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BIOLYTICA

Growth of r-CHO Cells in Serum-free Medium

Objective

Will the three dimensional matrix of CultiSpher have any influence on the cell growth, cell yield, cell detachment etc. in a 1 liter fermentor system using a serum free medium, when compared to a solid microcarrier consisting of the same material (gelatin coated)?

Culture conditions

Vessel: 1 liter fermenter (SGI)
Microcarrier: CultiSpher-G 4 g/L and solid gelatin coated microcarriers 3 g/l, both prepared according to instructions.
Medium: Day 1-4, MEM α2000 supplemented with 10% dialysed FBS and 30 nM methotrexate, day 4-80, Iscove/F12 (50/50) supplemented with 6 mg/L of bovine insulin. All media contained 100 units/ml of Penicillin G and 100 µg/ml of Streptomycin. pH was maintained at 7.2±0.20.
Cell line: CHO cells secreting von Willebrand factor (Transgene)
Agitation speed: 30-40 RPM.
Oxygenation: Bubble-free through 2 m silicone tubing/l with pure oxygen.

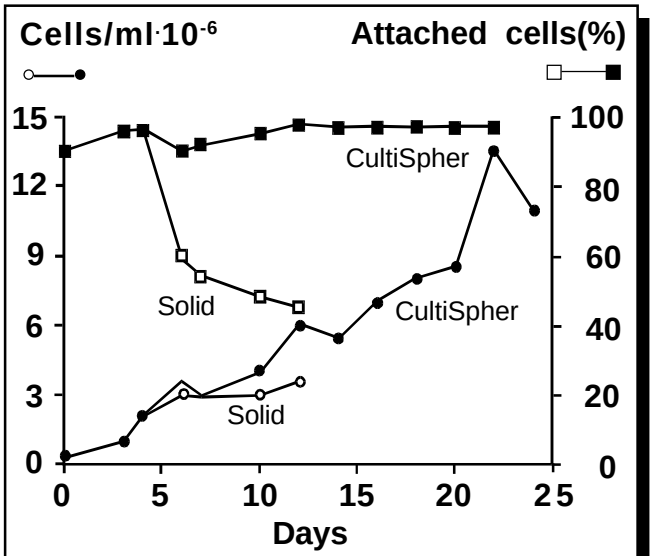

Figure 1. Growth and attachment of cells to CultiSpher and solid microcarriers

Results

After 2-4 hours, more than 90 % of the cells were attached to both type of carriers. Recombinant CHO cells grew exponentially after a 24 hour lag phase, reaching $1.85 \cdot 10^6$ cells/ml and $1.70 \cdot 10^6$ cells/ml on day 4 on CultiSpher and solid microcarriers respectively. The cell doubling time was approximately 24 hours. After a 4 day long growth phase, media was switched to a serum-free formula. Cell-densities reached a tresh-hold at $3.0 \cdot 10^6$ cells/ml on solid microcarriers while cell growth continued on CultiSpher-G to attain $13.5 \cdot 10^6$ cells/ml on day 22. Less than 5% detached from CultiSpher-G beads, even in a serum and attachment-factor-free medium. On solid microcarriers more than 40% of the cells growing in multilayers fell off the beads and were lost during medium change as early as day 6. For this reason that experiment had to be stopped at day 12.

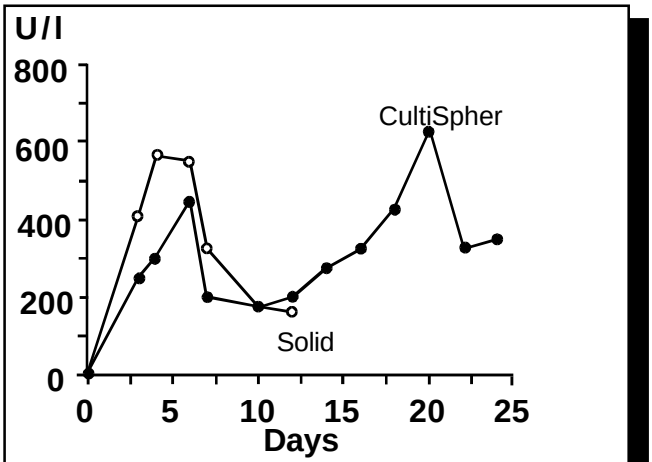

Figure 2. von Willebrand factor production

Discussion

According to these experiments, the available surface area of CultiSpher-G is at least 3-fold that of solid microcarriers $(13.5 \cdot 10^6/4g)/(3.0 \cdot 10^6/3g) = 3.4$.

The stress imposed on cells in a serum-free environment makes them more sensitive to shear forces. The sponge-like bead structure of CultiSpher-G protects them from these forces.

When cultivated under these conditions on a conventional solid microcarrier type, the cells cannot be maintained more than 10 days. Detachment can be as high as 60%.

Reference

Mignot, G. et al. (1990) "Production of recombinant von Willebrand factor by CHO cells cultured in macroporous microcarriers". Cytotechnology 4, 163-171

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