APPLIED MICROBIOLOGY, Jan., 1967, p. 201-202

Vol. 15, No. 1
Printed in U.S.A.

Large-Scale Cultivation of Mammalian Cells in Vitro[1]

A. HELLMAN,[2] J. D. REGAN, AND D. H. MARTIN
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Received for publication 27 July 1966

During the past decade various methods have been employed to propagate mammalian cells in suspension cultures. These techniques range from the use of Erlenmeyer flasks on a rotary shaker (W. R. Earle et al., Ann. N.Y. Acad. Sci. **58**:1000, 1954) to the use of stainless steel fermentors (D. W. Zeigler et al., Appl. Microbiol. **6**:305, 1958).

Certain biochemical studies require several hundred grams of cells, and it is advantageous to have these available with minimal effort. However, the quantity of cells that one can propagate with the former method is limited by the size of Erlenmeyer flasks available and the size of rotary shakers in commercial use; on the other hand,

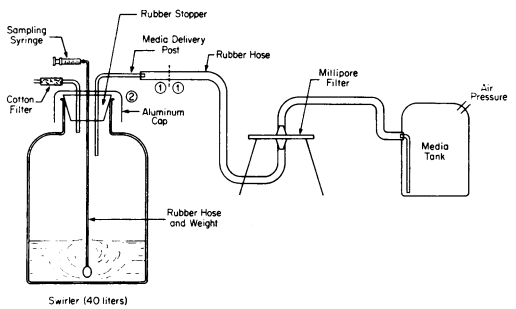

FIG. 1. *Swirler and filter equipment.*

fermentors can at times be difficult to operate and do involve appreciable cost.

In this paper we present a method for relatively large-scale propagation of HeLa cells by use of a 40-liter carboy ("swirler"). The swirler is aseptically filled with 10 liters of growth medium [A. Hellman et al., Acta Virol. (Prague) **9**:224, 1965] and agitated on a rotary shaker at 100 rev/min. By this method we obtain yields of approximately 50 g of cells (wet weight) after 5 days of growth, from an initial inoculum of 1.5×10^5 cells per milliliter.

The setup for the swirler and associated 144-mm membrane filter (Millipore Filter Corp, Bedford, Mass.) is depicted in Fig. 1.

[1] Research jointly sponsored by the National Institutes of Health and the U.S. Atomic Energy Commission under contract with the Union Carbide Corp.

[2] Assigned from Office of Director, Etiology Group, National Cancer Institute.

The swirler is autoclaved as a unit, being detached from the filter at point 1. This separate sterilization is necessary to prevent charring of the filter by residual heat in the swirler. After cooling, these components are aseptically connected.

The procedure for preparation of a swirler culture is as follows. (i) Mix appropriate nutrients in a large flask and refrigerate at 4 C for several hours. (ii) Filter this preparation through a 144-mm Millipore filter (not sterile), with prefilter, RA (120 μ), and HA (0.45 μ) being used in sequence if serum is present in the medium. (iii) This material is then filtered through a sterile Millipore filter (G. S., 0.22 μ) into a sterile swirler containing an appropriate quantity of sterile methylcellulose.

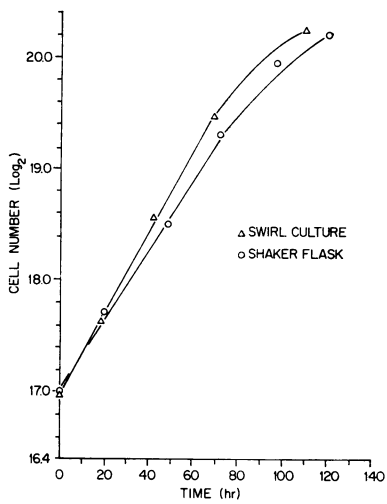

FIG. 2. *Population doubling time of HeLa cells.*

After filtration of the medium, the connection between the filter and swirler is clamped off at point 1 and cut. The swirler is then incubated for 3 days to determine the sterility of its content. It is then inoculated with approximately 1.5×10^5 cells per milliliter, by lifting the cover over the opening of the swirler (point 2).

The swirler is then placed on a rotary shaker (model 6140; Eberbach Co., Ann Arbor, Mich.).

The culture is agitated for 5 days with a rotary motion, describing a 2.5-cm diameter circle in the horizontal plane. The platform of the shaker is equipped with a rubber mat on which the carboy rests. To monitor the culture, samples are taken by the attached syringe at 24-hr intervals. The cells are counted by an electronic cell counter, and viability is determined by the erythrocin B dye-exclusion technique (H. J. Phillips et al., Exptl. Cell Res. 13:341, 1957). There is essentially no difference in the rate of cell doubling between swirler culture and Erlenmeyer suspension shaker culture (Fig. 2). Harvesting cells at 5 days gives the optimal yield, prior to entering the plateau phase.

Cell viability remains between 90 and 97% during the 5-day interval. One does not have rimming or clumping of cells, as is often the case with Erlenmeyer-flask cultures. The cell yield after 5 days of doubling ranges between 40 and 52 g of cells (wet weight) per 10 liters of medium. This amounts to an approximate 10-fold increase in cell weight during this interval. The cells are harvested on a Sharples centrifuge (laboratory model), which is operated at 7,500 $\times$ g with a flow rate of 450 ml/min.

We believe the procedure to be an efficient and reasonably inexpensive method of propagating rather large quantities of mammalian cells in vitro with a minimum of effort.

The help of E. F. Phares is gratefully acknowledged.