O9-3 **Microencapsulation of tumor cells as a useful tool for anticancer drug screening**

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INTRODUCTION

Since Sutherland developed the multicellular tumor spheroid (MTS) model to mimic the 3D-structure of small size solid tumors (Sutherland 1970), MTS have been found to be useful in several aspects of tumor biology, including screening and development of a novel anticancer agents (AA).

However, a simple and reproducible technique to generate MTS is a prerequisite for spheroid-based applications. The general criteria for selecting MTS production methods include efficiency, MTS unimodality, convenience and suitability for subsequent applications. Microencapsulation seems to be one of the simplest methods to generate MTS. Tumor cell microencapsulation provides several advantages over all classical techniques commonly used, such as generation of significant spheroid quantities, production of MTS with desired sizes, generation of MTS based on tumor and non-tumor cells which normally can't form aggregates in suspension culture, and finally, co-cultivation of tumor and normal cells in one microcapsule.

In the field of cell microencapsulation alginate (as polyanion) and poly-L-lysine (PLL) (as polycation) are usually used to form microcapsules. In our work we propose a replacement of PLL with chitosan based polycations, since chitosan is known as smart and pH-sensitive polymer. In fact, most commercial chitosans are soluble only at acidic pH value while cell encapsulation procedure should be carried out under physiological conditions (pH 6-8). Oligochitosans (MW below 20 kDa) are soluble at physiological pH values and can be used to form polyelectrolyte microcapsules. Furthermore, the grafting of chitosans with biocompatible water-soluble synthetic polymers, e.g. poly(vinyl alcohol) (PVA), could be also a promising approach.

In the present study we aimed to elaborate biocompatible microcapsules using alginate and chitosan based polycations, with entrapped MTS, and to test these MTS for estimation of the efficacy of anticancer therapies (e.g. photodynamic therapy and chemotherapy).

MATERIALS AND METHODS

Chemicals

Sodium alginate (medium viscosity, approx. 3,500 cps at 25 °C), EDTA and $CaCl_2x2H_2O$ were from Sigma (Germany). Oligochitosan (3.5 kDa, DD 98%) was kindly provided by A. Bartkowiak. Two graft copolymers of chitosan and PVA, i.e. chit-g-PVA_1 and chit-g-PVA_{20}, having PVA/chitosan molar ratio 0.92 and 20.5, respectively, were obtained by solid-state synthesis as described earlier (Ozerin 2006). All solutions for cell microencapsulation were prepared using 0.9% NaCl. Anticancer agents: Chlorin e6 (Ce6) was supplied by Porphyrin Products (Logan, UT, USA), Photosens© (NIOPIC, Russia), doxorubicin (Dox) and its derivatives with palmitic acid and 5-fluorouracil were synthesized Institute of macromolecular compounds (St. Petersburg),.

Cells and Culture Conditions In our study human cervical adenocarcinoma cell line (HeLa) and 2 human breast adenocarcinoma cell lines (MCF-7 and dox-resistant MCF-7/DXR) were cultured in DMEM (PanEco, Russia) and RPMI-1640 medium (GIBCO, USA), respectively, supplemented with 10% fetal bovine serum (FBS) purchased at HyClone (USA), at 37 °C in a 5% CO_2 atmosphere and were reseeded every 2-3 days.

Microencapsulation of Tumor Cells Microencapsulation of tumor cells was performed as described earlier (Zaytseva-Zotova 2011). Briefly, cell precipitate ($6x10^6$ cells) obtained by trypsinization of monolayer cell culture was mixed with 2 ml of a sterilized sodium alginate solution (1.3% w/v), and the mixture was added into $CaCl_2$ solution (0.5% w/v) using an electrostatic bead generator. The obtained hydrogel Ca alginate microbeads were incubated with oligochitosan or copolymer solution for 10 min, in order to form an alginate-oligochitosan membrane on the microbead surface. To get hollow microcapsules, the microbeads were incubated in a 50 mM EDTA solution for 10 min and then transferred to the culture medium. To form MTS within microcapsules the previously encapsulated cells were cultivated in cell culture medium supplemented with 10% FBS using 75 cm^2 flasks (Corning Inc.) at 37°C in 5% CO_2 for 2-4 weeks. Empty microcapsules (without cells) were prepared in the same way as described above. The microcapsule size distribution, the membrane thickness and the cell overall morphology were characterized using optical microscopy (Reichert Microstar 1820E, Germany). A non-specific sorption of Ce6, Photosens© and Dox derivatives by microcapsules as well as microcapsules permeability was analyzed using spectrophotometer Backman DU-70 (USA) and spectrofluorimeter Hitachi F-4000 (Japan).

Cytotoxicity study An aliquot of MMTS (100 µL) was incubated with AA solution (0 - 50 µm) in 24-well plates for 24 h in a darkness. Each well contained culture medium (0.5 mL) supplemented with FBS (2%). Monolayer cell culture (10^5 cells per well) was used as a control. Cell viability was assessed using the MTT colorimetric assay. To study photocytotoxicity of Ce6 and Photosens© the MMTS aliquots (100 µL) and the monolayer culture (10^5 cells) were incubated with the photosensitizer solution in 24-well plates for 24 h. Then, MMTS and the cells were washed 3 times with PBS, then 0.5 mL of the culture medium was added to each well, and the samples were irradiated at 650 nm diode laser light (light power density was 30 mW cm^{-2}, and light energy densities were varied within a range of 0.5 to 70 J cm^{-2}). The cell viability was measured by MTT-assay in 24 h after irradiation.

RESULTS AND DISCUSSION

In this study chitosan-g-PVA copolymers were obtained by a novel Solid-State Reactive Blending method. It was found that microcapsules based on the polyelectrolyte complex between alginate and chitosan-g-PVA copolymers are pH-sensitive and could be easily dissolved (opened) by slightly rising pH value of the culture medium up to pH 8.0 – 8.2. Nevertheless, the microcapsules were stable during long-term cell cultivation and provided cell growth and proliferation. To get MTS, microencapsulated tumor cells were cultivated for 2 – 4 weeks. The cells grew in aggregates which have been increasing in their sizes with the cultivation time until they fill all microcapsule volume (fig. 1).

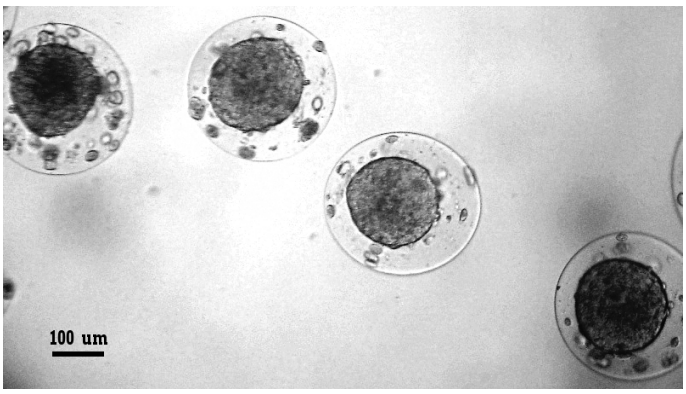

Figure 1 : Microencapsulated MTS based on MCF-7 cells, 2 weeks

The obtained encapsulated MTS were used to estimate cytotoxicity of the AA. And it was shown, that for all the AA the cytotoxicity increased with increasing of drug concentration in both encapsulated MTS and monolayer culture (control), but the viability in encapsulated MTS was considerably higher than that one in monolayer culture. For instance, the results obtained for Photosens© are presented in Fig. 2.

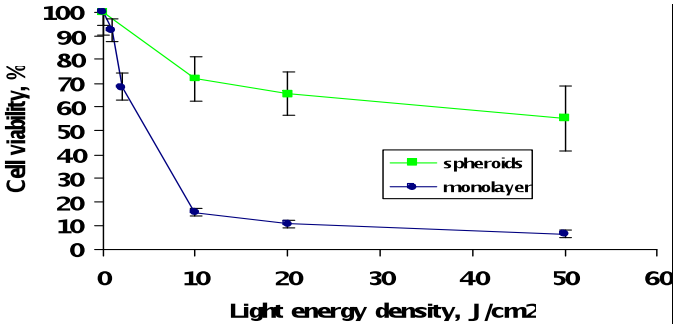

Figure 2 : MCF-7 cell viability in monolayer and spheroids 24h after photodynamic treatment with Photosens© (5 mg/ml) at various light energy densities

CONCLUSIONS

The smart microcapsules based on the polyelectrolyte complex between alginate and chitosan-based polycations were developed. The microcapsules were found to support growth of tumor cells and MTS formation. The use of novel chitosan-g-PVA polycations was shown to make possible simple MTS release from the microcapsules after long-term cell cultivation by slight increasing pH of cultivation medium up to 8.0 – 8.2. A number of AA was tested on encapsulated MTS. Our results confirmed that 3D organization of MTS leads to the remarkable decrease in anticancer therapy efficacy, compared to 2D monolayer cell cultures. Thus, encapsulated MTS could be considered as a promising 3D *in vitro* model to estimate the doses of drugs *in vitro* before carrying out preclinical tests. This work was partially supported by the bilateral Russian-French program (the joint CNRS-RFBR PICS project N 10-04-91056).

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