Percell
BIOLYTICA

Vesicular Stomatitis Virus(VSV) Production by Vero Cells

Objective

A comparable study between macroporous and solid microcarriers was set up to investigate if viral particles could infect cells growing inside the CultiSpher-G's macroporous matrix. Would the penetration process of viral particles in to the matrix slow kinetics of virus production? The majority of the cells were entrapped inside the CultiSpher-G beads.

Culture conditions

Vessel: A 300 ml (working volume) spinner(Bellco).
Microcarriers: CultiSpher-G 0.5 g/L, solid microcarriers 1.0 g/L. Both prepared according to instructions.
Cells: Vero (African Green Monkey Kidney, Praxis Biologicals).
Agitation speed: 30 rpm
Media: DME supplemented with 5% FBS, streptomycin sulfate (100 µg/ml) and penicillin G(100 U/ml).

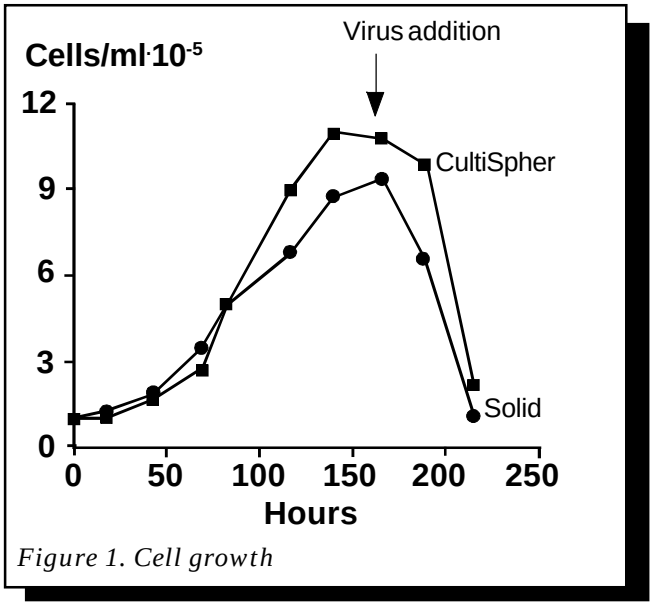

Figure 1. Cell growth

Virus infection: After Vero cells had reached stationary phase, carriers were allowed to settle and 150 ml of media was removed to reduce the volume in the reactor. Multiplicity of infection (MOI) was 0.01 (Viral plaque forming units to the final cell number). After addition of VSV stock, 166 hours after inoculation, the pH was reduced to 6.8 by CO_2 injection. A one hour attachment period was allowed, during which pH was maintained at 6.5-6.8 and intermittent agitation was used(1 min/15 min). Following the adsorption period, 150 ml of media was added, pH was re-adjusted to 7.2 and continuous agitation was resumed.

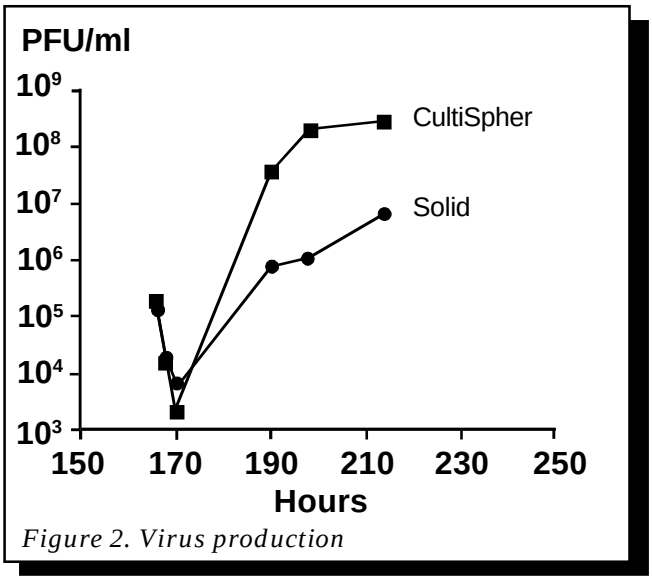

Figure 2. Virus production

Discussion

CultiSpher-G's larger available surface area permitted the use of a lower bead concentration (two-fold), figure 1. The viral particles quickly attached to the cells with over 99% of the infectious particles leaving the supernatant within 5 hours. The maximum VSV titer was observed 48 hours after infection at $3.25 \cdot 10^8$ PFU/ml. Expressed on a per cell basis the yield corresponds to 315 PFU/cell. The value for solid beads was 85 PFU/cell. The kinetics of VSV production is unchanged using macroporous microcarriers, figure 2.
Virtually all cells are utilized by the virus for replication(based on yield figures). The VSV yield on CultiSpher-G approached that seen in roller bottles(340 PFU/cell) using chick embryo fibroblasts.

Reference

Nikolai, T. J. & Hu, W-S. (1990) "Personal Communications". University of Minnesota.

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