Percell
BIOLYTICA

Growth of Vero and GMK Cells

Objective

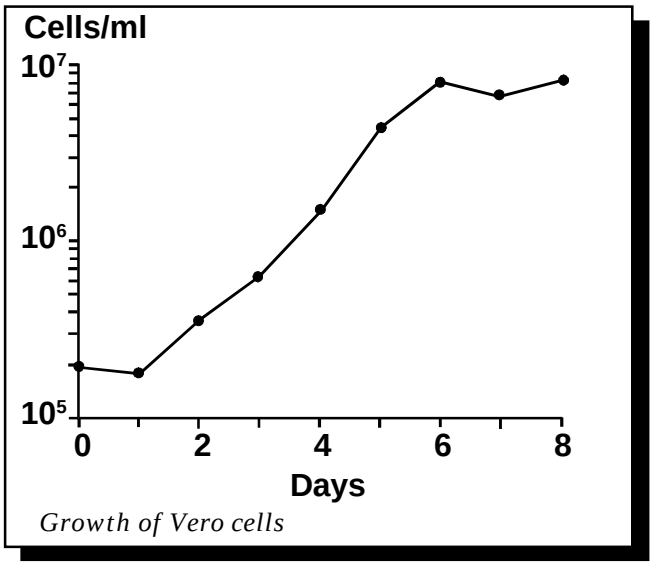
Vero and GMK cells are cell lines widely used for vaccine production. Both are fibroblasts and strictly anchorage dependent. They have also strong intercellular binding properties, which makes routine harvest with Dispase impossible. This application note describe a modified procedure for cell enumeration and shows that high cell densities can be obtained for both cell lines with CultiSpher-G.

Culture Conditions

Vessels: 50 ml spinners(Techne).
Microcarrier: CultiSpher-G prepared according to instructions used at either 2 g/l(Vero) or 1 g/l(GMK).
Cell line: Vero(African Green Monkey Kidney fibroblast, PHLS) and GMK(Green Monkey Kidney, Institute of Virology, University of Gothenburg, Sweden).
Inoculum: Cultures were innoculated at 200,000 cells/ml.
Cell counting: Duplicate samples of 0.5 ml were taken from the spinner. After sedimentation of the beads, 0.3 ml supernatant was withdrawn and 0.8 ml Dispase(5 mg/ml in PBS) added. Beads were completely dissolved after 30 minutes at 37°C. Cells were collected by centrifugation and 1.0 ml of citric acid(0.1 M) containing Triton X-100(1%, w/v) and crystal violet(0.01%, w/v) added. Stained nuclei were counted in a hematocytometer.
Agitation speed: 45 RPM.
Media: DME supplemented with 10% FBS, penicillin G(100 U/ml) and streptomycin(100 µg/ml). pH was controlled through CO_2 atmosphere. Media volume was varied according to the following scheme; day 0-3: 50 ml and day 4-10: 60 ml.

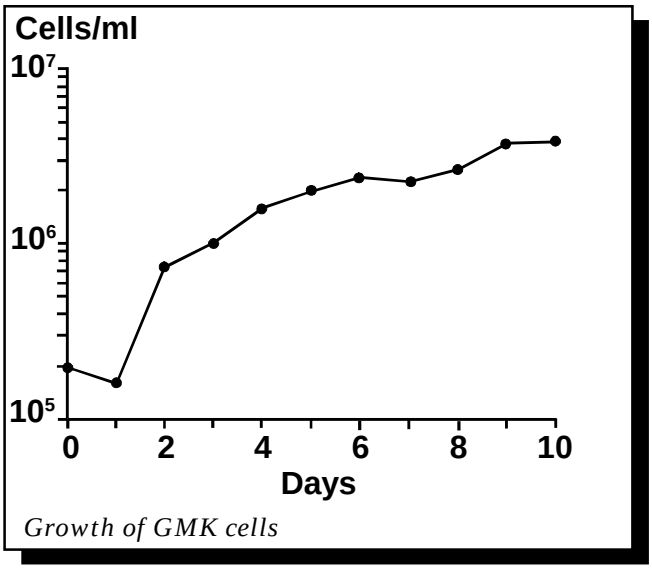
Results

Vero cells grew to a density of $8.2 \cdot 10^6$ cells/ml, which corresponds to a yield of $50 \cdot 10^8$ cells/g dry weight CultiSpher-G. Cell doubling time in exponential phase was 18 hours.



Growth of Vero cells

GMK cells grew to a density of $3.9 \cdot 10^6$ cells/ml, which corresponds to a yield of $47 \cdot 10^8$ cells/g dry CultiSpher-G. In exponential growth cell doubling time was 37 hours.



Growth of GMK cells

Discussion

Despite that Vero cells was cultured with a higher concentration(2 g/l versus 1 g/l) of CultiSpher-G than GMK cells, almost the same yield of cells were obtained. This indicates that the surface of CultiSpher-G is the limiting factor and if GMK cells have been cultured at 2 g/l of CultiSpher twice the cell concentration should have been obtained. As both this cell lines are fibroblastic they have considerably higher requirements for available surface area. When compared to CHO-K1 cells, the yield of cells is almost 3 times lower, $50 \cdot 10^8$ for VERO and GMK versus $136 \cdot 10^8$ for CHO-K1(Application Note 108).
It is not possible to use Dispase for harvesting and/or counting of cells, as most cells will be recovered as aggregates. The most convenient method for cell enumeration is nuclei counting after digestion of the matrix by Dispase. It is also possible to use trypsin for cell enumeration as this enzyme at the same time as it dissolves the matrix also dislodge the cells from each other. Harvesting can be done with high yield with a modified method using trypsin(Application Note 101).

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