Zentrum Angewandte Chemie
Institut für Technische Chemie

Partner in der Forschung

Institut für Technische Chemie, Callinstr. 3, 30167 Hannover

Universität Hannover

Online analysis of microcarrier cultivations

Arne Burzlaff, Thomas Brückerhoff, Cornelia Kasper, Jan-Gerd Frerichs, Thomas Scheper

Institut für Technische Chemie der Universität Hannover,
Callinstr. 3, 30167 Hannover

1 Introduction

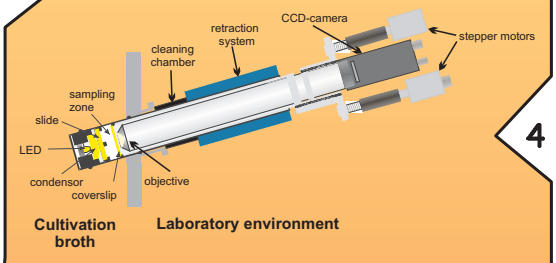
Microcarriers are being used for high-density cell cultures in several bioprocesses, which can be divided in three categories, a) high-yield production of cells or cell products, b) studies of cells in vitro, c) routine cell culture techniques. The cell densities achieved by this process can be 20 fold per culture volume compared with a monolayer bottle, resulting in a significantly higher yield.
Online analysis during a mammalian cell cultivation is still one of the major problems in bioprocess regulation.

2 Aim of this work

For the regulation of microcarrier cultivations, important process parameters are the cell density and the plating efficiency. In most cases the determination of cell density is time consuming and dangerous due to infection. This work should evaluate the applicability of in-situ microscopy and flow cytometry to gain process parameters fast and reliable. The first step was to evaluate different micro-carriers towards their suitability. For flow cytometry measurements micro-carriers with a size smaller than 200 µm had to be chosen.

3 Instrumentation

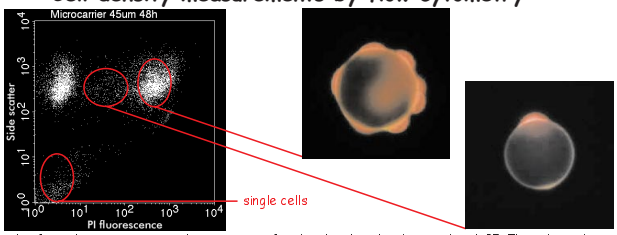
Cell line: NIH-3T3 mouse fibroblast
Microcarrier: Cytodex 1 and 3 (130-215 µm, Amersham), Glass and Plastic (90-150 µm, 150-210 µm, Cellon SA), Polystyrene Beads (45 and 73 µm, Polyscience)
Cultivation: RPMI, 5 % NCS, 37 °C, 5 % CO_2
Cytometer: BD FACS Vantage SE with Macro Sort option, 200 and 400 µm nozzle
Fluorescent dye: Propidiumiodide (PI; Molecular Probes)
Microscope: Olympus BX41 with reflected fluorescence option



4 Experimental

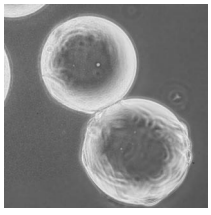
We tested several microcarriers towards their suitability for online analysis using in-situ microscopy and flow cytometry. Small scale cultivations were performed in 500 ml spinner flasks. A large scale cultivation was performed in a 5 l steel reactor. Samples were analysed directly by in-situ microscopy and after DNA staining with PI by flow cytometry. As the large microcarriers could not be analysed by flow cytometry due to size limitations, smaller model carriers were used.

5 Cell density measurements by flow cytometry

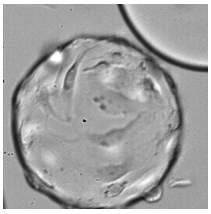


Samples from the microcarrier cultivation were fixed with ethanol and stained with PI. The sidewards scattered light was plotted against fluorescence intensity, showing 4 populations corresponding to the cell density on each microcarrier. The pictures show PI stained cells with a high and low cell density on the microcarriers.

6 Microscopic and in situ microscopic pictures of microcarriers in animal cell cultivation



Phase contrast picture of Cytodex 3 65 h after inoculation with 3T3 cells mag 200X



in situ picture of Cytodex 3 65 h after inoculation with 3T3 cells mag 400X

Summary

The experiments have shown that in-situ microscopy is capable of analysing a wide range of microcarrier cultivations, whereas flow cytometry is limited to small size microcarriers. Both systems have proved to be suitable methods for gaining informations about cell density and plating efficiency fast and reliable.

Outlook

In further work FITC labeled microcarriers will be used so that the plating efficiency can be monitored, by plotting the FITC fluorescence against PI fluorescence of the cells.
Furthermore adjustments of the flow cytometer tubing and nozzle will enable the analysis of bigger microcarriers.