Swine Vaccines

Introduction

Pigs and pork meat constitute more than one-thirds of meat produced worldwide and is an important component of the world economy pertaining to agriculture, trade and global food security. As such, pig health is an important consideration globally. However, infectious diseases pose constraints and challenges to swine production and outbreaks of swine diseases occur commonly – increasingly so with rapid globalization of the pig industry. There are a multitude of swine pathogens and preparedness for an outbreak is imperative. Vaccination of pigs could vastly contribute to enhancing pig health and could impact greatly on improving agricultural economy in swine - producing nations. Vaccines using mammalian cell substrates are an effective solution and need to be produced in a timely manner to handle an epidemic and also for pandemic preparedness.

In addition, a rapid, cost-efficient and large-scale production of Vaccine is essential. Therefore, in the following studies, an evaluation of Bioreactors using the Tide motion principle – the CelCradle™-500A and the TideXcell™-002 was undertaken as a viable option to potentially replace the use of conventional 2D-culture systems. This report focuses on 3 important pathogens that pose a threat to swine health globally – Hog Cholera, Porcine endemic diarrhea virus (PEDV), and Porcine reproductive and respiratory syndrome virus (PRRSV).

Hog Cholera Virus

Classical swine fever (CSF), otherwise known as hog cholera (HC) or just swine fever, is a specific viral disease of pigs. CSF is caused by CSF virus (CSFV) which can be the source of substantial morbidity and mortality events in affected swine. The disease occurs in much of Asia, Central and South America, and parts of Europe and Africa. This disease is reportable to the World Organization for Animal Health and viral detection can severely diminish pork exports. The medical prophylaxis for this disease is through Vaccination with modified live virus strains and is effective in preventing losses in countries where classical swine fever is enzootic. This method in conjunction with sanitary prophylaxis is a good method to eliminate or contain the prevalence of Hog Cholera.

Virus Description

The CSF virus (CSFV), the etiological agent of CSF, is an icosahedral and enveloped positive stranded RNA virus that, together with bovine viral diarrhoea virus (BVDV) and border diseases virus, belongs to the *Pestivirus* genus of the *Flaviviridae* family.(Munoz-Gonzalez *et al.*,2015) The genome contains 12,300 base pairs and comprises four structural and seven non-structural proteins.

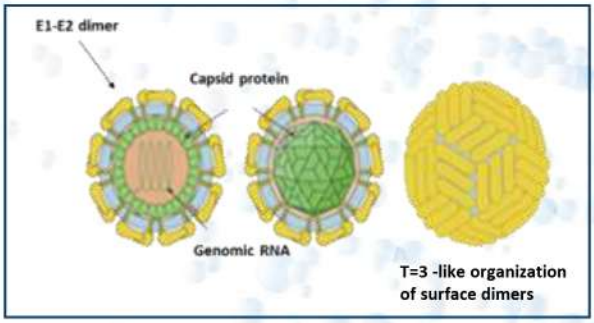

Figure 1: The Pestivirus genus of virus to which CSF virus belongs; the virus is enveloped, spherical, and about 50nm in diameter. Mature virions contain 3 virus-encoded membrane proteins (Erns, E1 and E2) in addition to the capsid protein.

Efficient live-attenuated vaccines against CSFV are used routinely in endemic countries. As with many other diseases affecting livestock, the most efficient vaccines currently available against CSFV are live attenuated vaccines. (Munoz-Gonzalez *et al.*, 2015) As such, a rapid and efficient platform to produce vaccines for prophylaxis is imperative. The TideMotion® Bioreactor, TideXcell™-002 , has been employed to generate High titre virus in swine testis (ST) cells which is achievable due to the high densities to which cell substrates can be cultivated to.

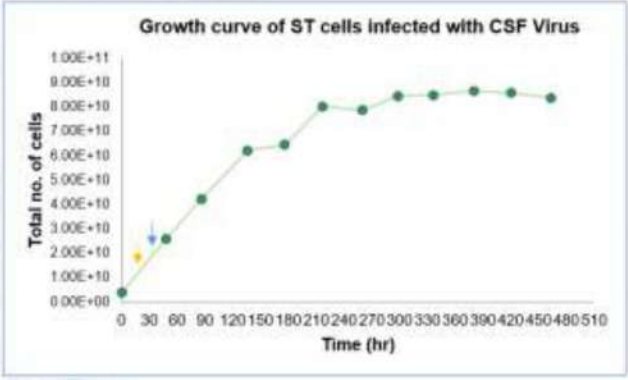

Figure 2: Growth curve of ST cells grown in the Tide Cell ™ -002 Bioreactor and infected with the CSFV The yellow arrow indicates a half-medium change and the blue arrow indicates time-point of Virus infection.

Virus harvest (h)	Virus harvest volume (L)	Virus titre (represented as virus-dilution fold)
86	8	200
136	9	700
176	9	700
217	9	700
260	9	700
298	8.5	700
336	8.5	400
380	8.5	<200
420	8.5	<200
463	8.5	<200

Table 1: Virus harvested at indicated time points was titrated by the Rabbit pyrogen method. The volume of harvested virus and the dilution required for a pyrogenic response in rabbits is indicated.

Titration of CSF Virus

Titration of the CSF virus was done by the Rabbit Pyrogen method. (Vipond *et al.,* 2016). Culture supernatants harvested from the Tide Cell Pro-002 bioreactor were diluted and injected into rabbits. Briefly, the higher the dilution that is required to induce a pyrogenic response, the higher the titre. As indicated in the table, the virus harvested from time points 136 to 298 have the highest titre.

PEDV

Porcine epidemic diarrhea (PED) emerged for the first time in Europe during the 1970s.The virus responsible for this disease is an *alphacoronavirus* known as PEDV. The PEDV has a single-stranded, positive-sense RNA genome. An infection with PEDV in pigs leads to severe liquid diarrhea, vomiting, and dehydration. In suckling piglet's population, PEDV causes high mortality. PEDV, the etiological agent of PED, is a large-enveloped RNA virus, which is a member of the genus *Alphacoronavirus* within the *Coronaviridae* family (Song *et al.*, 2015).

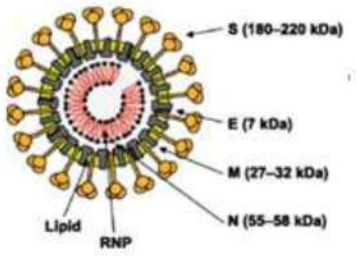

Figure 3: Schematic organization of the PEDV virion structure. Inside the virion is the RNA genome associated with the N protein to form a long, helical ribonucleoprotein (RNP) complex. The virus core is enclosed by a lipoprotein envelope, which contains S, E, and M proteins. The predicted molecular sizes of each structural protein are indicated in parentheses.

PED virus production

The CelCradle™-500 Bioreactor was used to cultivate Vero cells in complete IMDM medium to high cell densities as indicated in ***Figure 4*** and the virus infection was done at indicated time points. The virus titre obtained is represented in ***Figure 5***.

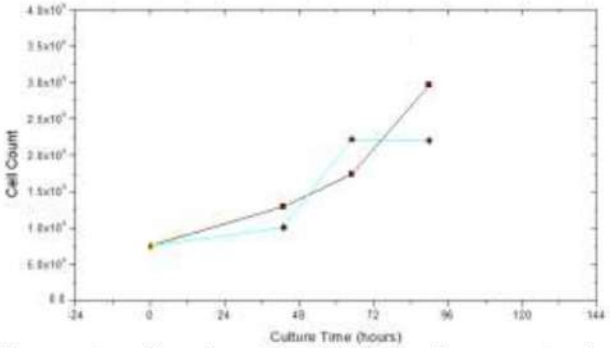

Figure 4: Growth curve of Vero cells grown in the CelCradle™-500 Bioreactor and infected with the PED virus (PEDV). The black line represents cells infected with 2.5% virus at a cell density of 2.97 E9 and the blue line indicates cells infected with 0.5% virus at a cell density of 2.2 E9.

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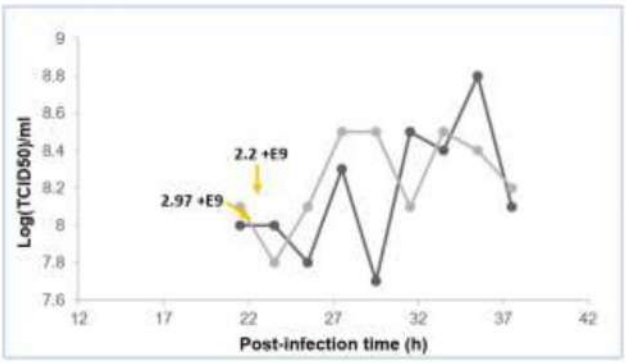

Figure 5: Virus titers of PED virus obtained from Vero cells. The black line represents the virus titer from cells infected with 2.5% virus at a cell density of 2.97 E9 and the grey line indicates cells infected with 0.5% virus at a cell density of 2.2 E9.

In addition, a rapid, cost-efficient and large-scale production of Vaccine is essential. Therefore, in the following studies, an evaluation of Bioreactors using the Tide motion principle – the CelCradle™-500A and the TideXcell™-002 was undertaken as a viable option to potentially replace the use of conventional 2D-culture systems. This report focuses on 3 important pathogens that pose a threat to swine health globally – Hog Cholera, Porcine endemic diarrhea virus (PEDV), and Porcine reproductive and respiratory syndrome virus (PRRSV).

Titration of PED virus

Titration of the virus obtained at the time – points indicated was done by the TCID50 method. (Reed and Muench, 1938) As represented, it was possible to cultivate Vero cells to high densities and infection with the PED virus resulted in extremely high titres of 1x10 $^{8.8}$ TCID50 /ml.

PRRSV

PRRS is one of the most important economically damaging diseases to the swine industry worldwide. It is an enveloped, single positive-stranded RNA virus and belongs to the family *Arteriviridae* of the order *Nidovirales*. (Liu *et al.*, 2017)The schematic of the virus structure is given below.

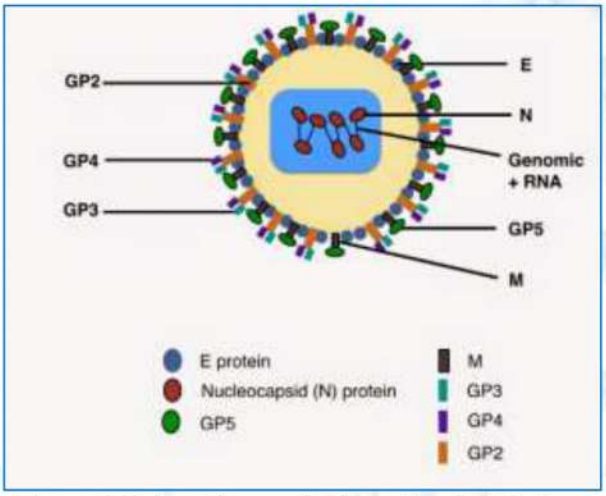

Figure 6: Schematic organization of the PRRS virion structure. It is an enveloped virus with a positive strand ssRNA genome of approximately 15kB in length, encoding for ten open reading frames (ORFs).Some of the encoded proteins and the nucleoprotein are represented here.

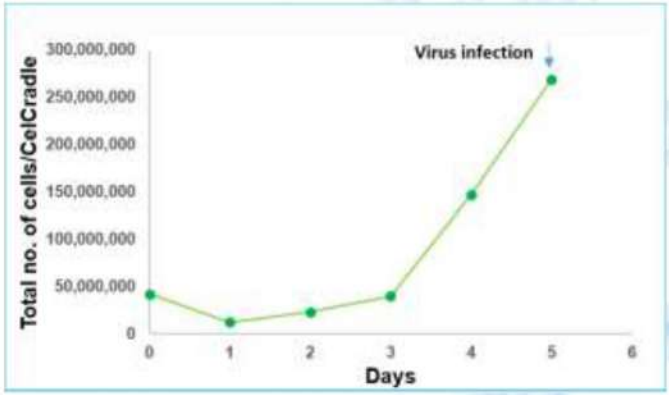

Figure 7: Growth of MARC-145 cells for production of Porcine Reproductive and Respiratory Syndrome virus (PRRSV) and recommended Virus infection time point

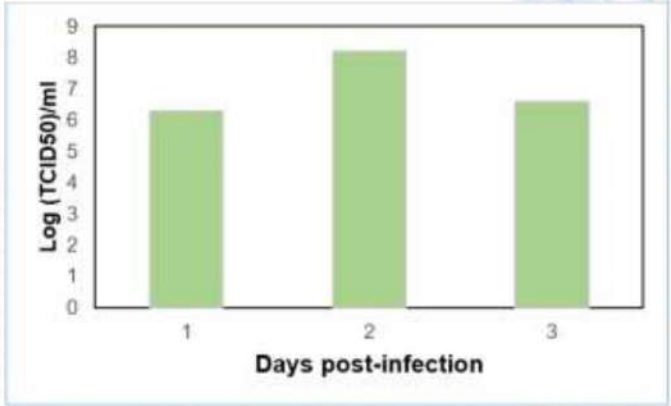

Figure 8: PRRSV titre at day 1-3 post-infection; the viral titre was a maximum at 1.4 × 10^8 $TCID_{50}$/ml at 2 dpi.

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Titration of CSF Virus

Multiple harvests of the virus were made and was titrated by the TCID50 method. The total viral titre of PRRSV in the CelCradle™ was 6.8 × 1010 TCID50/500ml.

In terms of equivalency, comparing the total virus yield from a CelCradle™ versus that from a T175- flask (which produced 5.1 × 10^8 TCID50/25ml of virus), the total viral yield a of 3D culture in the CelCradle™-500A was 132-fold higher than that of the 2D culture.

Comparison of 2-D culture systems VS TideMotion® Bioreactors for production of viruses against pig diseases

2-D culture system	3-D culture systems (TideMotion® bioreactors)	Equivalency
Roller bottles (1500 cm^2)	TideXcell™-002	123
T-150 flasks (150 cm^2)	CelCradle™-500A	285
T-175 flasks (175 cm^2)	CelCradle™-500A	132

More information on
www.vaccixcell.com (Bioprocessing tools) or
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Conclusion

The above findings indicate that TideMotion® Bioreactors are an excellent system for producing high titre viruses and are a very viable alternative to 2-D culture systems. With an aim of ensuring a reliable supply of vaccine supply and immunization for pigs, we introduce the means of achieving high-titre viruses to support scaleable production of Pig vaccines using TideMotion® Bioreactors.

Challenges to Vaccine production for dealing with an outbreak of Hog diseases can be easily met by the usage of TideMotion® Bioreactors and are indeed an excellent option to replace conventional 2-D culture systems.

References

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