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Propagation of *Peste des petits ruminants* (PPR) Virus in Vero Cells for Vaccine Production using Tide Motion Bioreactor

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ABSTRACT

Peste des Petits ruminants (PPR) is considered as one of the major constraints to the productivity of small ruminants in India. Currently PPR control is done by vaccination with an attenuated PPR strain (Sungri/96) produced in monolayers of vero cells grown in roller bottles or static flasks. This work focuses on the production of PPR vaccine using tide motion bio reactor as an advanced option for process scale-up. Both the roller and bio reactor cultures were propagated under same cultural conditions and infected with same multiplicity of infection of PPR vaccine virus. Assessment of infectivity titres determined at periodic intervals in both cultures revealed that the bio reactor culture produced higher amount of virus than the stationary cultures post infection. The results provide further insights into the feasibility of applying tide motion bio reactor cell culture technology to produce PPR vaccine in vero cells significantly simplifying the existing production process.

HIGHLIGHTS

- The *peste des petits ruminants* virus is grown in vero cells in roller culture bottles for regular vaccine production.
- In the present study, the tide motion bio reactor was used as an advanced option for the production of PPR virus for vaccine production.
- The study yielded higher titre of virus compared to roller culture bottle simplifying the existing procedure.

Keywords: PPR virus, vero cells, vaccine production, CelCradle-500AP, tide motion bioreactor

Peste des Petits Ruminants (PPR) is an acute, highly contagious and fatal disease of sheep and goats and is considered as one of the major constraints to the productivity of small ruminants in Africa and Asian countries (OIE, 2013). The disease is characterized by necrotic stomatitis, catarrhal inflammation of the occular and nasal mucosa, pneumonia followed by diarrhoea and death (Singh and Bandyopadhyay, 2015). Current control of the disease mainly includes isolation of animals and disinfection of the contaminated environment, and administration of a live-attenuated vaccine, which provides a strong immunity (Santhosh *et al*., 2013). Vaccine is produced with an attenuated PPR strain (Sungri/96) produced in monolayers of vero cells grown in roller bottles or static flasks (Hegde *et al*., 2008; Silva *et al*., 2008). High titers of the vaccine have also been obtained using Cytodex 1 microcarrier cultures in bench top fermenter (Mohan *et al*., 2009).

This study focuses on the production of a PPR vaccine using tide motion bio reactor as an advanced option for process scale-up. Tide motion bio reactor is a single use benchtop bioreactor system capable of supporting the high density culture of adherent or anchorage dependent cells. It is designed based on the concept of bellow induced intermittent flow of media and air through porous matrices where cells reside. This provides a low shear stress, high aeration and foam free culture environment.

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MATERIALS AND METHODS

Cells

Vero cells between 130 to 150th passage grown in Minimum Essential Medium (MEM) with earle's salts and 6 % foetal bovine serum is used for the propagation of PPRV.

Vaccine virus

The vaccine virus developed at the Rinderpest Laboratory, Division of Virology, IVRI, using an indigenous isolate of PPR virus (PPRV Sungri/96) was used for vaccine production.

Culture bottles/bio reactor

A CelCradle-500A single use bioreactor filled with BioNOCTM II carriers and Roller bottles were used.

Propagation of cells for vaccine production

The PPR vaccine was produced in vero cells by coculture method. In brief vero cell monolayer in a roux flask was trypsinized using trypsene versene glucose, centrifuged for 3 min at 400 × g and the centrifuged cells were inoculated into 120 ml of growth medium and transferred to a single CelCradle-500AP bottle. Virus with known titre was added to the cultures at m.o.i of 0.01. Cell seeding was performed by inverting the CelCradle bottle to allow all carriers to be submerged in cell slurry. "Inverted cell seeding" was performed in a CO_2 incubator at 37 ºC with gentle swirling every 15-30 min for 3 hours. To allow expansion of attached cells, bottle was topped up to 500 ml with growth media and placed in a CelCradle stage in CO_2 incubator with 5 % CO_2 with the following tide motion parameters: Uprate- 1 mm/sec, Uphold- 10 sec, Downrate-1 mm/sec and Down hold: 30 sec. Following, a part of the virus-containing medium was sampled at every day over a 8 day production period for monitoring pH, residual glucose concentration and virus titer. Carriers were sampled every day to perform: (1) CVD (Crystal Violet dye) nuclei count and; (2) visual observation of cell growth and distribution across matrix using Fluorescein diisothiocyanate (FDA) staining. Infectious viral titre per harvest was determined by the titration using 96 well plate methods. Media containing virus was harvested and fresh maintenance media was replaced on day 3 and on day 6 and 7. The final harvest of medium and cells (to obtain intracellular virus) was made at Day 8. In parallel, roller bottles were used for a control infection experiment.

RESULTS AND DISCUSSION

In the present study a laboratory scale tide motion bioreactor was used for the production of peste des petits ruminant virus for production of vaccine and its growth was compared with respect to the roller culture system. During operation the tide motion bio reactor was filled with culture medium and the culture medium was raised and lowered alternatively, to and expose the matrices to oxygen creating a dynamic interface between air and media on the cell surface to maximise nutrient uptake and oxygen transfer. When matrices expose to air, cells uptake oxygen while protected by thin layer of media in matrices. The reactor consists of 850 number of 5.5 g carriers providing

Table 1: The daily action carried out, pH of the media, cell count, glucose concentration of the media and virus titer

Day	Action	pH	Cell count (per strip)	Glucose concentration (g/L)	Virus titer (per ml)
Day 0	Cell seeding and virus cocultivation	6.8	5×10^8 (total cells seeded)	74.3	—
Day 1	—	6.8	6.7×10^4	0.42	—
Day 2	—	7.4	9×10^4	0.33	—
Day 3	Media change	7.85	1.5×10^5	0.28	$10^{4.75}$
Day 4	—	7.8	1.8×10^5	0.35	$10^{6.5}$
Day 5	—	7.92	1.12×10^5	0.22	$10^{6.5}$
Day 6	Harvest and media change	7.2	8.4×10^4	0.2	$10^{7.5}$
Day 7	Harvest and media change	7.9	5.34×10^4	0.59	$10^{6.5}$
Day 8	Complete harvest	7.85	5.34×10^4	0.43	$10^{6.5}$

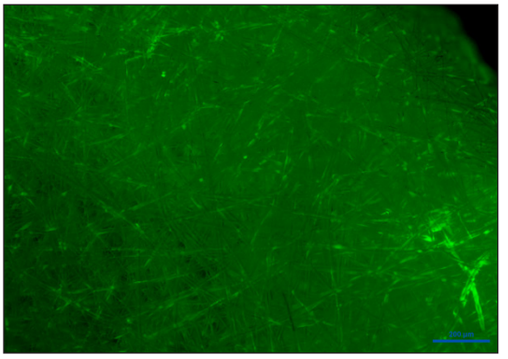

Fig. 1A

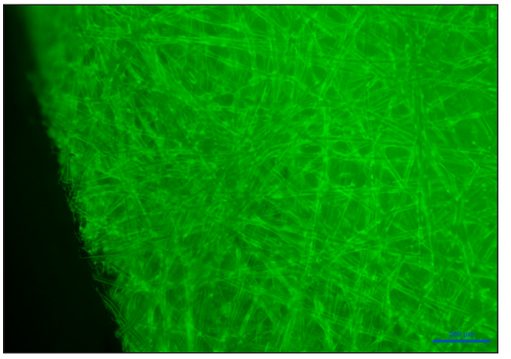

Fig. 1B

Fig. 1: Cell growth and distribution across matrix on visual observation under the microscope using Fluorescein diisothiocyanate (FDA) staining (**Fig. 1A:** Day 2 post cell seeding, **Fig. 1B:** Day 4 post cell seeding)

13,200 cm^2 surface area for attachment and growth of cells. the surface area of each carrier strip is 17.6 cm^2. The cells obtained from roux flask were inoculated onto a CelCradle bottle at a cell density of 5×10^8 cells/bottle. A seeding efficiency of 80% was achieved after 3 hours. The action carried out, pH of the media, cell count, glucose concentration of the media and virus titer of the sample collected every day are described in table 1. Cell growth and distribution across matrix on visual observation under the microscope using Fluorescein diisothiocyanate (FDA) staining is shown in Fig 1. Highest cell attachment was achieved 4 days of post incubation with 1.8×10^5 cells/strip, The optimal cell density achieved at different time points of the culture is shown in table. 1. PPR virus titers however started to increase from $10^{4.75}$ to $10^{7.5}$ TCID50/ml as represented in table 1. Whereas the control experiment with roller bottles yielded a virus titer of $10^{6.83}$ TCID50/ml. In the present study the surface area of the bioreactor was equal to 8 roller bottles of 1700 cm^2 and the virus titer was considerably higher than the roller bottle system there by reducing the limitation of surface area and handling.

Peste des Petits Ruminants is one of the most frequent and devastating diseases of small ruminants in India. The only way to control the disease is preventive vaccination. PPR vaccine is produced using vero cells. Currently, these cells are cultured in open systems using roller bottles, Cell factories or micro carriers which are labour -intensive as well and involve lengthy handling operations. Conventional culture systems for adherent cells tend to reduce cell growth because of their space limitation (surface area) and design. The CelCradle™ system provides a low shear stress, high aeration, and foam-free culture environment proving to be ideal for high-density growth of adherent cells such as vero that were used in this study. It is also a cost-effective bioprocessing solution that can speed up the large scale manufacturing process as compared to conventional systems.

CONCLUSION

Tide motion bioreactor can be explored as simple yet effective alternate option for growth of virus like peste des petits ruminants in mammalian cells like vero which can be used for vaccine production with higher yields of both cells and the virus.

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