BIOENCAPSULATION OF TUMOR CELLS IN NOVEL ALGINATE-(CHITOSAN-PVA) MICROCAPSULES

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Introduction

Presently multicellular tumor spheroids (MTS) are being widely used in various aspects of tumor biology, including studies in the field of radiation biology, photodynamic therapy as well as for testing effects of various chemotherapeutical drugs. As well known, cells cultured in monolayer or suspension can not mimic the cells *in vivo* properly. An *in vivo* mature tumor with an extensive vasculature has a very complex structure, consisting of regions of regularly dividing cells, hypoxic cells and necrosis zones, at increasing distances from blood vessels with 3D pattern bearing structural heterogeneity. To mimic the 3D-structure of small solid tumors, the multicellular tumor spheroid model was developed in the 1970s (Sutherland R.M. et al., 1970). However all classical *in vitro* methods, such as liquid-overlay, spinner flask and gyratory rotation systems, are time consuming and can not provide the production of spheroids with narrow size ranges. More over, some tumor cells can not form spheroids in suspension at all. Recently cell encapsulation method was proposed by us as a novel technique to quickly and easily prepare a large number of spheroids with narrow size distribution within a desired diameter range (Markvicheva et al, 2003). The idea of tumor cell encapsulation for generating tumor spheroids was developed and extended to testing chemotherapeutical drugs (Xulang Zhang et al., 2005). The authors of this paper proposed a new name for this kind of *in vitro* models, namely microenncapsulated multicellular tumor spheroid (MMTS). However, a spectrum of polymer materials which can be used for preparation of microcapsules is still very poor. Most of the researches used alginate (as a polyanion) and poly-L-lysine (as polycation) to prepare microcapsules.

The aim of the current paper is to propose a novel polycations (graft copolymers based on chitosan and poly(vinyl alcohol) (PVA); to elaborate a technique for preparing mechanically stable microcapsules using them, and to demonstrate a possibility for generating multicellular spheroids from various tumor cell lines (differed in morphology and origin) within these microcapsules.

Materials and Methods

Chemicals. Sodium alginate (Alg), medium viscosity, was from Sigma. Oligochitosan (MM 3.5 kDa, DD 89%) was kindly provided by Prof. Bartkowiak. Copolymers based on chitosan and poly (vinyl alcohol) (15 and 80 % of chitosan, wt) was obtained by solid-state synthesis from chitin (MM 650 kDa) and poly(vinyl acetate) (MM 50-100 kDa), as described earlier (Akopova et al, 2005). The method is based on joint action of high pressure and shear strain onto mixtures of solid polymers (powders). EDTA and $CaCl_2$ were from Sigma. All solutions for cell immobilization were prepared using 0.9% NaCl.

Cells and cell cultivation media. In our study a series of tumor cell lines were used : mouse melanoma cells M3, human breast adenocarcinoma cells MCF-7, mouse myeloma Sp2/0 cells, human leukemia CCRF-CEM and CEM/Cl cell lines. Cells we cultivated in DMEM or RPMI-1640 (Flow Laboratories) supplemented with foetal calf serum (FCS), BioClot.

Bioencapsulation of tumor cells in microcapsules A precipitate of cells (10^6 cells) were mixed with 2 ml of a sodium alginate solution, and the mixture was added to 25 ml of polycation solution (oligochittosan or chitosan-PVA copolymer) using a peristaltic pump and an air flow device. After incubation of obtained calcium alginate (CaAlg) microbeads in polycation solution for 4-7 min they were washed 3 times with physiological solution. Then the microbeads were incubated in 50 mM EDTA solution for 10 min and washed 3 times with 0.9% NaCl. After the followed incubation of the microbeads in 0,2 % AlgNa they were again washed and transferred into cultivation medium.

Cultivation of bioencapsulated cells to generate tumor multicellular spheroids Cells entrapped in alginate-oligochitosan or alginate-chitosan-PVA microcapsules were cultivated in 25 cm^2 (Corning Inc.) flasks in a 5% CO_2 atmosphere at 37°C.

Results and Discussion

The microcapsules were obtained in two steps : 1) preparation of CaAlg beads coating with polycation membrane and 2) dissolving the alginate core using any chelate compound (sodium citrate, EDTA). In order to develop stable microcapsules of the desired size with the desired membrane thickness (80-100 μm), we varied copolymer concentration and the incubation time of previously obtained CaAlg beads in polycation solution. As polycations 3 samples of copolymers and oligochitosan (as a control) were studied (Table 1). As can be seen, for all 3 samples used the conditions allowing to prepare stable microcapsules with desired membrane thickness of 80-100 μm were found.

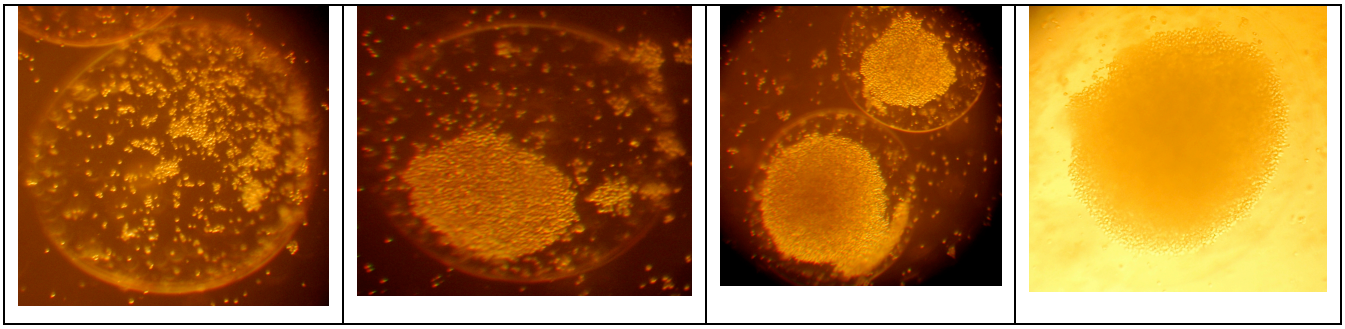
Table 1. Optimization of preparation of microcapsules with various membrane thickness.

Polycation sample	*Concentration of polycation , %*	*Membrane thickness at various incubation time, μm*		
		5 min	***7 min***	***10 min***
Oligochitosan (control)	**0.10**	78 ± 5	112 ± 6	Membrane was not observed
	0.20	89 ± 5	135 ± 6	Membrane was not observed
	0.40	Membrane was not observed		
Chit-PVA (1) (chitosan 80% wt)	**0.20**	27 ± 5	-	54 ± 5
	0.25	51 ± 5	76 ± 5	Membrane was not observed
	0.50	81 ± 6	-	Membrane was not observed
Chit-PVA (2)* (chitosan 15% wt)	**0.75**	-	37 ± 5	66 ± 5
	1.15	-	42 ± 5	87 ± 5
Chit-PVA (3)** (chitosan 15 % wt)	**0.20**	-	-	30 ± 6
	0.40	45 ± 5	81 ± 5	108 ± 6

(2)* polymer fraction soluble in cold water, MM 30 kDa; (3)* fraction soluble in hot water, MM 60 kDA.

The growth of mouse myeloma Sp2/0 cells encapsulated in alginate-(chitosan-PVA-1) microcapsules is demonstrated in Fig.1. As can be seen, the cells (which belong to suspension cultures, and therefore this is not

possible to generate multicellular spheroids by using common methods) formed small multicellular spheroids within microcapsules already on day 5. These multicellular spheroids increased in size and filled the whole microcapsule volume in 2 weeks after bioencapsulation (Fig.1 d).



(a) **(b)** **(c)** **(d)**

Fig.1. Growth of mouse myeloma Sp2/0 cells in alginate-(chitosan-PVA-1) microcapsules : a) day 1; b) day 5; c) day 10; d) day 14.

In order to test microcapsules for their capability to support the growth of different tumor cell lines, we have chosen for bioncapsulation the culture cell lines which differ in their morphology (suspension culture or monolayer) and their origin (human, mouse).

Table 2 shows that we succeeded to generate multicellular spheroids within microcapsules using 4 various mouse and human tumor cell cultures (both suspension and monolayer lines), but we failed

Table 2. Generation of multicellular spheroids in alginate- polycation microcapsules

Cell line	Oligochitosan	Copolymer chitosan-PVA-1	Copolymer chitosan-PVA-2	Character of cell growth in microcapsules	Time needed for generating multicellular spheroids, days
Sp2/0 (Ag14)	+	+	n.d.	within microcapsule volume	14
MCF-7	+	n.d.	n.d.	within microcapsule volume	28
M-3	–	–	–	on the surface of inner membrane	–
CCRF-CEM	n.d.	+	+	within microcapsule volume	18
CEM/C1	n.d.	+	+	within microcapsule volume	22

n.d. not determined

+ spheroids were generated within microcapsules; - spheroids were not generated within microcapsules.

in the case of mouse melanoma M3. Although both human MCF-7 and mouse M3 cells grow in monolayer under common conditions, it was not possible to get spheroids which could fill the whole

microcapsule volume in the case of melanoma M3 cells. The cells grew attached to the inner microcapsule membrane but did not fill the microcapsule volume.

The Table 2 demonstrates that the ability of cells to generate spheroids within microcapsules depended upon the properties of cell line (its origin and morphology), but did not depend greatly on the type of polycation we used.

Conclusion

Thus, we developed mechanically stable microcapsules based on alginate and new polycations (copolymers of chitosan and PVA), and demonstrated that they can be successfully used for development of novel microenncapsulated multicellular tumor spheroids (MMTS). These *in vitro* models will be employed to study anticancer treatment mechanisms.

References

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