SUBCULTURING MONOLAYER CELL CULTURES WITHOUT THE USE OF CENTRIFUGATION

(UDC 576.8.097.3.093.2)

E. P. Ugryumov

Department of Immunobiology (Head-Active Member AMN SSSR Prof. Zhukov-Verezhnikov), Institute of Experimental Biology (Director-Prof. I. N. Maiskii) AMN SSSR
(Presented by Active Member AMN SSSR I. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 59, No. 2, pp. 123-124, February, 1965
Original article submitted August 9, 1963

The method of monolayer cultures of human and animal cells has a number of advantages over the old methods of tissue culturing. Monolayer cell cultures were used in the investigation of space-flight factors [3].

All methods of subculturing monolayer cultures include the centrifugation of the cell suspension after separating the cells from the surface of the glass by solutions of trypsin or versene [1, 7]. In certain experiments this procedure was undesirable because the viability of the cells after centrifugation was lowered, and upon decanting the supernate some of the cells were lost. Furthermore, centrifugation prolongs the subculturing procedure, complicates it, and it is not always possible to remove the solutions of trypsin and versene, especially when they are overcharged with cellular material.

To reduce the subculturing time, simplify the subculturing procedure, eliminate loss of cellular material, and retain the cells in a maximally viable condition, we worked out a procedure for subculturing monolayer cell cultures without the use of centrifugation.

The developed schemes of subculturing cell cultures have been used for a long time when working with various strains of human cells: HeLa [6], CaVe [2], A-1 (obtained in 1959 by T. G. Orlova at the Moscow Research Institute of Viral Preparations), SK [4], No. 580 [8], and others.

The cells were cultivated in 65-mm diameter Carrel dishes. They were grown at 36° in a thermostat for 7 days with one change of the medium on the 3-4th day. The complete nutrient medium consisted of 90% synthetic medium No. 199, 10% bovine serum from young stock, and antibiotics (penicillin + streptomycin). We inoculated 30,000-50,000 living cells per 1 ml of medium. The volume of the medium on the dish was 10 ml. The cells were separated from the glass by trypsin or versene. We used standard solutions of trypsin (0.25%) and versene (0.02%) prepared at the Moscow Research Institute of Viral Preparations. The scheme of work with trypsin differed somewhat from that with versene.

Subculturing with removal of the cells by trypsin was done in the following manner.

1. The old medium was removed by decanting over the edge.

2. The layer of cells was covered with a small quantity of trypsin (3-5 ml).

3. The dishes were left on the experiment table at room temperature for several minutes.

4. The trypsin was poured off before the cell began to separate from the glass.

5. For better separation of the cells from the glass and from one another, the dishes were placed in a thermostat (this stage is not mandatory: the cells can be kept at room temperature). By the end of the stay in the thermostat the cell layer was a loosened film covered with a network of fine cracks.

6. The dishes were extracted from the thermostat, covered with a fresh portion of complete nutrient medium (5 ml), shaken, and the cell suspension ready for subculturing was obtained.

7. The number of cells was counted, the dose determined, and the new dishes inoculated.

Subculturing with removal of the cells by versene was done in the following manner.

1. The old medium was removed by decanting over the edge.

2. The cell layer was covered with versene (5-10 ml).

3. The dishes were placed in the thermostat together with the versene solution and extracted before the cells began to separate from the glass.

4. The versene solution was extremely carefully poured off, the dishes covered with a fresh portion of complete nutrient medium (5 ml), shaken, and the cell suspension ready for subculturing was obtained.

5. The number of cells was counted, the dose determined, and the new dishes inoculated.

As we see from the description, the maximal removal of the trypsin and versene is easily achieved, since during decanting the cells remain attached to the glass.

Subculturing with the use of versene required greater care and more experience. Subculturing with the use of trypsin is more simple and reliable.

The SK cell strain required particularly attentive carrying out of the subculturing procedure. When working with this strain, contact with trypsin and versene was kept to a minimum up to the moment of their removal.

If it is necessary to obtain a unicellular suspension the first portion of the trypsin and versene solutions were poured off and replaced with fresh. In these cases better cell separation was achieved.

Removal of clone strains of cells by the single-cell cultivation method in test tubes [5] requires disaggreggation of the cells of the colony when it consists of a small number of cells. Incomplete separation of the cells of the colonies from the glass made it possible to cultivate the first passages of the clone lines in the starting test tubes.

For this purpose the cell colony was covered with a solution of trypsin or versene, after contact the solutions were decanted, the complete nutrient medium poured into the test tube, sealed with a rubber stopper, and shaken. The cell colony after such treatment easily broke up into individual cells. Then the test tube was placed in a thermostat in a slanted position, and the first passage of the clone line was cultivated. The use of the described method increased the number of favorable results of separating clone lines of cells.

LITERATURE CITED

1. O. G. Andzhaparidze, V. I. Gavrilov, B. F. Semenov, et al.,Tissue Culture in Virological Investigations [in Russian], Moscow., (1962).
2. Ya. V. Dobrynin and R. P. Dirlugyan, Vopr. onkol., No. 5, (1961), p. 47.
3. N. N. Zhukov-Verezhnikov, I. N. Maiskii, V. I. Yazdovskii, et al., In the book: Artificial Earth Satellites [in Russian], Moscow., No. 2, (1961), p. 44.
4. T. G. Orlova, In the book: Viral Infections and Antiviral Preparations [in Russian], Moscow. (1961), p. 330.
5. V. N. Sadovskii and A. D. Timofeevskii, Review of Reports of the Third Summary Scientific Conference of the Institute of Experimental and Clinical Oncology AMN SSSR [in Russian], Moscow., (1959), p. 77.
6. E. P. Ugryumov, Byull. Éksper. biol., 3, (1964), p. 120.
7. G. O. Gey, W. D. Coffman, and M. T. Kubicek, Cancer Res., 12, (1952), p. 264.
8. D. Pol, Cell and Tissue Culture [in Russian], Moscow., (1963).