Chitosan with MM 3.8 kDa was kindly provided by Prof. A. Bartkowiak.
Cell lines: the Siberian mountain goat continuous kidney continuous cell line (SMGK-60); murine hybridoma clone SS-3 of IgG1 isotype, producing monoclonal antibodies, specific to porcine IgM.
Immobilization of cells in alginate/chitosan microcapsules. The cell sediment ($3x10^6$ cells) was resuspended in 2 ml of a 1.5% (w/v) sodium alginate solution prepared in physiological solution. The suspension was sprayed into a calcium chloride solution (0.5-1% w/v). The obtained granules were washed with physiological solution 3 times, with their following transfer into 25 ml of 0.1% (w/v) chitosan solution and mixed vigorously, the granule incubation time covering from 0.5 to 5 minutes. Then they were washed with physiological solution, put into 50 mM EDTA solution and incubated for 10 min at stirring. The obtained microcapsules were washed 3 times with physiological solution and nutrient medium without serum successively, then transferred into cultivation medium.
Cultivation of immobilized cells. The cells were cultivated in 500-ml flasks at 37^oC on a roller set (12 to 15 r.p.m.), the medium filling volume was 100 ml. Nutrient medium change was conducted when pH value decreased to 6.7-6.8. The cell concentration was calculated with a routine method in Goryaiev chamber with preliminary destruction of the microcapsules.

Results

As it had been shown before [4], alginate-chitosan membrane width at microcapsulation was dependent on the duration of alginate granule incubation in chitosan solution. Mechanical strength of microcapsules also depended on pH index of chitosan solution in the course of its contact to calcium-alginate granules [5]. The research works were conducted at pH ranging from 6 to 7 to keep maximal amounts of vital cells in the course of incapsulation. Significant drop in mechanical strength of microcapsules was observed at pH indices ranging from 6.2 to 7.0, when as early as

within 10 minutes' incubation the number of destructed capsules comprised 20% while following a 20 minutes' incubation it comprised more than 80% of their total numbers. At pH 6.0 microgranule strength rate was satisfactory and the amount of destructed microcapsules did not exceed 3% following a 10-minute incubation.

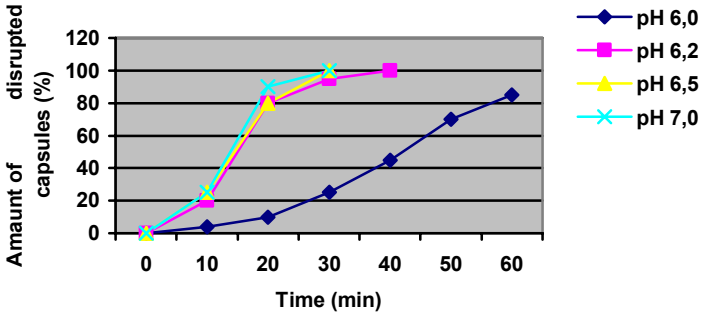

Fig.1. Dependence of mechanical strength of microcapsules from chitosan solution pH indices.

Optimal initial concentration of cells in sodium alginate solution was (1-2)x10^6 cells/cm^3. At this concentration about 70 to 150 cells per a capsule were seen in the formed capsules (Fig. 1A). As a result, the cell concentration following the immobilization was 50x10^3 cell per a cm^3 of nutrient medium. Within the first five days the hybridoma cell concentrations increased 10 times and by day 18 the general concentration of hybridoma cells reached 8x10^6 cells per a ml of nutrient medium which is 1000 times as higher of its initial concentration. At the same time, on day 12 concentration of living cells started its dropping while concentration of dead cells increased which might be connected with high cell density in a microcapsule and as a consequence with the decrease in nutrients & oxygen delivery to incapsulated cells (Fig. 2A).

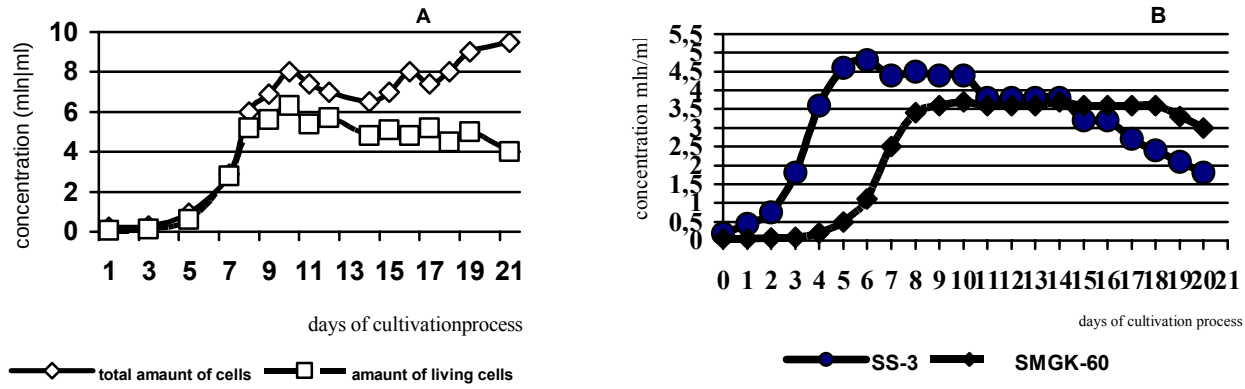

Fig. 2. Kinetics of immobilized cell growth:
A- hybridoma cells SS-3 in microcapsules;
B – hybridoma cells SS-3 and SMGK-60 in microgranules**.**

The basic sign of incapsulated hybridoma cells functional activity preservation is the stability of monoclonal antibody production. To examine the cell-produced monoclonal antibody accumulation the samples of cultural liquid were daily selected. Activity and specificity rates of the obtained monoclonal antibodies were determined through indirect ELISA. One or two days following medium change the monoclonal antibody activity in cultural medium was 1:1600. The total volume

of the collected medium containing monoclonal antibodies was 1500 ml within 18 days which was 15 times as higher as the antibody amounts derived at a single cultivation in a 1-ml matrass.

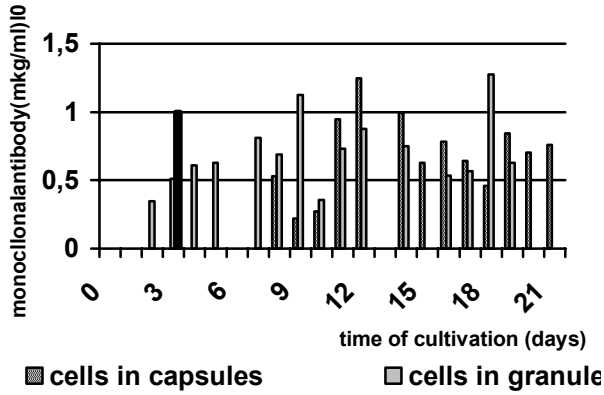

Fig. 3. Kinetics of hybridoma cell SS-3-secreted monoclonal antibody accumulation in medium.

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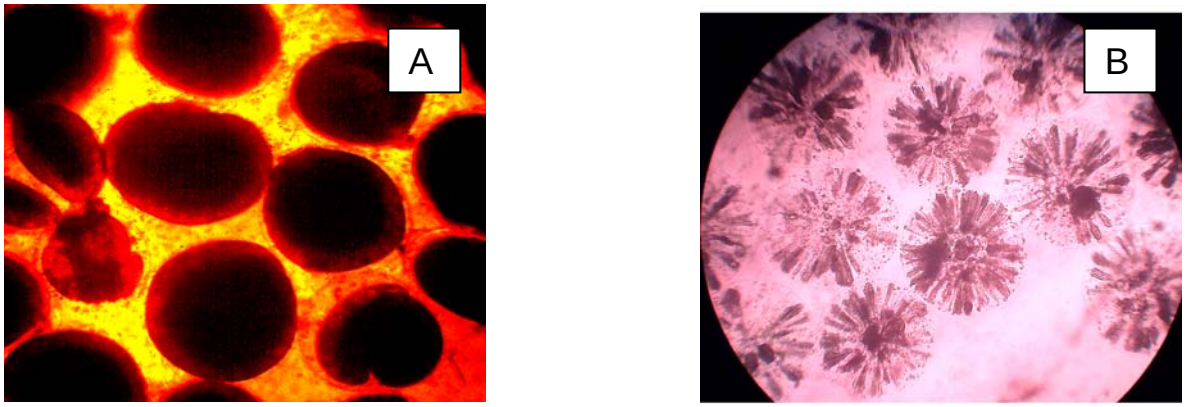

Fig.4. Hybridoma 1D2cells in microcapsules (A) and microgranules (B).

The optimal seeding concentration for cells at their insertion into microgranules was 2.5-3x10^6 cells per a ml of polymer mixture. At a lower concentration (i.e. 1x10^6 cells per a ml of polymer mixture) the cells did not get into all pores of the microgranules; at a higher concentration (4-5x10^6 cells per a ml of polymer mixture) filling of the granules and extinction of cells occurred within 7 to 10 days which did not answer the purposes of immobilized cell long-term cultivation. Similar results were obtained with cells SK and 1D2. The developed method of immobilization provides growth of various cell lines in microgranules and maintaining their vitality up to 20 days (Fig. 2B).

Direct infection of nutrient medium containing microsphere-immobilized cells with Teschen disease, bluetongue, classical swine or Aujeszky's disease viruses showed that the microspheres

were impermeable for viruses, and the cells sensitive to the above viruses, encapsulated inside them, were protected from a infected agent. Some attempts of infection into immobilized cells were made by treatment of the microgranules with 0,02% versene solution for 1 to 3 min. The example of the researches with Teschen disease virus showed that its accumulation indices in immobilized cells were a bit higher than those obtained at the infection with a stationary culture of these cells, but gaining of the maximal infectious activity rates of the virus shifted 2 to 3 days (Table 1).

Table 1.

Comparative activity rates for Teschen virus at stationary and granular culture methods.

Method of virus cultivation	Infectious dose (lg $TCID_{50/ml}$)	Accumulation of virus on days (lg $TCID_{50/ml}$)					
		1	2	3	4	5	6
Stationary	0,04	7,8± 0,32	8,3± 0,25	7,2± 0,16	6,0± 0,5	NT	NT
In granules	0,003	NT	6,0± 0,31	6,2± 0,25	8,75± 0,25	9,2± 0,27	7,8± 0,42

The 2-day shift of virus maximal accumulation as compared to the stationary culture might be due to the infection of cells located deep in the granule occurs much later than of the ones located near its surface.

Conclusions

Analysis of earlier results [2,3] and presented investigation data allow making a conclusion that chitosan may be used for production of microcapsules aimed at cultivation of various substrates within. Class G immunoglobulins (IgG) are able to enter through alginate-chitosan membrane. The encapsulated hybridoma cells were the case showing that their cultivation allows collecting the produced MAbs within a long period of time (i.e. 18 days). Inoculation of the microcapsules containing heterologous cells (SMGK-60) into murine organism provides their reproduction and prevents from implantant rejection.

References

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