CELL CULTURE IN MICROGRAVITY CONDITIONS.

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Abstract

Results of "Fibroblast-1" and "Fibroblast-2" experiments carried out in space flight on board biosatellites "Bion 10" and "Foton-10" in collaboration with specialists of European Space Agency (ESA) with the use of "Biobox" board equipment manufactured by "Dornier" by order of ESA are adduced. The subjects of investigations were cell cultures *in vitro* obtained from mice embryo and human praeputium. It is demonstrated that under microgravity conditions cell division rate, growth and motility of monolayer cultures developing on solid substratum decrease. It is supposed that the main cause of the reduction of cell functional activity is the diminishing of cell-cell and cell-substratum interactions (adhesive properties). An inclusion of growth factors into substratum-collagen and fibronectine- improves cell growth properties. Possible molecular mechanisms of gravity influence on growth and motility of cells in culture *in vitro* are discussed. The data obtained are of both theoretical and practical interest for space biology and medicine.

Introduction

For a long time unicellular organisms living in liquid medium or cells of different organs and tissues within multicellular animal or plant organisms served as the subjects for space studies. Far fewer experiments have been conducted on cultures of isolated cells and tissues functioning in liquid or on solid substrata/1,2,3,4.5/.

Two experinments on connective tissue cultures - fibroblasts- with the use of "Biobox" device in space were carried out by us.

The experiment "Fibroblast-1" was conducted on board specialized biosatellite "Cosmos-2229" in December 1992. As a subject of studies we used fibroblasts isolated from mice embryos and cultivated both in monolayer on glass and in three-dimensional histoculture. Afterflight analysis of the results obtained demonstrated that the space flight conditions significantly affect morphological characteristics of cell culture. Considerable aggravation of the quality of monolayer and the general state of the culture was observed to the end of the experiment. After autoradiography we have analysed a number of nuclei, the ratio of the total nuclei number to the number of nuclei labelled with ^{3}H-thymidine in control and flight specimens of monolayer culture. The results are presented on figure 1. Note that the number of cells per 1 mm^2 of the glass in the flight variant is markedly lower than in the control; cells were less sprawling, leading to a twofold decrease in the mean nucleus area. Nuclei become more oval. The data adduced show that in monolayer culture samples exposed on board biosatellite the number of nuclei (both total and of labelled with ^{3}H thymidine) is significantly lower than in control. A comparative analysis of the total nuclei number and of the number of nuclei labelled with ^{3}H thymidine in a histoculture revealed no significant difference between flight and control samples. The data obtained provide evidence that microgravity conditions have a negative influence on the general state of the culture. We suggest that the main reason for worsening of the state of monolayer culture to the flight end may be the weightlessness . On the basis of this assumption, a hypothesis was suggested claiming that adhesive cell properties may be changed under space flight due to a sharp decrease in microgravitation /6/.

To verify this hypothesis, it was proposed to conduct an experiment in which films of different nature and molecular composition would be used as substrates for developing monolayer, together with traditional cover glass. Moreover, it was intended to carry out an experiment on human fibroblasts with the view of evaluating the results obtained more precisely and enhancing their

scientific and applicable significance.

The "Fibroblast-2" experiment was prepared in the laboratory of gravitation biology of the Institute of Biomedical Problems in cooperation with the laboratory of dermatology (Liege University, Belgium). The experiment was financially supported by European Space Agency (ESA) and was being carried out during a period from February 17 to March 3, 1995 on board Russian spacecraft "Foton-10".

Materials and methods

Fibroblast cultures were obtained from postnatal skin of human extreme flesh (praeputium) by growing monolayer from pieces placed between two glasses. The culture was grown on DMEM medium with the addition of 10% embryonic serum on plastic glass of 10 x 20 mm^2. Three variants of the experiment were worked out. In the first variant, the culture was grown on pure glass; in the second variant, glasses covered with sorbed collagen and in the third, those covered with sorbed fibronectine were used. Suspension (2.5 ml) containing 2.5 x 106 cells per 1 ml of the medium was poured into Petri dishes with glasses prior to filling up the devices.

The experiment was conducted in the "Biobox" device (manufactured by "Dornier") by order of European Space Agency. The needed automatic temperature regime was maintained in the device; media were exchanged and biomaterial fixed. The biomaterial was placed into cultivation chambers (each of 1 ml volume) in special plunger-containers (PC). In total, eight PC were used: four for performing flight experiments and four for earth control. One Pc of each four was used for biochemical analysis by specialists from Belgium. The PC have two modules separated from each other, and each contains one cultivation chamber connected with three polyethylene containers (1 ml volume). The first container was filled with cultivation medium with ^3H thymidine, the second was filled with Henks medium, and the third was filled with fixator consisting of 80% alcohol in six plungers; in two plungers (one in control and the other experimental) were filled with GITS fixator for specialists from Belgium. PC had mechanical arrangements for media exchange in chambers activated by electrical impulses sent by commanding arrangement "Biobox".

Prior to transfer into PC chambers, cells, which did not develop monolayer, were placed on noncovered glass. Monolayer was placed on glasses covered with collagen and fibronectine. A portion of monolayer on narrow glass sides was taken using teflon scratcher (a wound was made). Also, culture specimens on collagen and fibronectine were fixed in Petri dishes. The biomaterial was placed into PC as follows. In the first pair of PC, both chambers were used for fibroblasts cultivation on a pure glass. In the second pair, fibroblasts growing on sorbed fibronectine were cultivated. In the third and fourth pairs of PC, cultures were grown on collagen. Cultivation chambers were filled in with nutrient medium so that the volume of air bubble in them was minimal. Filled and hermetically covered electromechanical PC were transferred into "Biobox" and stored at 20 °C for 2.5 days. The experiment started after 30 min following satellite entrance to orbit. The commanding device of "Biobox" increased the temperature during 2 h from 20 to 37 °C, and then it was commanded that the initial medium in chambers should be changed for a medium with ^3H thymidine. The active phase of the experiment lasted 48 h. After that, the Henx medium was given to the chamber to wash the culture from the medium with ^3H thymidine, and after 10 min. fixator was placed to the chamber. The control experiment was conducted synchronously with the flight experiment. After fixation, the material was stored at 17 °C in the "Biobox" and then in refrigerator at 8 °C. In the process of the afterflight treatment, cells were stained with hemotoxilin. Next, the total number of nuclei per 1 mm^2 of glass was calculated using VIDS system including NIKON microscope, IBM PC and a monitor with digitizer, then, the nucleus area was calculated. Autoradiography is being conducted at present.

Results and Discussion

At the end of the experiment (landing of the satellite) and the delivery of "Biobox" to the laboratory, all PC were thoroughly inspected. Unfortunately, not all fixations were successful. In the control experiment, leakage from the plunger containing glass with fibronectine occurred,

leading to the loss of a portion of the material. Inspection of glasses in flight and control variants revealed the absence of cells on glasses not covered with collagen or fibronectine. The state of monolayer on remaining glasses cannot be considered as satisfactory. A portion of cells are absent, wound sides are uneven. Cells output into the wound was observed only once, in the flight experiment on a collagen basis.

Results of the experiment are shown in Tables 1 and 2.

The mean number of nuclei per 1 mm^2 of glass calculated in all experimental variants is presented in Tables 1 and 2. As seen from these data, the state of monolayer on the collagen and fibronectine bases is different. Cell density in the material fixed prior to filling in plungers (background) on the collagen base is four times higher than cell density on the fibronectine base. Apparently, this may be explained by a decrease in the cell number (nuclei per 1 mm^2 of glass) on the collagen base in the process of the experiment. In the flight material, this decrease is nearly 50% and in the control it is 25%. Thus, in this case, the flight conditions aggravate life activity on the collagen base. The number of cells on glass covered with fibronectine increased in the experiment by approximately 80%. No significant difference between the values in the flight and control variants was observed.

Table 2 presents results of morphometry of fibroblas nuclei. As can be seen from the table, the nuclei area is larger by nearly 30% in a more rare background culture grown on fibronectine than in the nuclei area in the background culture grown on collagen. The size of the form factor indicates equal proportion between nuclei area and their perimeter, though nuclei of background cells grown on collagen have more elongated shape than nuclei of cells grown on fibronectine. At the end of the experiment, nuclei area in cells of the flight variant on collagen increased by approx. 30%, compared with the background, they become more oval; at the same time young cells entering the wound have nuclei area equal to the background and width/length ratio remains unchanged, compared with the background. Also, the area of nuclei grown on collagen base in the control is equal to the background area. The width/length ratio of nuclei of cells grown on collagen during the flight and in control is the same. The nuclei area does not increase on glass covered with fibronectine in the flight variant, whereas in the control variant, the area is significantly decreased and reaches the value obtained in the control on the collagen base. In all three variants, no change in the ratio of cell area to the perimeter was observed. However, cell nuclei become more elongated, compared to the background, both in flight and in control. Note that no differences were obtained between the width/length values obtained during the flight and in the control.

Conclusion

The data obtained indicate that the molecular composition of the substrate, with which cells interact during the preflight growth and in the process of the experiment significantly affects the results of the experiment. Cells, which did not form monolayer during the preflight growth on a pure glass, did not proceed their growth in the process of the experiment and completely died. The number of cells grown on collagen up to very dense monolayer in the experiment decreased. The nucleus shape was changed becoming oval. Cells grown on fibronectine and having not dense monolayer in the background developed in the experiment to reach equal size both in control and experiment; their number was increased by approx. 1.8 times.

The shape of cell nuclei changed, giving elongated cells. The base composition affected differences between afterflight area in control and flight variants to a lesser extent, although background values of nuclei areas differed considerably. Cells developed in wound during the flight were morphologically nearer to the background morphological parameters than to the control ones.

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Tabl.1 Average of nuclei in fibroblast cell culture per 1 mm^2 area.

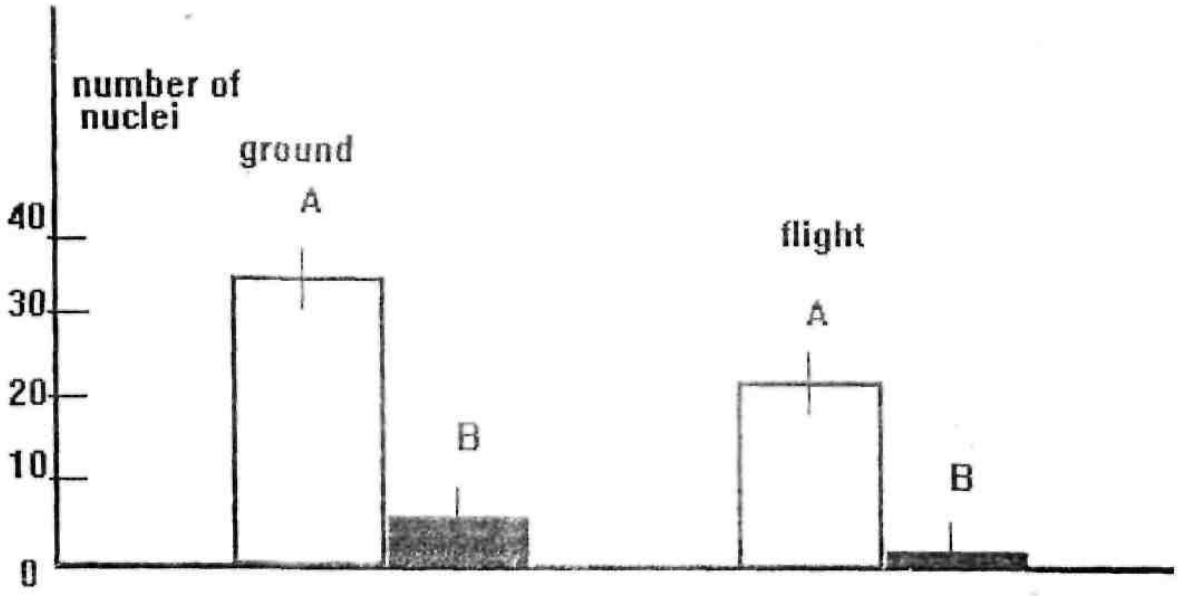
Variants	Prior state	Flight	Ground	P
Cells on collagen	2357 ± 98	1198 ±56	1768± 115	00,1
Cells on fibronectine	843 ± 50	1599± 103	1422± 96	incor

Tabl. 2 Morphological characteristics of nuclei in fibroblast cell culture.

Variants	Parameters	Prior state	Flight	Ground	P
Cells on collagen	Area ($10^{-4}mm^2$)	0.32 ± 0.01	0.46 ±0.04 *0.31 ±0.04	0.31 ± 0.02	0.01
	Form factor**	0.85 ±0.01	0.87 ±0.01 *0.82±0.01	0.87± 0.01	incor
	Axial ratio	0.60 ± 0.01	0.70 ±0.02 *0.60±0.02	0.68 ± 0.02	incor
Cells on fibronectine	Area($10^{-4}mm^2$)	0.42 ±0.02	0.44±0.02	0.30 ±0.03	0.01
	Form factor**	0.89 ±0.03	0.87 ±0.01	0.81 ±0.02	incor
	Axial ratio	0.69 ± 0.02	0.60 ±0.02	0.63 ± 0.02	incor

* nuclei of cells in the wound

** form factor - 4 *n* X area / $perimeter^2$



The number of nuclei in monolayer cell culture in the view of microscope (magnification 7*90)

A - the total number of the nuclei

B -the number of the nuclei containing ^{3}H thymidine