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Development of suspension adapted Vero cell culture process technology for production of viral vaccines

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ABSTRACT

Vero cells are considered as the most widely accepted continuous cell line by the regulatory authorities (such as WHO) for the manufacture of viral vaccines for human use. The growth of Vero cells is anchorage-dependent. Scale-up and manufacturing in adherent cultures are labor intensive and complicated. Adaptation of Vero cells to grow in suspension will simplify subcultivation and process scale-up significantly, and therefore reduce the production cost.

Here we report on a successful adaptation of adherent Vero cells to grow in suspension in a serum-free and animal component-free medium (IHM03) developed in-house. The suspension adapted Vero cell cultures in IHM03 grew to similar or better maximum cell density as what was observed for the adherent Vero cells grown in commercial serum-free media and with a cell doubling time of 40–44 h. Much higher cell density (8×10^6 cells/mL) was achieved in a batch culture when three volume of the culture medium was replaced during the batch culture process.

Both adherent and suspension Vero cells from various stages were tested for their authenticity using short tandem repeat analysis. Testing result indicates that all Vero cell samples had 100% concordance with the Vero DNA control sample, indicating the suspension cells maintained their genetic stability. Furthermore, suspension Vero cells at a passage number of 163 were assayed for tumorigenicity, and were not found to be tumorigenic.

The viral productivity of suspension Vero cells was evaluated by using vesicular stomatitis virus (VSV) as a model. The suspension cell culture showed a better productivity of VSV than the adherent Vero cell culture. In addition, the suspension culture could be infected at higher cell densities, thus improving the volumetric virus productivity. More than one log of increase in the VSV productivity was achieved in a 3L bioreactor perfusion culture infected at a cell density of 6.8×10^6 cells/mL.

1. Introduction

Most cell culture processes for vaccine production are mainly based on adherent cells: *e.g.*, MRC5 human lung cells, Vero simian kidney epithelial (Vero), Madin-Darby Canine Kidney (MDCK) cells, and chicken embryo fibroblast (CEF) cells [7]. Vero cells are considered as the most widely accepted continuous cell line by the regulatory authorities (such as WHO) for the manufacture of viral vaccines for human use [10]. Vero cells are susceptible to a broad range of viruses and have been widely and commercially used, after propagation on microcarriers, for the production of rabies, polio, enterovirus 71, influenza and other vaccines [11,17,2]. Vero cell culture technologies have been also explored for productions of many more viral vaccines over the last two decades [14].

The growth of Vero cells is anchorage-dependent and Vero cells can only proliferate when provided with a suitable surface. Anchorage-dependent cell cultivation formats include many "2D" planar approaches, such as roller bottles and multiple- or stacked-surface based systems of many types, and microcarrier technology. Presently, microcarrier technology, which employs small beads of glass, plastic or other composition having various densities, porosities and surface treatments that are dispersed in a suspension within stirred tank reactors, provides the most popular platform for cell production by providing a large surface area for cell growth.

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Scale-up of cell mass for large-scale adherent cell-based manufacturing is of particular concern or challenging as attachment-dependent culture processing generally requires more steps (the required detachment and reattachment of cells during passaging) and time than suspension [13]. As a result, the scale-up of adherent cell culture process is not only complicated but also labor intensive involving higher cost of goods [16,22].

Adaptation of Vero cells to grow in suspension will simplify handling of cultures such as inoculation, cell passaging and process scale-up significantly, and therefore, reduce the production cost. Recently, several research teams have attempted to adapt adherent Vero cells to grow in suspension culture [18,12], however, some obstacles, such as low cell viability, relatively longer cell doubling time (>50 h), still remain to be solved in the development of adaptation process.

Work on adaptation of adherent Vero cells to grow in suspension started several years ago at our research facility. The objective of this work was to adapt adherent Vero cells to grow in suspension in serum-free media, to characterize the adapted cell in terms of genetic stability and tumorigenicity, and to improve the cell growth and virus productivity of suspension adapted Vero cell under different culture processes through process intensification.

2. Material and methods

2.1. Cell line, culture media and viruses

Adherent Vero cells originated from ATCC CCL-81 were grown and maintained in either ProVero 1 (Lonza, MD), VP-SFM (Gibco, NY) or IHM03, a serum-free and animal component-free medium developed in-house, in T-flasks in a humidified incubator with 5% CO_2 and at 37 °C. The culture was diluted and passaged twice a week.

Recombinant vesicular stomatitis virus expressing the green fluorescent protein (VSV-GFP) [20], a generous gift of Dr. John Bell, Ottawa Hospital Research Institute, was selected as a model virus to evaluate the virus productivity of adherent and suspension adapted Vero cells.

2.2. Adaptation of adherent Vero cells to grow in suspension and maintenance of suspension adapted Vero cell culture

The adherent Vero cells recovered from a cryo-preserved vial and grown in ProVero 1 or IHM03 medium in T-flasks were transferred to shake flasks at a concentration of 0.25×10^6 cells/mL in serum-free IHM03 medium or commercial media to start the suspension adaptation process. Half of spent medium in the suspension culture was replaced twice per week during the period of adaptation until the cells started to grow in suspension at a reasonable rate such as at a double time of less than 72 h. Once the cells were adapted to grow in suspension, the suspension adapted Vero cells were serially passaged by sub-culturing Vero cell suspension culture twice a week in plastic shake flasks agitated at 120 rpm in a humidified incubator with 5% CO_2 and at 37 °C. Each passage was accomplished by diluting suspension cell cultures at inocula ranging between 0.13 and 0.25×10^6 cells/mL in serum-free IHM03 medium. Cell banks were developed at different passages and stored for future evaluation.

2.3. Authentication of suspension adapted Vero cells

DNA samples were extracted from adherent and suspension adapted Vero cells at various passages and shipped to the National Institute of Standards and Technology (Gaithersburg, MD) for short tandem repeat (STR) analysis according to the method developed by Almeida et al. [1]. Control Vero DNA was also used in the authentication test.

2.4. Tumorigenicity testing of suspension adapted Vero cells

The tumorigenicity assay of suspension adapted Vero cells at a passage number of 163 was contracted out to BioReliance Corporation (Rockville, MD), a contract research organization. The evaluation of tumor formation in nude (nu/nu) athymic mice following subcutaneous injection of Vero cell suspension was conducted according to the "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals", a test system recommended by The Center for Biologics Evaluation and Research, FDA [6]. Briefly, 30 female four weeks old Athymic nude (nu/nu) mice were assigned to three treatment groups (10 mice each group) in the test. Each mouse in Group 1 (Test article) was injected subcutaneously between the scapulae with 10^7 suspension VERO cells in 0.2 mL of serum-free IHM03 medium. While each mouse in Group 2 (Positive control) and Group 3 (Negative control) was respectively injected with 10^7 18Cl-10T cells (a Syrian hamster tumor line derived from a chemically transformed Syrian hamster embryo cell line) and 0.2 mL of serum-free IHM03 medium. All animals were observed every working day and the injection site palpated twice a week for lesion development for a period of up to 84 days. Each lesion was observed and the diameter was measured. On day 18, all animals in Group 2 were euthanized and subjected to necropsy in accordance with their study protocol due to size of tumors. On day 84, all animals in Groups 1 and 3 were euthanized and subjected to necropsy. Gross lesions, lung, scapular lymph nodes, and the injection site from all animals in all groups were removed for histopathological analysis.

2.5. Batch, fed-batch and batch culture with medium replacement

Suspension adapted Vero cells were seeded at a range between 0.13 and 0.25×10^6 cells/mL in IHM03 medium in 125 mL plastic shake flasks with a working culture volume of 25 mL. Samples were taken regularly for cell count and analysis of metabolites and residual medium components. Some cultures were fed with commercial feeds such as HyClone™ Cell Boost 5 (GE Healthcare), HEK FS (Xell AG, Bielefeld, Germany) when the cell density reached $1.5–2 \times 10^6$ cells/mL. In addition, spent medium in some cultures was replaced with fresh IHM03 medium for a few times over a batch culture process to examine the effect of medium replacement on the cell growth and maximum cell density.

2.6. Bioreactor culture

The culture was performed in a controlled 3.5 L Chemap CF-3000 bioreactor (Mannedorf, Switzerland) equipped with three surface baffles and two marine impellers, and also with probes to measure and control the temperature and dissolved oxygen (DO) at 37 °C and 40% air saturation. pH of the culture was controlled in the range of 7.1–7.2 by the addition of CO_2 via the surface or sodium bicarbonate solution (7.5% (w/v)). Agitation rate was maintained at 100 rpm. In perfusion mode, cells were retained in the reactor using a BioSep 10L acoustic filter (Applikon Inc., Foster City, CA). Masterflex L/S peristaltic pumps (models 7521-40, Cole-Palmer, Vernon Hills, IL) were used to add fresh medium, harvest cells, and pump the culture through the recirculation loop.

2.7. Cell count and analysis of metabolites

The culture sample was first mixed with an equal volume of Accumax solution (Innovative Cell Technologies, Inc.) in a 1.5 mL vial, the mixture was then incubated at 37 °C and with agitation

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for 30 min. Cell counts were performed with an automated cell counter (Cedex Automated Cell Counter) or using a hemacytometer and erythrosine B. Metabolites such as lactate and ammonia were analyzed by Cedex Bio Analyzer (Roche CustomBiotech). Residual amino acids in spent culture media were quantitated by HPLC.

2.8. Production of recombinant-vesicular stomatitis virus expressing the green fluorescent protein (VSV-GFP)

Adherent or suspension adapted Vero cells were grown in IHM03 medium in T-flask, shake flask or bioreactor to desired densities and then infected with VSV-GFP at MOI of 0.1 with or without medium replacement. Supernatant of infected cultures was harvested at different times of post infection and stored at −80 °C for further analysis. Infection of suspension Vero cells at high densities (such as 5×10^6 cells/mL) in shake flask or 6 well plate cultures was accomplished by concentrating cells from a low cell density (such as 1×10^6 cells/mL) culture and resuspending cell pellet in fresh medium before the virus infection. Extra cellular virus titer (titer of virus in culture medium after clarification) was measured in duplicate by 50% tissue culture infective dose/ml ($TCID_{50\%}$/ml) on HEK293A as described by Elahi et al. [5]. $TCID_{50\%}$ was calculated using Spearman-Karber method and expressed as $TCID_{50\%}$/ml [8].

3. Results and discussion

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

Adherent Vero cells previously cultured in serum-containing media were easily adapted to grow in either serum-free ProVero, VP-SFM or IHM03 media in T-flasks. Once the adherent cells resumed a normal growth in the serum-free media, they were then transferred to plastic shake flasks with the commercial media mentioned above or serum-free IHM03 medium to initiate the adaptation of adherent cells to grow in suspension culture. The Vero cells started to grow in suspension only in serum-free IHM03 medium after 5–8 weeks of adaptation process to reach a doubling time in a range between 40 and 44 h. While in the commercial serum-free medium cultures, the Vero cells aggregated and the adaptation was not successful. Experimental data indicated that the culture media (starting media) used to grow the cells in adherent culture stage did not affect time required to adapt the adherent cells to grow in suspension. The suspension culture primarily consisted of single cells but some aggregates were also observed. Later work showed that the formation of cell aggregates was mainly due to the attachment of cells to the surface of shake flask and formation of a cell ring. Cell aggregates were generated when the cell ring was detached from the surface. Far fewer cell aggregates were observed in bioreactor culture due to the reduced ratio of bioreactor wall surface to the culture volume. Morphologies of Vero cells grown in adherent culture in T-flasks or in suspension culture in shake flasks are shown in Fig. 1.

The doubling time (40–44 h) of suspension adapted Vero cells in IHM03 medium was longer than the about 24 h reported in serum-containing and actively growing adherent culture [15]. The increased cell doubling time of suspension adapted Vero cells in serum-free cultures was more likely and partially due to the removal of serum, as the removal of serum usually increased the cell doubling time, such as from 31 to 43 h [17,19]; Also shear stress in the suspension culture, especially in the culture without supports of microcarrier, might also reduce the cell growth rate. This might be evidenced by a relatively longer cell doubling time of 28–38 h in a study carried out by Yang et al. [22] to examine Vero cells bead to bead transfer in spinner flasks with serum containing medium. Therefore, it appears that, when the Vero cells were cultured in serum-free media especially in stirred cultures, the doubling time of Vero cells was usually longer than 24 h [9,3,4].

3.2. Growth of suspension adapted Vero cell in batch cultures

The suspension adapted Vero cell grew to a density slightly higher than $2x10^6$ cells/mL in shake flask cultures (Fig. 2). This cell density was higher than or similar to what reported in microcarrier

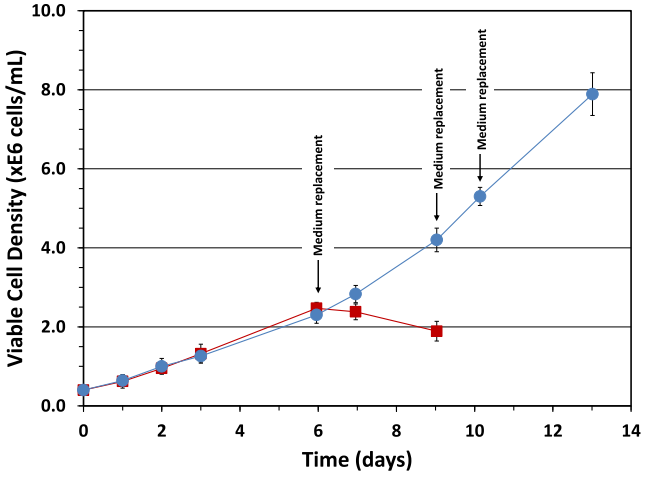

Fig. 2. Growth of suspension adapted Vero cell in batch cultures (■) with serum-free IHM03 medium and also in batch cultures (●) with three volumes of medium replacements during the culture process. The cell viability was always higher than 98% during the both culture processes.

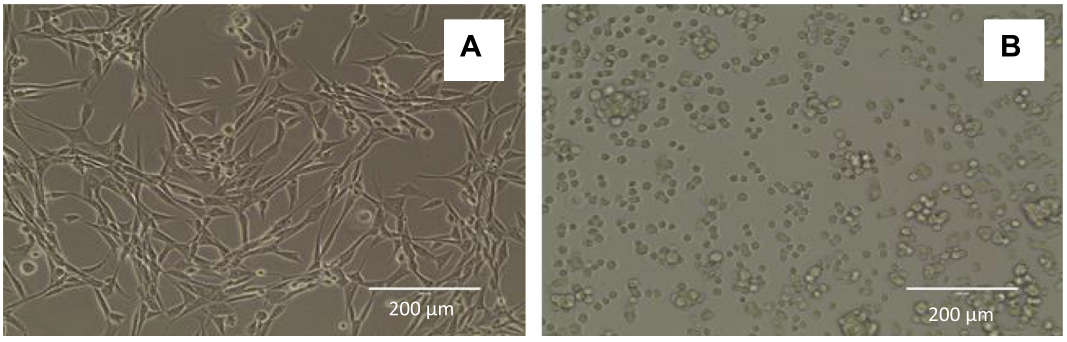

Fig. 1. Morphologies of Vero cells grown as adherent culture (A) in T-flasks or as suspension culture (B) in 125 mL plastic shake flask. The suspension culture primarily consisted of single cells but also some aggregates were also present.

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cultures [17,3,21]. The maximum cell density in the batch cultures was not significantly improved by feeding the batch cultures with Cell Boost 5 (Hyclone, Logan, UT) or HEK TF (Xell, Bielefeld, Germany) (data not shown). These feeds have been reported to contain complex nutrients required to support substantial growth of HEK293 cells. In addition, mixing IHM03 with other high performance commercial media such as HEK GM (Xell, Bielefeld, Germany) did not either improve the maximum cell density in the batch culture or cell doubling times. Analysis of residual nutrient components in the spent media taken from the batch culture process revealed that there were still substantial amounts of glucose (almost 20 mM/L) and amino acids in the spent media at the end of culture. While the concentration of lactate (about 20 mM/L) and ammonia (4–5 mM/L), the two common inhibitory metabolites, were at normal ranges observed in other types of cell cultures. Thomassen et al. [21] also found the growth of Vero cells in stirred culture was not inhibited by the lactate and ammonia at the above concentration ranges. These data indicate that the cell growth in the batch culture might not be limited by the availability of nutrients or inhibited by the common metabolites, lactate and ammonia. Accumulation of unknown metabolites in the cellular microenvironment was probably responsible for the density-dependent inhibition of cell growth observed under the optimized environmental condition [15].

Medium replacement was explored as a mean to improve the cell growth and maximum cell density in the batch culture by removing inhibitory metabolites/replenishing nutrients in the culture. The data in Fig. 2 showed the viable cell density in shake flask cultures reached 8×10^6 cells/mL after 3 complete medium replacements during the batch culture process. Similarly, a higher cell density was achieved by Thomassen et al. [21], when their culture was operated under a recirculation mode, where cells were retained in the bioreactor while 5 culture volumes of fresh medium per day was circulated.

3.3. Genetic stability of adherent and suspension adapted Vero cells over the culture passaging and maintenance process

DNA samples were extracted from adherent Vero cells having been maintained in IHM03 medium for 5 and 37 passages respectively, and from suspension adapted Vero cells having been cultured in IHM03 medium for 33 and 59 passages. These DNA samples along with other DNA samples extracted from various Vero cell clones were profiled by a short tandem repeat analysis. Electropherogram results of all samples showed 100% concordance with the Vero DNA control sample, indicating that the adherent and suspension adapted Vero cells retained their genetic stability after passaging and/or adapting to grow in suspension in serum-free IHM03 medium for many passages. Fig. 3 showed the electropherograms of adherent Vero cell after 5 passages in IHM03 and suspension adapted Vero cell after a total of 59 passages in IHM03.

3.4. Tumorigenicity of suspension adapted Vero cells

To evaluate the tumorigenicity of the Vero cells following the adaptation in suspension culture, nude mice were injected subcutaneously with 1.0×10^7 Vero cells at a passage 163. As positive control the mice were injected with the same number of 18C1-10T cells. Mice injected subcutaneously with culture medium served as negative control. All of the positive control mice (Group 2) developed tumors at the injection site as expected, although tumors were not found in other sites examined. These mice were scheduled sacrificed and necropsied by 18 days post-injection, because they had masses (>1 cm) detected at the injection sites. None of the test article (Group 1, suspension adapted Vero cell) treated mice or the negative control article (Group 3) treated mice had any palpable masses noted at the injection site at terminal sacrifice and were necropsied on day 84 post-injection. Clinical findings on gross necropsy are presented in Table 1.

Table 1
Summary of Gross Necropsy Findings.

	Test Article	Positive Control	Negative Control
No. examined:	10	10	10
Tissue/Finding			
Gross Lesions			
No visible lesion	10	10	10
Injection Site			
No visible lesion	10	0	10
Discoloration; red; roughened; multiple	0	1	0
Mass	0	2	0
Mass, dark	0	1	0
Mass; red; roughened	0	3	0
Mass: roughened	0	4	0
Lungs			
No visible lesion	10	10	10
Lymph Node, Scapular			
No visible lesion	10	10	10

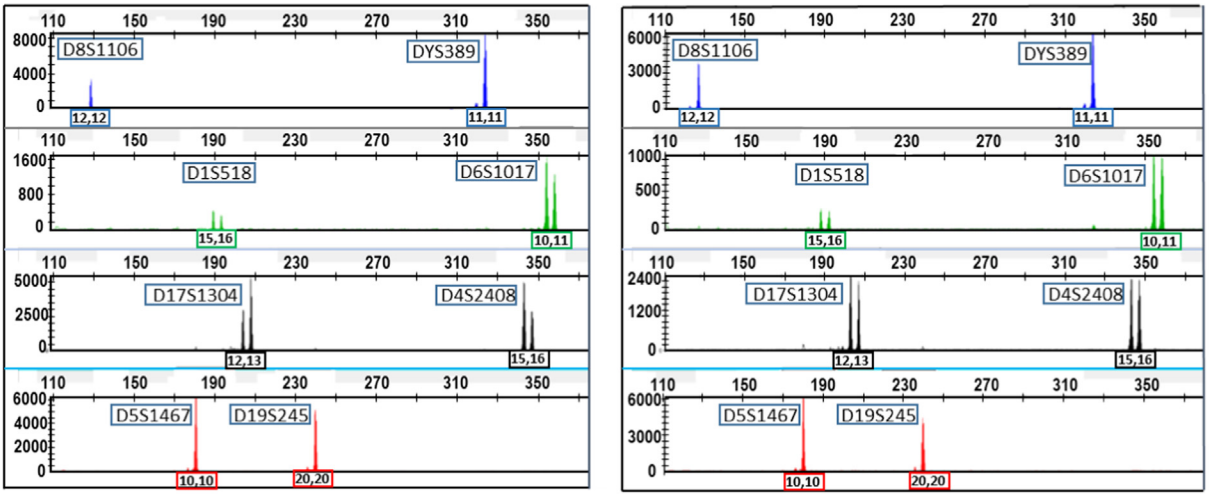

Fig. 3. DNA from adherent and suspension adapted cells at various passages was profiled by STR analysis. Left figure, electropherogram of adherent Vero cells having been maintained in IHM03 for 5 passages; Right figure, electropherogram of suspension adapted Vero cells having been cultured in IHM03 for 59 passages.

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The injection site masses found in all mice in Group 2 were examined by microscope, and showed that they were composed of a population of neoplastic cells that likely arose from the injected 18C1-10T cells that served as the positive control material. These masses were assigned a microscopic diagnosis of Xenograf, and were found only at the injection site, and did not spread to other organs or tissues examined. None of the mice receiving the test article (Group 1), or serum free cell culture media only (Group 3) had evidence of neoplastic cellular proliferation, i.e. tumors, at the injection site or in other organs or tissues examined. In conclusion, under the conditions of this study, the test article, suspension adapted Vero cell at a passage number of 163, was not found to be tumorigenic.

3.5. Production of vesicular stomatitis virus (VSV) in adherent and suspension adapted Vero cell flask cultures

The production of VSV in adherent Vero culture in T175 flask and in suspension adapted Vero cells in 125 mL shake flask cultures was conducted to compare their virus productivity. Both adherent and suspension cultures were infected at respective cell density of about 0.4 and 1×10^6 cells/mL with a medium replacement before the viral infection. The data in Fig. 4 showed that the volumetric virus productivity of the suspension adapted Vero cell was higher than the titer achieved by the adherent Vero cell in T-flask. In addition, the VSV production increased with the increasing cell density in the suspension shake flask cultures, while the volumetric virus productivity declined in the adherent Vero culture when the cell density at infection increased to 1.0×10^6 cells/mL from 0.30×10^6 cells/mL in the adherent cultures. The slightly declined virus productivity could be due to increased confluence percentage of monolayer or availability of nutrients in the T-flask culture.

Experiments were carried out to explore if the volumetric productivity of VSV could be improved by infecting the suspension cultures at higher cell densities. To achieve this objective, suspension culture grown to 0.87×10^6 cells/mL was centrifuged and the cell pellets were resuspended in fresh IHM03 at respective higher cell density of 2.5 and 5.0×10^6cells/mL. The data in Fig. 5 showed the volumetric productivity improved from 2.3×10^9 $TCID_{50\%}$/mL to 8.9×10^9 $TCID_{50\%}$/mL, or 3.8 folds of increase, when the cell density at infection increased from 0.87×10^6 cells/mL to 2.5×10^6 cells/mL. However, further increase in the cell density to 5.0×10^6 cells/mL at infection resulted in no further noticeable increase in the VSV production, indicating a potential limitation in nutrient availability. These results indicated that it is possible to increase the volumetric productivity of VSV through infection at higher cell density. However, the culture process needs to be optimized to avoid limitation of nutrients and other parameters.

3.6. Production of vesicular stomatitis virus (VSV) in suspension bioreactor batch cultures

The growth of suspension Vero cell and production of VSV were scaled-up to a 3L controlled bioreactor. The suspension Vero cell showed a similar growth rate (data not shown) as those achieved in the small shake flask cultures, but with much less cell aggregates. The culture was infected at a cell density of 1.2×10^6 cells/mL without medium replacement. Fig. 6 showed that the VSV productivity achieved in the 3L bioreactor culture was similar to the titer obtained in the small shake flask cultures infected at 1×10^6 cells/mL (Fig. 4), indicating the availability of nutrients might not be a limiting factor when the culture was infected at a low cell density such as 1×10^6 cells/mL, as medium replacement before the viral infection did not dramatically improved the virus titer.

The data in Fig. 6 also revealed that the production kinetic of VSV in the 3L bioreactor culture was faster than in the shake flask control culture. The volumetric productivity of VSV peaked at 28 h post infection in the 3L bioreactor culture, while the highest titer of VSV appeared in the shake flask culture after 45 h post infection.

3.7. The growth of suspension Vero cell and production of VSV in 3L bioreactor perfusion culture

Perfusion culture was explored as an alternative to the batch culture with medium replacement to achieve high cell density for infection in order to increase volumetric virus productivity. As shown in Fig. 7, the perfusion started at a rate of 0.5 vol of media per bioreactor volume per day (VVD) when the cell density in the bioreactor reached $2x10^6$ cells/mL. The cell density reached 4.2×10^6 cells/mL after 2 days of perfusion at a rate of 0.5 VVD, and further increased to 6.8×10^6 cells/mL when the culture was operated at a perfusion rate of 1 VVD for one additional day before the virus infection. These data showed that the perfusion was an effective mean to increase maximum cell density of the culture. The cell density increased by $2.2–2.5 \times 10^6$ cells/mL after perfusing

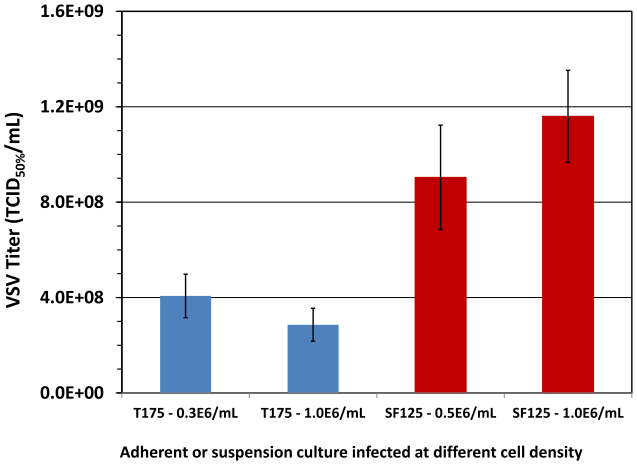

Fig. 4. Production of VSV in adherent (T175) and suspension adapted (SF125) Vero cell cultures. Culture medium in T-175 flask and 125 mL shake flask were replaced before the viral infection at cell densities from 0.3×10^6 cells/mL to 1.0×10^6 cells/mL.

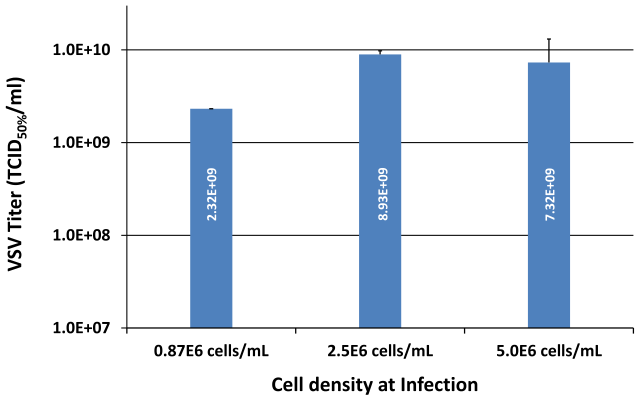

Fig. 5. Effect of increasing cell density at infection on the VSV production. The volumetric virus productivity increased by over 3.5 times when the cell density was increased from 0.87×10^6 cells/mL to 2.5×10^6 cells/mL. However, further increase in the cell density to 5.0×10^6 cells/mL resulted in a decline in the virus titer instead of improvement.

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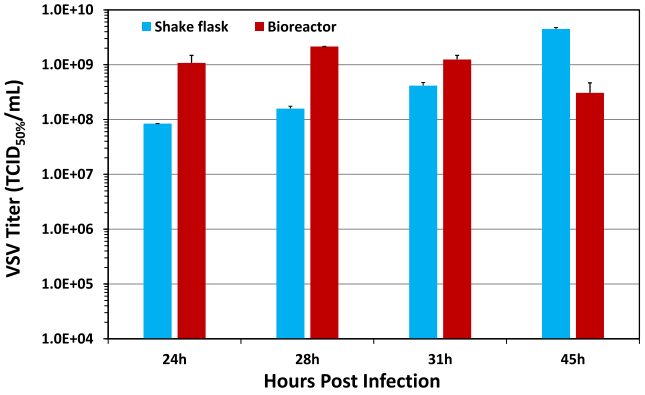

Fig. 6. Production of VSV in a 3 L bioreactor and control shake flask cultures infected at a cell density of 1.2×10^6 cells/mL without medium replacement before virus infection over the time course.

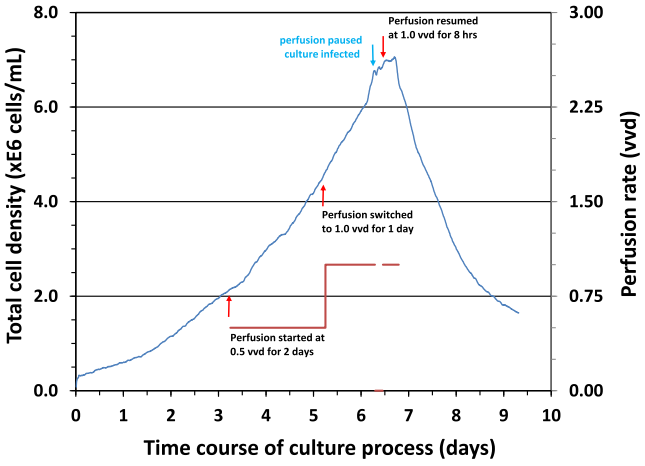

Fig. 7. Profile of cell density monitored by a capacitance probe in a perfusion bioreactor. The cell density reached 6.8×10^6 cells/mL after 2 days of perfusion at 0.5 vvd and another day at 1 vvd before the virus infection. The cell density was verified by daily cell counts, and the cell viability was always higher than 98%.

each bioreactor volume of medium, which was roughly equivalent to the maximum cell density achieved in the batch culture.

The culture in the bioreactor was infected at the cell density of 6.8×10^6 cells/mL. The infected culture was taken from the bioreactor and maintained in shake flasks as bioreactor controls (BioR-CTL-6.8E6). In parallel, cells grown in shake flask (never been in bioreactor) with medium replacement were also infected as shake flask controls (SF-CTL-6E6). The above infected cultures were also diluted with fresh medium to 1×10^6 cells/mL as references (BioR-CLT-1E6 and SF-CTL-1E6) of cultures with unlikely nutrient limitation and metabolite inhibition during the phase of virus infection and production. Comparison of viral productivity between the references and the bioreactor and shake flask cultures provides some insight into potential nutrient limitation and/or metabolite inhibition in the bioreactor and shake flask cultures infected at higher cell density (6.8×10^6 cells/mL). Data in Fig. 8 showed that the VSV production in the bioreactor and control shake flask cultures all peaked at 22 h of post infection. The virus productivity in the bioreactor (Bioreactor) was slightly better than the infected control shake flask cultures (BioR-CTL-6.8E6 and SF-CTL-6E6) with the cells either from the bioreactor or from the shake flask with medium replacement, indicating a possible favorable condition in the bioreactor for the virus production. The virus productivity in either the bioreactor or shake flask cultures

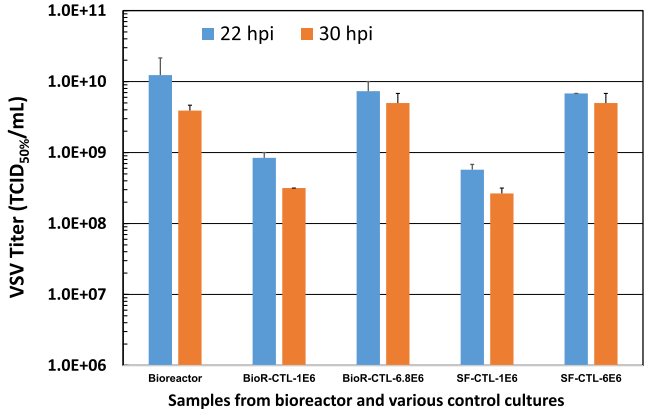

Fig. 8. Production of VSV in the perfusion bioreactor culture. Infected culture taken from the bioreactor was maintained in shake flask as bioreactor controls (BioR-CTL-6.8E6). In parallel, cells grown in shake flask (never been in bioreactor) with medium replacement was also infected as shake flask controls (SF-CTL-6E6). The above infected cultures from the bioreactor or shake flask were also diluted with fresh medium to 1×10^6 cells/mL as references (BioR-CLT-1E6 and SF-CTL-1E6).

infected at 6×10^6 cells/mL showed nearly one log higher than that in the control culture infected at 1×10^6 cells/mL (BioR-CTL-1E6 and SF-CTL-1E6). This clearly demonstrates that the perfusion culture combined with infection at high cell density was a feasible approach to increase the volumetric virus production.

4. Conclusions

The experimental results from this study demonstrated that adherent Vero cells were successfully adapted to grow in suspension in an in-house developed serum free medium. The suspension adapted cells retained their genetic stability and were not found to be tumorigenic. These adapted cells could be used for high yield productions of viruses, such as VSV, in at high cell density and in suspension cultures at reduced production cost.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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