Evaluation of a new suspension MDCK cell line for influenza vaccine production

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Introduction

Current world wide research on influenza vaccine production focuses on the characterization and evaluation of different mammalian cell lines as host systems. 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.

Advantages of suspension cells are an easier scale-up and the reduction of costs due to microcarriers. Possible disadvantageous include difficulties of cell retention.

Here, we present the successful adaptation of an adherent MDCK cell line[1,2] (ECACC #841211903) to growth in suspension in a chemically defined protein-free medium, giving us the opportunity for a thorough comparison of "suspension versus adherent cells".

Methods

Cultivation of MDCK cells in a 1 L-stirred tank bioreactor or 1 L-Wave bioreactor. Medium with different additions (see table 1), direct infections with human influenza at different time of infection (toi) according to cell numbers reached (at least 2 x 10^6 cells/mL).

Tab. 1: Overview on cultivation conditions used

	▲	●	●	△	■
cells	MDCK.SUS1	MDCK.SUS2	MDCK.SUS2	MDCK.SUS1	$MDCK_{adh}$
bioreactor	STR	STR	STR	wave	STR
rpm	75	75	75	-	50
pH	7.2	7.2	7.2	7.3	7.2
medium	SMIF8	AEM* + gluc+ gln	SMIF8	SMIF8	Episerf + gluc + gln + pyr + 2 g/L MC
start cell nr	3.5 x 10^5	4.0 x 10^5	4.0 x 10^5	4.0 x 10^5	2.5 x 10^5
vol	1 L	1 L	1 L	1 L	1 L
aeration	40%	40%	40%	14-10%	40%
preculture**	P35	P7	P10	P21	P8
virus	A/PR/8/34	A/PR/8/34	B/Malaysia	A/PR/8/34	A/PR/8/34
moi	0.025	0.025	0.025 + gluc + gentamycin	0.025	0.025 + gluc
trypsin (U/cell)	1 x 10^{-5}	1 x 10^{-5}	1 x 10^{-5}	5 x 10^{-6}	5 x 10^{-6}
toi	145 h	118 h	97 h	94 h	96 h

* STR cultivation in AEM, preculture in SMIF8!
** passage number of preculture

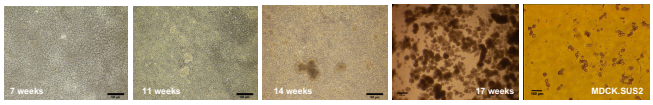
Cells: $MDCK_{adh}$ (ECACC #841211903), MDCK.SUS1 & MDCK.SUS2: $MDCK_{adh}$ adapted to growth in suspension (see below).
Virus: $MDCK_{adh}$ seed from human influenza A/PR/8/34 (H1N1, RKI) and influenza B/Malaysia/2506/2004 (NIBSC).
Medium: Episerf (serum-free) (Gibco), SMIF8 (peptide-free, protein-free, chemically defined) (Gibco by contact through K. Scharfenberg), AEM (serum-free) (Gibco).
Equipment: STR (Sartorius), Wave (Wave Biotech AG), ViCell (Beckman Coulter), Bioprofile 100 Plus (Nova Biomedical), anion exchange chromatography (Dionex).

Results

Generation of MDCK.SUS1 & MDCK.SUS2

$MDCK_{adh}$ cells were adapted in a biphasic strategy to SMIF8 medium and further to growth in suspension[3].

- growth to hyperconfluency in T175 (Greiner) in SMIF8 with 10% FCS (Gibco): medium exchange; no trypsinization
- reduction of serum and detachment of aggregated cells to suspension culture
- adaptation to suspension culture (MDCK.SUS1)
- selection to shorter doubling time and higher RPM (MDCK.SUS2) in spinner flask (Techne) by slowly raising stirrer frequency



- ❑ good growth in suspension for MDCK.SUS1
- ❑ adaptation successful, although very long (20 weeks to MDCK.SUS1) with more media exchanges & conditioned medium
- ❑ reduction of doubling times for MDCK.SUS2 (around 31 h)

Cultivations

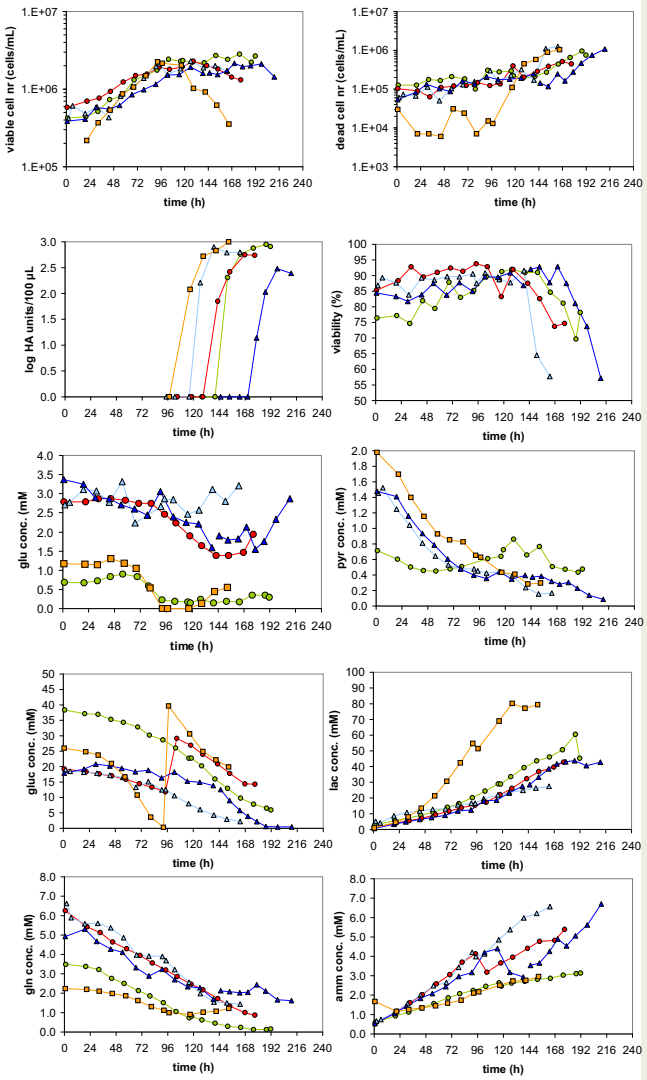


Fig. 1: Profiles of cell numbers, metabolites & virus titer during cultivation of MDCK cells and during influenza virus production (cultivation conditions see table 1).

- ❑ MDCK.SUS2 (2.4 x 10^6 cells/mL in 104 h, $t_{1/2}$: 34 h) grew faster than MDCK.SUS1, viabilities of 85-90%
- ❑ SMIF8 & AEM show good cell growth in suspension, but different metabolite profiles - SMIF8 is chemically defined, therefore preferred !
- ❑ similar virus titers as in $MDCK_{adh}$ cells
- ❑ comparable data in stirred tank & wave bioreactor,
- ❑ surprisingly high glucose/lactate metabolism in $MDCK_{adh}$ compared to typical data in 5 L STR[1,2]

Conclusions

- ➢ MDCK.SUS2 show a good potential for influenza production
- ➢ further comparison with respect to glycosylation, proteomics, cell physiology and downstream processing

References

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

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