Percell
BIOLYTICA

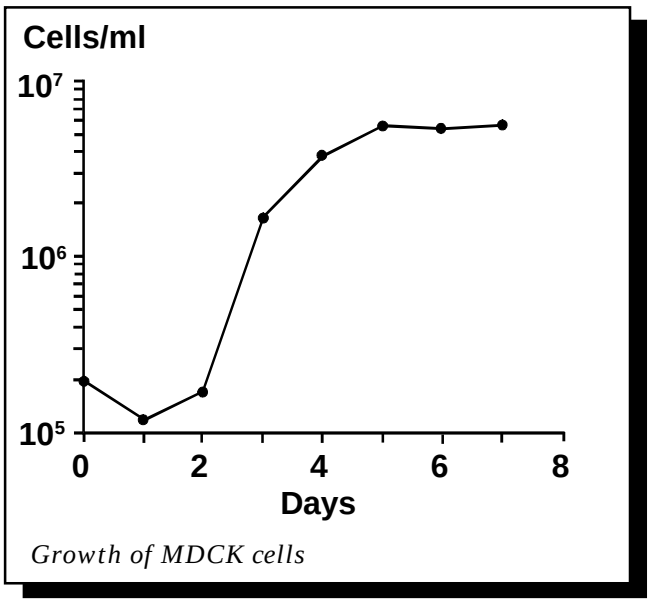
Growth of MDCK Cells

Objective

Due to their adhesive properties, both intercellularly as well as to surfaces, MDCK cells possess several challenges when they are to be used in large scale cell culture. This application note describes several alternatives to reduce the difficulties usually encountered in the culture of these cells.

Culture Conditions

Vessels: 50 ml spinners(Techne).
Microcarrier: 2 g/l CultiSpher-G prepared according to instructions.
Cell line: MDCK(Canine kidney epithelial, PHLS).
Inoculum: Culture was inoculated 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 After sedimentation of the beads, 0.3 ml supernatant was withdrawn and 0.8 ml Dispase(5 mg/ml in PBS) added.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) was 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 4-7: 60 ml.



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Results

MDCK cells showed a lag phase of 2 days during which cell concentration decreased to 120,000 cells/ml. After this lag phase, cells entered the exponential growth phase with a mean doubling time of 11 hours. The stationary phase was reached after 5 days of growth. Maximum cell concentration was $5.7 \cdot 10^6$ cells/ml which corresponds to a cell yield of $34 \cdot 10^8$ cells/g dry weight of CultiSpher-G. The obtained growth span was 34 times.

Discussion

MDCK cells adhere very strongly to each other. It is not possible to enumerate the cells by standard Dispase-techniques. The gelatin matrix will be dissolved by Dispase but only a few of the cells will appear as single cells. More than 90 % of the cells will be collected as aggregates. We therefore recommend the use of nuclei counting.
MDCK cells adhere very strongly to tissue culture plastics. Cells are usually subcultured by prewashing with PBS-EDTA and strong trypsin solutions. This treatment reduces cell yields significantly. A more convenient way to subculture these cells is to add a small amount of CultiSpher directly into the tissue culture flask. The cells will colonize the beads which can be easily transfered to another tissue culture flask. If the cells are going to be used for innoculation of spinners or fermenters we recommend the use of our modified trypsin method(see also Application Note 101).

Percell Biolytica AB
Ji-Te gatan 9, S-265 38 Åstorp, Sweden
Phone: 46-42-502 82, Fax: 46-42-501 91