Vaccine 27 (2009) 6464–6467

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Suspension-Vero cell cultures as a platform for viral vaccine production

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ARTICLE INFO

Article history:
Available online 24 June 2009

Keywords:
Vero cells
Vaccines
Suspension culture

ABSTRACT

Since Vero cells are currently considered as an acceptable cell substrate to produce a wide range of viruses, we developed a virus production platform using Vero cells adapted to grow in suspension in serum-free media. After adapting anchorage-dependent Vero cells to grow as a free-cell suspension, vesicular stomatitis virus, herpes simplex virus 1 and polio virus 1 production rates were evaluated in batch cultures using spinner flasks and perfused cultures in a bioreactor. The achieved results constitute valuable information for the development of a low-cost high-productivity process using a suspension culture of Vero cells to produce viral vaccines.

1. Introduction

For virus production using cell cultures, as well as for other cell-based processes such as recombinant protein production, research is forwarded in the development of suitable methodologies to sustain high yields. The adaptation of anchorage-dependent cells to grow in suspension cultures is a valuable tool for industrial applications due to scale-up limitations associated with monolayer cultures. Suspension cultures can be easily scaled up to the production level in bioreactors, which are designed to reduce hydrodynamic forces and heterogeneity [1].

Furthermore, it is known that the addition of serum to the cultures presents some disadvantages, such as the risk of inducing hypersensitivity, variability of serum batches and the possible presence of contaminants (mycoplasma, bovine viruses, etc.) [2]. Therefore, the ever-increasing need for improved biological safety is pushing industries forward in the development of serum- and protein-free media formulations.

Based on these concepts, the production of the subunit influenza vaccine (OPTAFLU®) in MDCK 33016 cells which were originated from the MDCK epithelial cell line after adaptation to grow freely suspended in serum-free, chemically defined media, can be cited as an example [3].

The continuous Vero (African green monkey kidney) cell line is recommended by the World Health Organization for vaccine production for human use [4] and has been used, after propagation on microcarriers, for the production of rabies, polio, enterovirus 71 and hantaan virus vaccines [2,5–7].

This work describes the adaptation of Vero cells to suspension growth (sVero) in serum-free media. We report the kinetic growth of adapted cells in batch and perfused cultures and the production of three different viruses (vesicular stomatitis virus, herpes simplex virus 1 and polio virus 1). These results were compared with those obtained when working with anchorage-dependent Vero cells. The insights gained in developing this viral production platform might benefit future actions to produce vaccines using this cell line.

2. Materials and methods

2.1. Cell lines

Adherent Vero E6 cells (ATCC CRL-15869) were grown in T-flasks at 37 °C, 5% CO_2 in MEM (Gibco) supplemented with 10% foetal calf serum (FCS, Bioser, Argentina). Vero E6 cells were simultaneously adapted to suspension growth and serum-free media using a multi-step method, progressively increasing the percentage of serum-free home-made media. Resulting sVero cells were grown in serum-free home-made media (SFM) in spinner flasks agitated at 50 rpm at 37 °C and 5% CO_2.

2.2. Viruses

Vesicular stomatitis virus (VSV, Indiana strain) was obtained from Instituto Nacional de Enfermedades Virales Humanas "Dr. Julio I. Maiztegui" (INEVH, Pergamino, Argentina), herpes simplex virus 1 (HSV1) was provided by Laboratorio de Virología, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (UBA, Buenos Aires, Argentina) and polio

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doi:10.1016/j.vaccine.2009.06.020

virus 1 (PV1) was obtained from Cátedra de Virología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires (UBA, Buenos Aires, Argentina).

2.3. Bioreactor cultures

Cultures were performed in stirred tank bioreactor (Bioengineering, Switzerland) with a 4.5 l working volume equipped with a spin filter used for perfusion. During the cell proliferation phase, pH was set at 7.2, pO_2 at 50% air-saturation, temperature at 37 °C and agitation rate at 100 rpm. Media exchange was started after the cell density reached 1.0×10^6 cells/ml and perfusion rate was regulated according to the growth profile. For the virus production phase the temperature was decreased to 34 °C. Samples were collected daily in order to determine cell density, cell viability, virus titer, ammonia, lactate and glucose levels.

2.4. Cell counting and metabolite analysis

Cells were stained with trypan blue and counted in a hemacytometer. Glucose and lactate concentrations were measured using an YSI model 2700 analyzer (Yellow Springs Instruments). Ammonium concentration was determined using a kit based on the Berthelot reaction (Sociedad de Bioquímicos, Argentina).

2.5. Virus production

VSV, PV1 and HSV1 were employed for infection of anchorage-dependent Vero cells growing in T-flasks and sVero cells growing in spinner flasks. Bioreactor experiments were performed with sVero cells using VSV and PV1. For each virus the production was optimized using different media compositions and cell density at infection.

Infected cell supernatants were harvested, clarified by low-speed centrifugation and maintained at −70 °C until titration.

2.6. Virus titration

Infectious virus titers were determined by plaque formation unit assays or by 50% tissue culture infective dose ($TCID_{50\%}$) assays, both on Vero cells monolayer. To minimize inter- and intra-assay variability, samples generated from the same experiment were tested on the same day when possible, and quadruplicates were used for each virus sample.

3. Results and discussion

3.1. Adaptation of Vero cells to suspension growth in serum-free media

The adaptation procedure lasted 120 days. Once adapted to this condition Vero cells were no longer adherent, they were named sVero cells and they grew as single cell suspension. Although it has been reported that Vero cells can grow in suspension as cell aggregates [8], to our knowledge these constitute the first data of Vero cells that grow without forming clumps. Adaptation of Vero cells to serum-free media was also successfully achieved by others [9].

3.2. Growth kinetic of sVero cells in serum-free media

As shown in Fig. 1, sVero cells were able to reach 2.5×10^6 cells/ml 7 days after starting the culture. This value is slightly higher than the one reported for Vero cells grown in serum-free media on microcarriers in spinner flasks under optimized conditions [10,11]. Lactate level reached 2.3 mM on day 4 and after that, the concentration decreased to 0.3 mM while

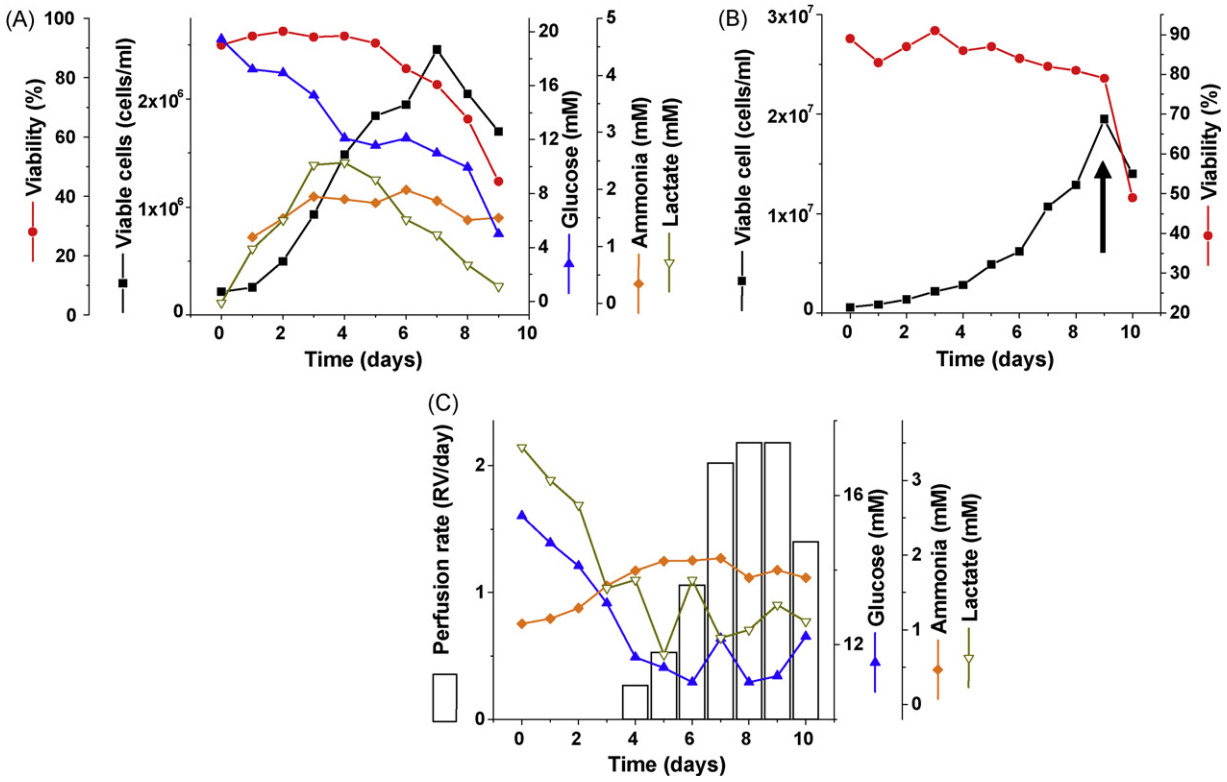

Fig. 1. Profiles of cell density, cell viability, glucose, lactate and ammonia concentrations for sVero cells grown in spinner flasks (A) and agitated bioreactor (B and C). Arrow indicates cell infection with PV1.

Table 1
Production and productivity of VSV in batch cultures for Vero and sVero in different culture media.

	Cell density at infection (cells/ml)	Culture media	Viral titer ($TCID_{50\%}$/ml)	Productivity ($TCID_{50\%}$/ml)
Vero	3.0×10^5	SFM	3.2×10^5	1
	3.0×10^5	SFM + 2% FCS	5.0×10^7	167
	3.0×10^5	MEM + 2% FCS	8.0×10^7	266
sVero	3.0×10^5	SFM	4.6×10^5	1.5
	3.0×10^5	SFM + 2% FCS	1.7×10^8	567
	3.0×10^5	MEM + 2% FCS	7.0×10^7	233

Table 2
Production and productivity of VSV in batch and in perfusion cultures.

	Cellular density at infection (cells/ml)	Viral titer ($TCID_{50\%}$/ml)	Productivity ($TCID_{50\%}$/ml)
Batch	0.42×10^6	4.6×10^8	1,105
	0.75×10^6	4.6×10^8	622
	2.44×10^6	3.2×10^9	1,295
	3.15×10^6	4.6×10^9	1,344
	4.78×10^6	2.1×10^9	449
	9.35×10^6	1.0×10^9	1
Perfusion	9.35×10^6	3.2×10^{10}	3,423

Table 3
Production and productivity of PV1 in batch and in perfusion cultures.

		Cell density at infection (cells/ml)	Viral titer ($TCID_{50\%}$/ml)	Productivity ($TCID_{50\%}$/ml)
Vero	Batch	3.0×10^5	6.1×10^8	2,033
sVero	Batch	3.0×10^5	6.8×10^8	2,267
	Perfusion	1.9×10^7	2.1×10^{10}	1,105

glucose level decreased from 19.4 to 5.1 mM. These results indicate that sVero cells metabolize glucose efficiently since only a minor proportion of the carbon source is converted to lactate. The glucose level in perfusion mode was stabilized around 12 mM while lactate level reached 2.2 mM at the moment of the infection (day 9). Ammonia concentration reached a maximum value of 1.6 mM.

For continuous cultures the perfusion rate was increased from 0.25 reactor volume/day (RV/day) on day 4 to 2.25 RV/day (day 8). After that, the perfusion rate was maintained constant until day 9, when cell density reached 1.9×10^7 cells/ml. The achieved cell concentration was higher than the one reported for Vero cells growing on microcarriers in perfused cultures [10,12].

3.3. Virus production

3.3.1. VSV production

First of all, we evaluated VSV production in sVero cells growing in spinner flasks in comparison with the original anchorage-dependent cell line growing in T-flasks using an MOI of 0.1 PFU/cell. In both cases, serum supplementation significantly affected viral production (Table 1). Maximum productivity of VSV was reached when infecting sVero cells growing in SFM + 2% FCS. We achieved a 2-fold increase in viral titer compared to the maximum titer reached when working with Vero cells in MEM + 2% FCS. Then we evaluated the influence of cell density on VSV production by infecting sVero batch cultures with 4.2×10^5, 7.5×10^5, 2.5×10^6, 3.2×10^6, 4.8×10^6 and 9.4×10^6 cells/ml and a multiplicity of infection (MOI) of 0.1 PFU/cell. In continuous cultures, the infection was performed after cell density reached 9.3×10^6 cells/ml with a MOI of 0.02 PFU/cell and both production and productivity were significantly higher. Attempts to increase virus titers in batch cultures by increasing the sVero cell concentration above 3.15×10^6 cell/ml at the moment of infection failed and resulted in lower specific yields and overall production (Table 2). This is often referred to as "cell density effect" [13], and in this case, it is probably related to nutrient limitations since in non-infected control cultures with higher cell concentrations the viability was compromised (data not shown).

3.3.2. HSV production

We evaluated the influence of FCS in production yields when working with different cell concentrations at the moment of infection. When sVero batch cultures with 2.5×10^5 cells/ml were infected, FCS did not have any effect on HSV production, reaching 3.2×10^8 $TCID_{50\%}$/ml within 47 h post-infection. In contrast, a 2-fold increase was observed using FCS when the initial cell density was 8.0×10^5 cells/ml (data not shown). Similar results were achieved with anchorage-dependent Vero cells.

3.3.3. PV1 production

sVero cells were infected with a MOI of 1 PFU/cell at 34 °C. The maximum viral titer achieved was 6.8×10^8 $TCID_{50\%}$/ml in batch cultures. A similar result was obtained for anchorage-dependent Vero cells (Table 3). In perfusion cultures, a higher virus titer was obtained when compared to batch culture experiences. The volumetric yield was 30-fold higher although the overall specific virus production rate per cell was 2-fold lower.

4. Conclusions

Vero cells were successfully adapted to grow in suspension mode, in serum-free media and in the absence of disaggregating agents. To establish whether this process affected Vero cells susceptibility to viral infection, and therefore their capability to produce viruses, we infected Vero and sVero batch cultures with three different kinds of viruses.

Under optimized conditions for each cell line, similar production yields were achieved with each virus. The addition of FCS increased VSV production, while the production of PV1 demonstrated to be serum independent. Even though results concerning FCS influence

on HSV production were inconclusive, the optimization of production parameters, such as cell density made the addition of FCS unnecessary.

Production yields of VSV in perfusion cultures were optimized reaching a 2-fold and a 10-fold increase in cell and volumetric productivity, respectively. Accordingly, volumetric yield of PV1 production in perfusion cultures was significantly higher when compared to batch cultures, even though viral production per cell was halved. This technology offers a solution to the requirements for high yield vaccine production processes by providing a fully scalable suspension-Vero cell-based platform.

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