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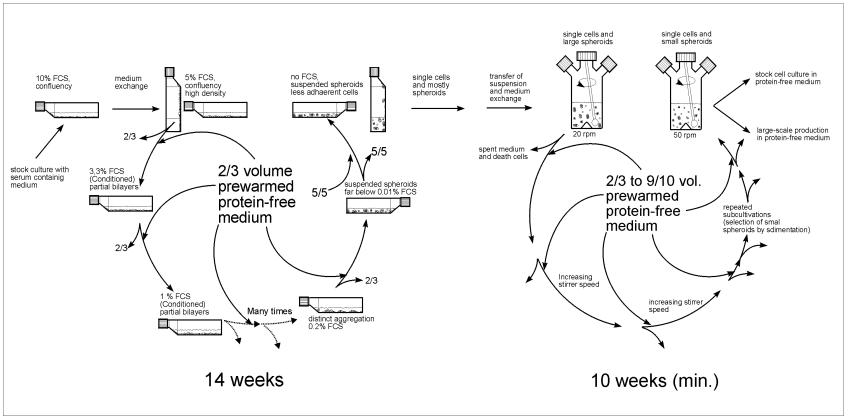
Cost-optimized Virus Production with MDCK Cells Adapted to Suspension Using Protein- and Peptide-free Chemically Defined SMIF-Medium

Introduction

Actually many vaccine production processes still use adherent cell lines with cost intensive microcarrier technology and often poorly defined protein-and peptide rich sometimes serum-containing media. Different mammalian cell lines as host systems are under evaluation for optimizing process conditions. Commercially available adherent cell lines like MDCK and Vero cells as well as new designer cell lines like PER.C6, AGE1.CR or EB66®, typically suspension cells, are considered.
In our way of process optimization we adapted cells of a very early passage of MDCK to suspension culture in protein- and peptide-free chemically defined SMIF-Medium using already described biphasic strategy[1]. Here we show the first positive results of of virus production with this optimized host systems.

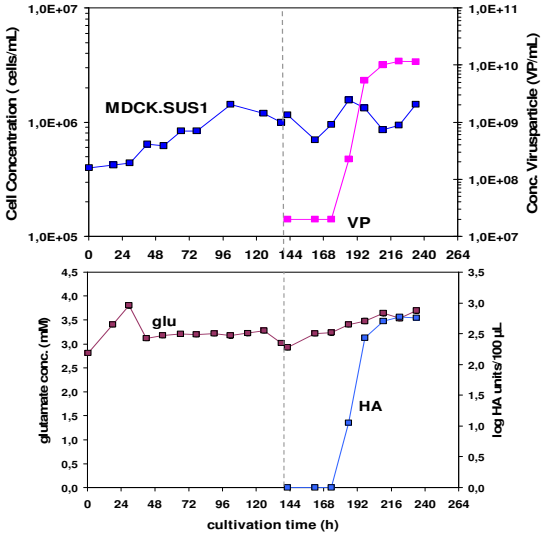
Methods

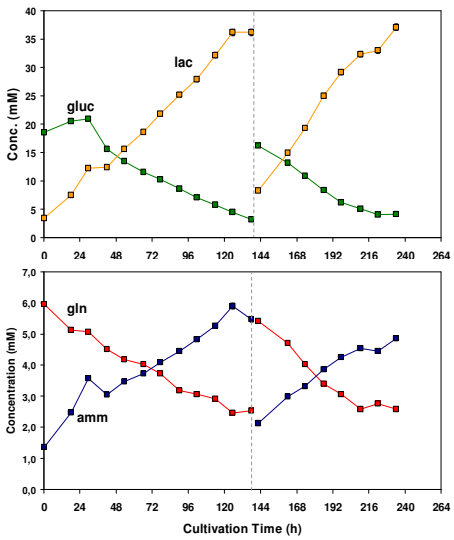
Cells: MDCK (ECACC nr. 841211903) were adapted to growth in suspension as MDCK.SUS1 & MDCK.SUS2 (see below).
Medium: SMIF08 (Gibco by contact through K. Scharfenberg)
Cell adaptation procedure: Adherent MDCK cells were adapted in a biphasic strategy to SMIF and further to growth in suspension. Therefore cells were grown to hyperconfluency in T175 (Greiner) in SMIF08 with 10% FCS (Gibco) by additional medium exchange and without further trypsinization. Further reduction of serum and detachment of aggregated cells to suspension culture resembles already described strategy[2] Adaptation to suspension culture (MDCK.SUS1) and selection to shorter doubling time and tolerance of higher RPM (MDCK.SUS2) were done in spinner flask (techne) by slowly raising stirrer frequency of spinner tablet (H+P) over weeks.
Virusproduction: Spinner with 150 mL culture in SMIF08 for cell growth, before infectionculture medium exchange (centrifugation stepp, cells resuspended in 20 % contitioned medium plus 80 % fresh medium) followed by infection with A/PR/8 RKI moi: 0.01 (Trypsin: 4 x 10-6 U/cell)



Results

MDCK.SUS1 shows already good growth in suspension in protein-free medium.
In comparison to former description for e.g. HeLa or CHO[1] the adaptation to a similar status requires clearly more time (around 20 vs. 8 weeks similar to VERO) including more media exchanges with higher portion of conditioned medium.
However doubling times were high (passage average above 50h for MDCK.SUS1). Therefore, these cells were further selected and doubling times could be reduced for MDCK.SUS2 (passage average around 36h; apparent in log 31 h). Both show good virus multiplication (MDCK.SUS1 & MDCK.SUS2 not shown)





Conclusion

The described strategy provides an adapted cell line useful for virus multiplication in suspension without expensive microcarrier handling.
Further adaptation and selection gives an improved host cell culture with potential for virus production processes (fast cell expansion + comparable virus multiplication).
The partial medium exchange facilitates the controlled reduction of the protein and serum content as well as the exploitation of the self conditioning effect.

HeLa	Human epitheloid cell line from cervix carcinoma	SMIF06
MDCK (ECACC) *1)	Madin Darby Canine Kidney cell line	SMIF08
Vero (WHO) *1)	African Green Monkey Kidney Passage-no. 140 (from WHO MCB)	SMIF08PG

Further cell lines useful for virus production and already adapted to SMIF medium

[1] Scharfenberg, K, Wagner, R., **1995**, Animal Cell Technology Developments towards the 21st Century, 619-623