Cultivation of mammalian cells within semi-permeable microcapsules

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INTRODUCTION

Today cell immobilization in microcapsules increasingly attracts the attention of the researches working in the field of biotechnology. Cell immobilization within semi-permeable microcapsules allows a free exchange of nutrients, oxygen as well as cell products between entrapped cells and cultivation medium. The main advantage of this technique is a production of a high quantity of cells within microcapsule volume. All these points make the microencapsulation technology widely used in biotechnology, biomedicine and tissue engineering, Orive G. (2003).

One of the most known applications of this method is a cultivation of genetically modified cells to obtain recombinant products (peptides, enzymes, proteins), Seifert DB (1999). The microencapsulated cells were shown to maintain their viability for a long time (20 days or more), providing high production of biologically active compounds. Moreover, cell microencapsulation is one of the promising strategies for tissue engineering and cellular therapy which has a potential to be used for development of a novel 3-dimentional (3-D) model of small size solid tumor for experimental oncology, Markvicheva E. (2003). The advantages of this method are generation of significant quantities of cells and production of multicellular spheroids of desired sizes based on various cell lines.

Thus, the aim of the current research was 1) to demonstrate an ability of various cell lines, different in their morphology and origin, to grow within polyelectrolyte microcapsules and 2) to show a capability of microencapsulated 1D2 cells to produce IgG monoclonal antibodies (MAbs) against an idiotypic determinant on 1D2 (anti-ricin A chain monoclonal antibody) and genetically modified H1299 cells to produce chimeric antibodies against Interferon-γ.

MATERIAL AND METHODS

Chemicals: Sodium alginate (medium viscosity), EDTA and $CaCl_2$ were from Sigma. Oligochitosan (MM 3500 Da, DD 98 %) was kindly provided by prof. A.Bartkowiak (Poland). All solutions for cell immobilization were prepared with 0.9% NaCl.

Cells and cell cultivation media: A number of mammalian cell cultures were used in this study, such as Siberian mountain goat kidney cells (PSGK-60); Baby hamster kidney cells (BHK-21); mouse hybridoma cells (1D2) producing IgG MAbs to an idiotypic determinant on 1D2; mouse melanoma cell line (M3); human breast adenocarcinoma cells (MCF-7); mouse myeloma cells (Sp2/0); human leukemia cell lines (CCRF-CEM and CEM/C1); mouse lymphoma cells (P388D1); and

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genetically modified human lung carcinoma cells (H1299) producing chimeric antibodies against Interferon-γ.
H1299 cells were cultured in DMEM-F12 medium with 5 µg/ml penicillin and 5 µg/ml of streptomycin; PSGK-60 cells were cultured in Eagle's medium (MEM) and the other cell lines were cultivated in Dulbecco Modified Eagle's Medium (DMEM). All culture media was supplemented with 10% foetal bovine serum (HyClone, USA). Cells were cultivated at 37^0C in a humidified 5 % CO_2 / 95 % air atmosphere.

Microencapsulation of cells: Cell precipitate (1 x 10^6 cells) was mixed with 2 ml of a sterilized sodium alginate solution, and the mixture was extruded using an air-flow device or an electrostatic bead generator into 0.5 % $CaCl_2$ by peristaltic pump. The obtained hydrogel microbeads were incubated with 0.2 % oligochitosan solution for 10 min, in order to form alginate-oligochitosan membrane on microbeads surface. Then microbeads were washed 3 times with physiological saline. In order to get hollow microcapsules, the microbeads were incubated in 50 mM EDTA solution for 10 min and they were again washed and transferred into cultivation medium.

Cultivation of microencapsulated cells: The cells entrapped in microcapsules were cultivated in 25 cm^2, 75 cm^2 (Corning Inc.) flasks or in 500-ml flasks on a roller set (12 to 15 rpm). The medium was replaced when its pH value decreased to 6.7-6.8. To measure cell concentration in the microcapsules, their aliquot (0.1 ml slurry) was mechanically destructed to release cells, and then the cells were calculated in hemocytometer.

Determination of antibody production: Monoclonal antibody quantification in cultivation medium of encapsulated cells was measured using ELISA. For hybridoma 1D2, 96-well immunoplates were coated with 100 µl of anti-mouse IgG (5 µg per well) and then incubated for 18 h at 4^oC. Then wells were washed twice in PPS-PSM (phosphate-buffered physiological solution (pH = 7.2-7.4) with 0.05 % polyoxyethylene (20) sorbitan monolaurate). The adsorption ability in wells then was blocked by incubation in of 200 µl 1 % BSA (in PPS-PSM) at 37 ^{o}C for 1h. After that wells were washed 4 times in PPS-PSM. Antibody supernatants 100 µl were added in each well and incubated for 1h and then were washed 6 times in PPS-PSM. MAbs added to the surface were detected with rabbit antibodies peroxidise conjugate against mouse IgG. After incubation for 45 min wells were washed 8-times in PPS-PSM, and a substrate solution was added. The absorbance was measured with microplate reader (Multiskan MCC/340 at 450 nm) after 20 min.

RESULTS AND DISCUSSION

In order to demonstrate cell growth and proliferation in polyelectrolyte microcapsules, the cells which differed in their morphology (suspension culture or monolayer) and their origin (human, mouse, etc) were chosen for microencapsulation. The optimal initial cell concentration used for microencapsulation was found to be (1.0 ± 0.2) x10^6 cells/ml of sodium alginate solution. This concentration provided formation of microcapsules with homogeneous cell distribution inside the microcapsules (70-150 cells per microcapsule).

Table 1 demonstrates an ability of various cell lines to fill microcapsule volume. This ability was found to depend upon the properties of cell line, namely its origin and morphology. We succeeded to get cell aggregates within microcapsules for almost all selected cell lines, except mouse melanoma M3. Melanoma cells grew on the inner microcapsule membrane but did not fill the microcapsule volume. Encapsulated M3 cell growth and proliferation were found to be suppressed,

in spite of the free volume inside microcapsule where cells could rotate and move freely. Nevertheless, 9 cell lines were successfully cultivated inside microcapsules being viable (more than 70 % cells) during all the cultivation time.

Cell line	Time needed to fill microcapsule volume, days
CCRF-CEM	18
CEM/C1	22
1D2	11
Sp2/0	14
P388D1	28
MCF-7	28
M-3	–
BHK-21	11
PSGK-60	14

Table 1 : The list of cell line grown in alginate-oligochitosan microcapsules.

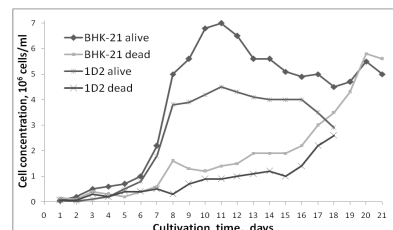

Figure 1 : Growth curves for hybridoma 1D2 cells and for BHK-21 cells encapsulated in alginate-oligochitosan microcapsules.

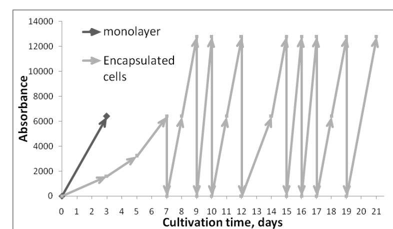

Figure 2 : Accumulation of IgG MAbs produced by 1D2 cells in cultivation medium.

A typical growth curves for encapsulated cells are shown in Figure 1. Initially, cells distributed evenly as single cells within the microcapsule. After being cultured for 5 days, the cells formed small aggregates within the microcapsules. These aggregates increased in their sizes and filled the whole microcapsule volume in 14-30 days after encapsulation. As can be seen from Figure 1, by day 10 the cell concentration reached 7 x 10^6 cells/ml and 4.5 x 10^6 cells/ml for BHK-21 and 1D2 cell cultures, respectively. On day 15 alive cell concentration decreased while dead cell concentration raised. This could be explained by high cell density within the microcapsule resulting in the decrease of diffusion of nutrients and oxygen supply into the encapsulated cells. Thus, the cell microencapsulation allowed to provide long-term cultivation (up to 20 days) of 9 various cell cultures and to maintain viable cells at very high concentrations within microcapsules.

The possibility to maintain high densities of viable cells in microcapsules due to the ability of semi-permeable membrane to provide a free exchange of bioactive substances between the entrapped cells and the cultivation medium, were the reasons to encapsulate hybridoma 1D2 and genetically modified H1299 cells for their further use to obtain recombinant products.

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Samples of culture medium were collected daily and MAb concentrations were determined by ELISA. Monolayer cell culture was used as a control. 1D2 cells entrapped in microcapsules presented a higher antibody production after first 9 days. As can be seen from Figure 2, MAbs with the activity rate of 1:13000 were tested in the medium after the medium replacement on day 1 or 2. Another H1299 cell line genetically modified for transient expression and production of chimeric antibodies to interferon-γ were encapsulated in microcapsules and cultivated for one month. Chimeric antibodies production per 1 ml of cultivation medium by cells growing in microcapsules was shown to be 1.5 times higher compared to monolayer culture. These results confirmed that encapsulation of transfected cells is an effective strategy for antibodies production.

CONCLUSIONS

The alginate-oligochitosan microcapsules were demonstrated to provide the growth and proliferation of 10 animal cell lines different in their morphology and their origin. For 9 cell lines the compact cell clusters within microcapsules were formed. The long-term cultivation of 1D2 and H1299 cells within microcapsules allowed to provide antibody production for 20 days. Thus, the biocompatible microcapsules embedded with animal cell lines could be successfully used in a number of biotechnological areas, whereas a variety of biocompatible polymers could expand the potential of the microencapsulation technique.

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