A METHOD OF OBTAINING CLONE LINES OF CELLS

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This work was undertaken in connection with the program of medico-biological research with Soviet satellite ships [2]. The object of this investigation was to continue the study of clone formation using a microcapillary [6, 8, 9, 10], with the aim of increasing the productivity of the method and the efficiency of seeding single cells. All work on clone formation requires aseptic conditions. An ordinary test tube was used as the vessel for cultivating single cells as being readily available and most convenient for the subsequent manipulations. The nutrient medium consisted of synthetic medium No. 199 + calf serum in proportion of 3 : 1 or 2 : 1, with antibiotics (pencillin + streptomycin, 100 units/ml of each).

Cells for seeding were trapped by a microcapillary. The latter was most conveniently made ex tempore in the course of the work in aseptic conditions, from ordinary sterile Pasteur pipets by heating the junction between the wide and narrow parts and then drawing out a fine microcapillary from the softened part of the pipet. The most useful method of working with the microcapillary was as follows. The newly made microcapillary was connected to a rubber tube, broken off at a distance of about 3 cm, and about 5 drops of nutrient medium was aspirated into it. The microcapillary was introduced into the field of vision of a microscope above a single free-lying cell, immersed in the fluid, and brought close to the cell. By gentle aspiration the cell was brought into the microcapillary. If any doubt arose, the contents of the microcapillary were again expelled into the nutrient medium and confirmation was obtained that the cell was single or that the cell which it was intended to take hand in fact been taken. Next, the cell was transferred to a test tube containing nutrient medium. The cell was seeded by expelling one drop of nutrient medium from the microcapillary over the test tube containing 1 ml of nutrient medium. The microcapillary was then ready again for trapping cells. The test tube with the seeded cell was incubated in an inclined position at 36°.

The Soviet MBI-3 microscope (objective 3.7 × and ocular 10 ×) was used in this investigation. A plexiglas shield was fixed over the objective by means of a rubber stopper to prevent airborne infection. An ordinary glass slide was fixed in the slide holder of the microscope and on it was placed a drop of the cell suspension containing a few cells. By moving the stage the required field of vision could be found quickly and easily, an important matter during large-scale seeding.

The working cell suspension was prepared by diluting the initial suspension obtained after removing the cells from the glass with trypsin or versene. Three- or four-day cell cultures were taken. Cells of malignant strains – carcinoma of uterus HeLa [7], carcinoma of stomach Cave [1] – and of normal strains – human amnion A-1 (obtained in 1959 by T. G. Orlova at the Moscow Research Institute of Viral Preparations) and human fibroblasts No. 630 [3] – were used in the experiments.

Preliminary experiments revealed that the method was perfectly reliable: 50 drops, made on a glass slide (for control purposes), each contained one cell. Consequently, the suggested technique enables single cells to be seeded. The optimal time for trapping and seeding a single cell is about 10 sec, the time for seeding 15 cells from one sample of working suspension 3-4 min.

During cultivation of single cells in test tubes it was possible to obtain a fairly high percentage of development of cells in colonies; the yield of colonies was 63% for cells of strain A-1, 50% for HeLa cells, 37% for cells

of strain No. 630, and 10% for cells of the Cave strain. The cells of the different strains gave different yields of colonies. Colonies of cells from the same strain differed from one another in size, shape, and cell composition. Colonies from different strains on the whole retained the differences existing between the strains [4, 5].

To isolate clone lines from the growing colonies, the nutrient medium was removed and the colonies treated with versene, and then centrifuged in the same tubes. The versene was then removed, the tubes filled with fresh nutrient medium, and again incubated at 36° in an inclined position. In this way several clone lines of cells could be obtained simultaneously.

The use of a microcapillary tube filled with nutrient medium considerably shortened the time taken to trap and seed the single cells. The whole procedure of clone formation now requires a minimal time, and this, in turn, means less trauma to the cells and more efficient seeding. The number of cells which can be seeded is increased immeasurably. The suggested practically solves, from the technical point of view, the problem of clone formation in accordance with the principle: one cell, one clone. It remains to study the nutritive requirements of single cells, after which is may be expected that the efficiency of seeding will rise to values approaching 100%. The use of test tubes for cultivating clones has simplified the technique of obtaining clone lines of cells.

SUMMARY

A method of using microcapillaries filled with a small amount of nutrient medium is suggested for trapping and planting single cells in test-tubes. Trapping is done from a drop of cellular suspension on a slide, which is fixed under a microscope. Since the time of cloning is minimized cloning, cellular injury decreases, and the planting efficiency rises. The yield of colonies for A-1 strain was 63%, HeLa strain – 50%, No. 630 strain – 37% and Cave strain – 10%. The number of planted single cells is unlimited. The use of test tubes simplified obtaining of clonal cell lines.

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