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ORIGINAL RESEARCH PAPER

Optimization of an adenovirus-vectored zoster vaccine production process with chemically defined medium and a perfusion system

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Abstract

Objectives Cells grown in chemically defined medium are sensitive to shear force, potentially resulting in decreased cell growth. We optimized the perfusion process for HEK293 cell-based recombinant adenovirus-vectored zoster vaccine (Ad-HER) production with chemically defined medium.

Methods We first studied the pseudo-continuous strategies in shake flasks as a mimic of the bioreactor equipped with perfusion systems. Using design of experiment (DoE) in shake flasks, we obtained the regression models between Ad-HER titer/virus input–output ratio and three production process parameters: time of infection (TOI), multiplicity of infection (MOI), and virus production pH (pH). We then confirmed the effect of Pluronic F68 (PF-68) at 3.0 g/L on HEK293 cell growth and Ad-HER production in shake flasks and a 2 L benchtop bioreactor.

Results The optimized process was scale-up to a 2 L benchtop bioreactor with the PATFP perfusion system, which yielded cell density of 7.4×10^6 cells/mL and Ad-HER titer of 9.8×10^9 IFU/mL at 2 dpi, comparable to the bioreactor with a ATF2 system.

Conclusion This optimization strategy could be used to develop a robust process with stable cell culture performance and adenovirus titer. Increasing PF-68 concentration in chemically defined medium could protect cells from shear stress generated by perfusion system.

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Keywords Adenovirus · Chemically defined medium · Perfusion cultivation · Shear force · Zoster vaccine

Introduction

Herpes zoster (HZ), or shingles, is caused by reactivation of the varicella-zoster virus (VZV) (Adams et al. 2010). The epidemiology of herpes zoster indicates an association between aging and vulnerability to VZV reactivation (Weinberg 2007). As the most common complication of herpes zoster, postherpetic neuralgia causes chronic and debilitating pain, which significantly impairs life quality of affected individuals (Adams et al. 2010; Lecrenier et al. 2018). Approximately one in three persons develop herpes zoster during their lifetime, so that vaccination for prevention is recommended (Harpaz et al. 2008; Lecrenier et al. 2018).

Adenovirus-vectored vaccine is able to infect a variety of dividing and non-dividing cells (Tatsis and Ertl 2004) and induce humoral and cellular immune responses (Zhu et al. 2020). Though the specific antibody response and T-cell response induced by type-5 adenovirus vectored vaccine could be partly reduced by the presence of high pre-existing immunity, the positive responders still have been identified even in the low dose group (Zhu et al. 2020). Thus the type-5 adenoviruses have been widely applied for the production of vaccines (such as Ebola vaccine (Li et al. 2017), COVID-19 vaccine (Zhu et al. 2020), rabies vaccine (Shen et al. 2012) and tuberculosis vaccine (Shen et al. 2016)) and hold the potential to be used for the HZ vaccine production.

HEK293 remains the primary host cell line for production of the modified replication-deficient adenoviruses for therapeutic purposes (Lusky 2005; Kovesdi and Hedley 2010) and be used for the production of Ebola vaccine (Li et al. 2017) and COVID-19 vaccine (Zhu et al. 2020). Through process optimization, the HEK293 cells have been adapted to serum-free suspension cultivation for human vaccine production, which yields comparable cell density and adenovirus titer with that of serum-containing medium (Shen et al. 2012, 2016). Compared with serum-containing medium, serum-free medium has the benefit of avoiding potential virus contamination risk and reducing costs of down-stream process (Toriniwa and Komiya 2007). However, batch-to-batch variability caused by protein hydrolysates in serum-free medium is still the major barrier preventing wide application of this medium (Sung et al. 2004). Use of chemically defined medium is an alternative solution to address this issue and achieve a robust and continuous production process. However, this causes increased sensitivity of cells to shear force, the resultant impaired cell growth and volumetric productivity (Shen et al. 2016), possibly owing to low cell protein content (Ritacco et al. 2018). Non-ionic surfactant Pluronic F68 (PF-68) is usually supplemented in chemically defined medium to reduce cell damage caused by shear forces (van der Valk et al. 2010; Xu et al. 2017).

Perfusion processes with continuous medium refreshment have been demonstrated to be superior over batch process to improve cell density and virus productivity (Genzel et al. 2014). Through continuous feed of fresh medium and removal of by-product metabolites, the perfusion cultivation avoids nutrition limitations to allow high cell densities. The resultant increased daily productivity reduces equipment footprints, especially suitable to handle large vaccine requirement due to emergent pandemics, such as COVID-19 (Ferreira et al. 2021). The commercially available XCell™ ATF system (Repligen, USA), which consists of a cell retention module (hollow fiber membrane) and an air-operated diaphragm pump, has several merits including low shear stress, high cell viability and easy scale-up. Another commonly used system for cell perfusion cultivation is the hollow fiber membrane-based tangential flow filtration (TFF) system, driven by peristaltic pump or centrifugal pump, which has merits of simplicity and scalability (Clincke et al. 2013; Coronel et al. 2019). However, the shear force generated by perfusion system may be the primary source of cell damage and should be solved during the process development.

In this study, we intend to optimize process parameters to produce recombinant adenovirus-vectored zoster vaccine (Ad-HER) with robust high titers, using chemically defined medium and a novel PATFP perfusion system by our group. Pseudo-perfusion processes in shake flasks were conducted first to study cell growth and metabolism. The Ad-HER production process was optimized efficiently in shake flasks with design of experiment (DoE) approach, providing data for perfusion cultivation in the bioreactor. The beneficial effect of PF-68 on HEK293 cell growth and Ad-HER

production was then confirmed in shake flasks. Finally, PF-68 was added into the chemically defined medium, reduced shear force generated by the PATFP system, and yielded comparable Ad-HER titer with that by the ATF2 system.

Materials and methods

Culture medium, cell line, and virus strain

The ready-to-use HEK293 chemically defined medium (HEKCD medium, TBD science, Cat. No. HEKCD01, China) with initial 6 g/L glucose, 3 mM glutamine, 3 mM L-alanine-L-glutamine, 1.5 g/L PF-68 is a customized medium developed by our laboratory. Some experiments add extra PF-68 (Gibco, Cat. No. 24040032, USA) into HEKCD medium. The HEK 293 cell line (Invitrogen, USA), adapted to suspension and serum-free culture, is pre-cultured in baffled shake flasks with HEKCD medium at 37 °C, 5% CO_2 atmosphere and 120 rpm. The viral stock, with concentration of 1.1×10^9 IFU/mL, used for infection is a recombinant adenovirus containing glycoprotein E of herpes zoster (Ad-HER) constructed using the Ad.MAX™ system (SignaGen Laboratories Inc., USA).

Cell culture in shake flasks

Cells are cultured with an initial cell density of 3×10^5 cells/mL and a 50 mL working volume in 250 mL baffled shake flasks (Corning, USA). The pseudo-perfusion process, manually renew medium daily, is conducted in shake flasks after day 4 to mimic perfusion process in bioreactor. The culture is transferred into 50 mL centrifugal tube and pelleted by centrifugation at 800×*g*, 5 min, room temperature. Then different amounts of cell-free supernatant are discarded and replaced with an equal volume of warm fresh medium to resuspend cells: 50% for 0.5 reactor volume per day (RV/day), 100% for 1 RV/day, and 100% and twice a day for 2 RV/day. Samples were taken every 12 h or 24 h.

Optimization of Ad-HER production with the DoE approach

Three key process parameters for Ad-HER production process, cell density at the time of infection (TOI), multiplicity of infection (MOI), and virus production pH (pH), are selected as the parameters in the DoE approach (Table 1). The experimental matrix is generated by MODDE software (MKS Data Analytics Solutions, Sweden). Virus titer and virus input–output ratio (IOR) during 3 days post infection (dpi) are used as the responses (Table 2). The cells were cultured in shake flasks with pseudo-perfusion process to obtain the cell density needed for infection. The perfusion operation was started at day 4 with a perfusion rate of 0.5 RV/day, then changed to 2 RV/day at day 6. Once cells were infected, a perfusion rate of 2 RV/day was used.

Cultivations in bioreactor with perfusion systems

In this study, the 5 L benchtop bioreactor (Applikon Biotechnology, Holland) with 2 L working volume is used for Ad-HER production. The culture conditions were set as: 37 °C, 120 rpm, pO_2 of 50% air-saturation, pH 7.2. A novel peristaltic pump driving PATFP perfusion system (made by our laboratory) and the low shear force ATF2 perfusion system (Repligen, USA) are used for cell retention and medium exchange. In both systems, the cell separation module is a hollow fiber filter (Repligen, USA) with inner diameter 1 mm, pore size 0.2 μm and filter area 0.13 m^2.

Cell and metabolites analysis

Cell density and viability are determined by using the trypan blue exclusion method. The average cell diameter is measured with the cell counter Vi-CELL BLU (Beckman Coulter, USA), using a previously validated measuring program for HEK 293 cells. The culture samples are centrifugated at 800×*g*, 2 min to

Table 1 Factors and responses evaluated in DoE for Ad-HER production process

Parameters	Unit	Abbreviations	Multilevel
TOI	10^6 cells/mL	TOI	2, 5, 7, 10
MOI	–	MOI	1, 5, 10, 15
Virus production pH	–	pH	6.9, 7.2
Titer	IFU/mL	Titer	–
Virus input–output ratio	–	IOR	–

Table 2 Experimental matrix and data of the DoE approach

No	MOI	TOI	pH	Titer (IFU/mL)			Virus input–output ratio		
				1 dpi	2 dpi	3 dpi	1 dpi	2 dpi	3 dpi
1	1	2	6.9	1.5E+08	1.5E+09	4.4E+09	75	754	2211
2	5	2	7.2	6.6E+07	1.9E+09	4.4E+09	6	179	415
3	10	2	7.2	1.3E+08	2.4E+09	4.6E+09	6	115	220
4	15	2	6.9	7.0E+08	3.7E+09	2.5E+09	22	117	79
5	1	5	7.2	1.6E+07	3.0E+09	6.4E+09	4	687	1465
6	5	5	7.2	5.1E+07	6.5E+09	6.2E+09	2	287	274
7	10	5	6.9	1.5E+09	5.8E+09	4.4E+09	35	136	103
8	15	7	7.2	5.0E+08	1.4E+10	6.2E+09	5	139	61
9	1	7	7.2	5.8E+07	4.5E+09	9.3E+09	8	643	1329
10	5	7	6.9	2.2E+09	5.0E+09	3.4E+09	57	130	89
11	10	10	7.2	5.1E+08	9.8E+09	7.1E+09	5	100	72
12	15	10	6.9	3.5E+09	5.0E+09	2.0E+09	21	30	12
13	1	10	6.9	7.9E+08	4.2E+09	3.3E+09	72	384	301
14	15	7	7.2	6.6E+08	1.5E+10	9.9E+09	6	132	87
15	15	10	6.9	3.1E+09	5.4E+09	1.9E+09	18	32	11
16	10	2	7.2	1.4E+08	3.4E+09	6.0E+09	6	148	261

remove the pellets. Concentrations of glucose, glutamine, lactate, ammonium and lactate dehydrogenase (LDH) in supernatants are measured by Cedex Bio analyzer (Roche Diagnostics, Switzerland). The virus samples were collected and stored at − 80 °C until measurement. The titer (infectious units, IFUs) of Ad-HER are detected by a QuickTiter™ Adenovirus Titer Immunoassay Kit (Cell BioLabs, USA).

The cell-specific rate (q) of glucose consumption, lactate production and ammonium production are calculated by the following equations:

in batch cultivation phase (before perfusion),

$$q_s = \frac{\mathrm{c}_1 - c_2}{t_2 - t_1} \cdot \frac{2}{X_1 + X_2} \tag{1}$$

$$q_\mathrm{p} = \frac{\mathrm{c}_2 - c_1}{t_2 - t_1} \cdot \frac{2}{X_1 + X_2} \tag{2}$$

and in perfusion phase,

$$\mathrm{q}_s = \left[\frac{Q}{V} \cdot \left(c_s - c\right) - \frac{dc}{dt}\right] \cdot \frac{1}{X} \tag{3}$$

$$\mathrm{q}_\mathrm{p} = \left[\frac{dc}{dt} - \frac{Q}{V}\left(c_s - c\right)\right] \cdot \frac{1}{X} \tag{4}$$

where X is viable cell density, the subscript (1, 2) represents sample point; the subscript S represents substrate (glucose or glutamine); the subscript P represents products of metabolism (lactate or ammonium); t is cultivation time; Q is perfusion rate; V is working volume; c_s is the concentration of a substrate or a product of metabolism in the perfusion medium; c is the concentration of a substrates or a product in the bioreactor.

Results

Cell culture in shake flasks with pseudo-perfusion

In this study, the pseudo-perfusion strategies were conducted in shake flasks, which provides data to optimize Ad-HER production processes in bioreactor with perfusion mode (Fig. 1). As shown in Fig. 1a, the cell batch mode yielded highest cell density of 2.6×10^6 cells/mL at day 7, and cell growth reached stationary phase at ~ day 5. By comparison, the pseudo-perfusion strategies (starting at day 4 with 0.5, 1 and 2 RV/day, respectively) yielded higher cell densities than the batch mode, reaching over 7.9×10^6 cells/mL at day 10. All three pseudo-perfusion strategies resulted in similar cell densities throughout the whole cultivation process, with 2 RV/

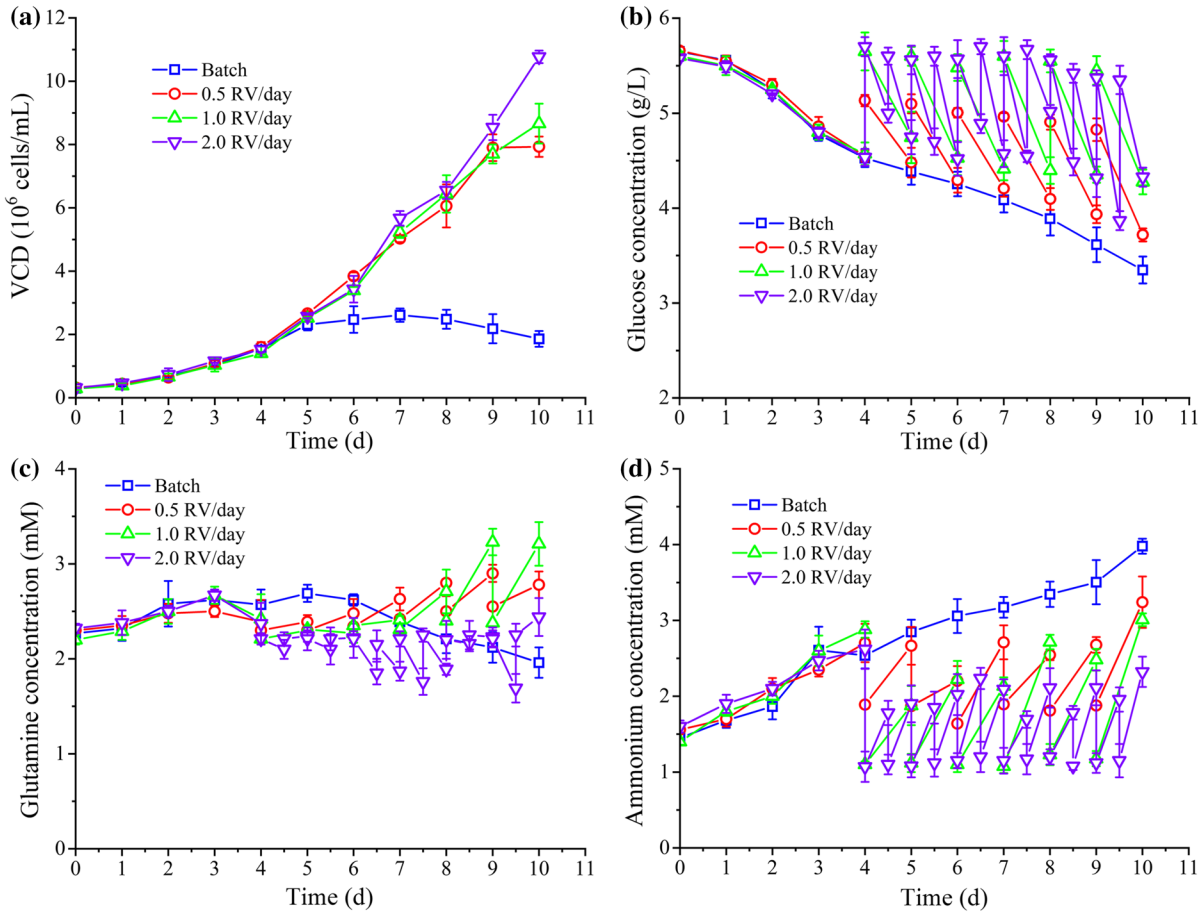

Fig. 1 Cell growth curves (**a**) and metabolite profiles of **b** glucose, **c** glutamine, **d** ammonium of HEK293 cells in shake flask with different pseudo-perfusion strategies. Experiments were run in triplicate and error bars represent standard deviations

day slightly better. The metabolite profiles are shown in Fig. 1b–d. Glucose remained higher than 3 g/L during the whole cultivation process for all strategies (Fig. 1b). L-glutamine as the nitrogen source is also provided from the hydrolysis of L-alanine-L-glutamine dipeptide by the peptidases secreted from cells. The glutamine was measured by a Cedex Bio analyzer equipped with the reagent kit for glutamine detection (reagent for L-alanine-L-glutamine dipeptide detection was unavailable). Thus, the glutamine gradually increased during the first 3 days, then in the late cultivation stage slightly decreased for batch mode and fluctuated for the pseudo-perfusion strategies, but remained above 1.5 mM for all groups (Fig. 1c). Lactate level maintained low for all groups, possibly due to low consumption of glucose and efficient aeration (data not shown). Ammonium maintained below 3.0 mM for all pseudo-perfusion strategies, while it accumulated steadily and reached ~ 4 mM at the end of cultivation for the batch mode. In general, use of pseudo-perfusion strategies resulted in significantly higher cell densities than the batch mode, possibly owing to supplementing fresh nutrients and removing metabolic byproducts. Based on this, the perfusion mode selected for the following perfusion studies is set as: perfusion starting at day 4 after inoculation, a perfusion rate of 0.5 RV/day for 2 days, then 2 RV/day thereafter.

Model analysis and design space explore for Ad-HER production

The reduced combinatorial design recommended by MODDE software was used to investigate the

quantitative relationship between key process parameters (TOI, MOI and virus production pH) and critical quality attributes (Ad-HER titer and virus input–output ratio). The Ad-HER titer and virus input–output ratio within 3 dpi of each run were collected as the responses (Table 2). There is a trend that Ad-HER titer decreases from 2 to 3 dpi at higher MOI (MOI = 10 or 15), while Ad-HER titer increases from 2 to 3 dpi at lower MOI. Considering the adenovirus replication cycle [volumetric virus productivity is maximum at 2 dpi (Altaras et al. 2005)] and relative higher titer at 2 dpi in Table 2, we built up the regression models of titer and IOR at 2 dpi in response to TOI, MOI and virus production pH, and statistics indicates the models well fit the experimental data ($R^2 = 0.962$ (adjusted $R^2 = 0.929$) and $Q^2 = 0.860$ for titer (Fig. 2a), and $R^2 = 0.988$ (adjusted $R^2 = 0.976$) and $Q^2 = 0.906$ for IOR (Fig. 2b), respectively). As shown in the coefficient plot (Fig. 2c, d), all variables evaluated are significant model terms ($P < 0.05$). Within the range of parameters tested, TOI and MOI have positive effects on titer and negative effects on IOR, respectively, and virus production pH has positive effect on both responses.

Robust setpoint and design space of three variables were explored based on the regression model (Table 3) and acceptable criteria. Low virus input–output ratio increases the need of virus seeds. In this study, virus input–output ratio higher than 180 and Ad-HER titer higher than 5×10^9 IFU/mL were set as criteria. Based on this, the robust setpoint was calculated by MODDE software as TOI of 7.3×10^6 cells/mL, MOI of 3.8 and virus production

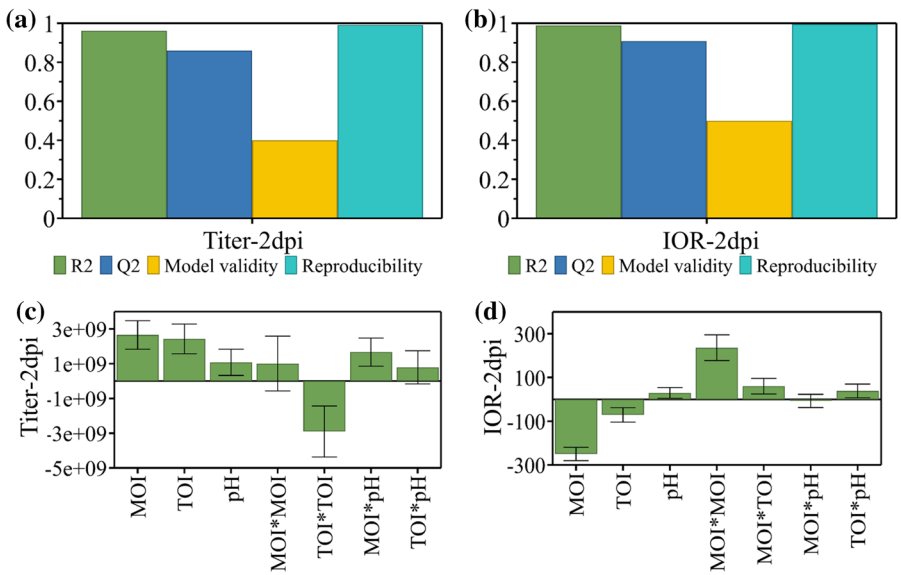

Fig. 2 Analysis of regression models at 2 dpi. Summary of model fit for Ad-HER titer (**a**) and virus input–output ratio (**b**). The coefficients (scaled and centered) for Ad-HER titer (**c**) and virus input–output ratio (**d**)

Table 3 Regression models for the Ad-HER production

Response	Equations of the responses	Adjusted R^2	Lack of fit
Titer (IFU/mL)	$9.0 \times 10^{10} - 1.1 \times 10^{10} \times \text{MOI} - 6.6 \times 10^9 \times \text{TOI} - 1.3 \times 10^{10} \times \text{pH} + 2.0 \times 10^7 \times \text{MOI} \times \text{MOI} - 1.8 \times 10^8 \times \text{TOI} \times \text{TOI} + 1.6 \times 10^9 \times \text{MOI} \times \text{pH} + 1.3 \times 10^9 \times \text{TOI} \times \text{pH}$	0.929	0.091
IOR	$1874.9 - 77.1 \times \text{MOI} - 485.3 \times \text{TOI} - 133.9 \times \text{pH} + 4.8 \times \text{MOI} \times \text{MOI} + 2.2 \times \text{MOI} \times \text{TOI} - 6.9 \times \text{MOI} \times \text{pH} + 63.9 \times \text{TOI} \times \text{pH}$	0.976	0.136

pH of 7.1. The design space hypercube, with Probability of model failure < 5%, was TOI from 6.8 to 8.4×10^6 cells/mL, MOI from 2.9 to 4.7, and virus production pH from 7.04 to 7.18.

Effect of PF-68 on cell growth and Ad-HER production

The pseudo-perfusion process optimized in shake flasks was scaled up into bioreactors with ATF2 system and PATFP system (Fig. 3). Both systems could achieve alternating tangential flow in hollow fiber membrane surface, with ATF2 system driven by peristaltic pump and PATFP system driven by diaphragm pump. Use of the ATF2 system yielded viable cell density of 7.4×10^6 cells/mL at 182 h and a decrease of cell density after infection (Fig. 3a). In contrast, use of the PATFP system resulted in decrease of cell viability upon initiation of perfusion (at day 3.5), and cell density started dropping after day 5 (Fig. 3b). We hypothesize such dramatically different modes of growth in two perfusion systems would be caused by the intense shear stress generated in PATFP system (Wang et al. 2017).

In order to verify the above hypothesis and reduce shear stress caused by the peristaltic pump of PATFP system, the shear protectant PF-68 was supplemented into chemically defined HEKCD medium. We first tested its effect on cell growth and Ad-HER production in shake flasks. The HEKCD medium was supplemented with PF-68 to the final concentration of 2.0 g/L (HEKCD + 0.5 g/L PF-68) and 3.0 g/L (HEKCD + 1.5 g/L PF-68) respectively. The cells were cultured with pseudo-perfusion operation to yield viable cell density of ~ 7.3×10^6 cells/mL and then infected with Ad-HER with MOI of 3.8. The experiment was run in duplicate. As shown in Fig. 4, during the cell growth stage, the addition of PF-68 had no effect on cell growth. However, a slight decrease of viable cell density from 4.63×10^6 cells/mL to 3.77×10^6 cells/mL was observed at 2 dpi when PF-68 at final concentration higher than 1.5 g/L. The PF-68 at a final concentration of 3.0 g/L showed no effect on cell viability during the whole process. The HEKCD medium with final PF-68 concentration of 1.5 g/L and 3.0 g/L yielded Ad-HER titer of $5.3 \pm 0.1 \times 10^9$ IFU/mL and $5.2 \pm 0.2 \times 10^9$ IFU/mL respectively, indicating the 3.0 g/L PF-68 had no significant effect on Ad-HER titer.

The perfusion process in bioreactor with 3.0 g/L PF-68

Finally, chemically defined HEKCD medium supplemented with shear protectant PF-68 final concentration of 3.0 g/L was used to produce Ad-HEK in bioreactor with PATFP system (Fig. 5). Indeed, addition of PF-68 restored cell growth after day 5. VCD in PATFP system reached to similar value of the ATF2 system (7.2×10^6 cells/mL), although additional 3 days were needed for cells to grow (Fig. 3a). Measurements of the metabolites showed glucose, glutamine, lactate and ammonium remained > 3.6 g /L, > 2.3 mM, < 1.8 g/L and < 2.0 mM, respectively,

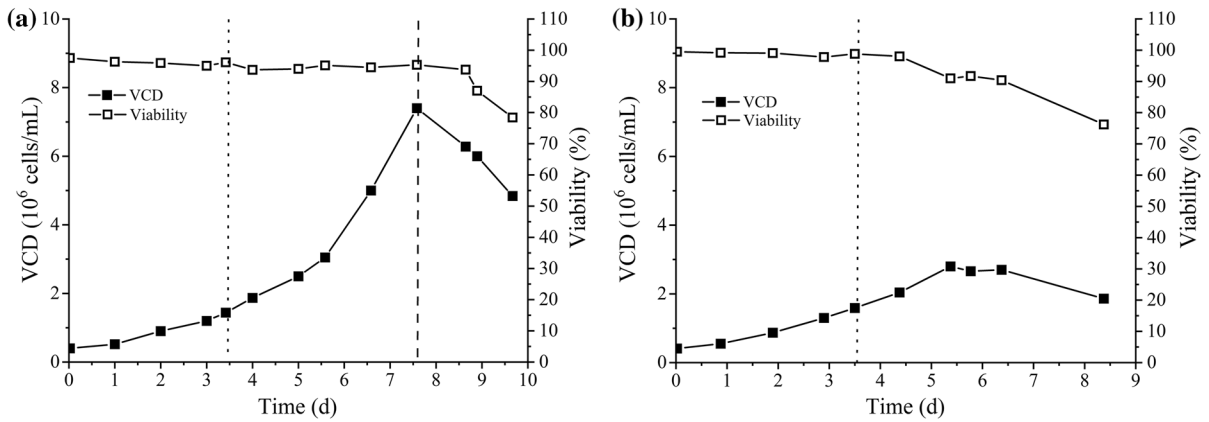

Fig. 3 Cell growth curves of HEK293 cells in bioreactors with ATF2 system (**a**) and PATFP system (**b**). Dot line indicates starting time of perfusion and dash line indicates time of infection

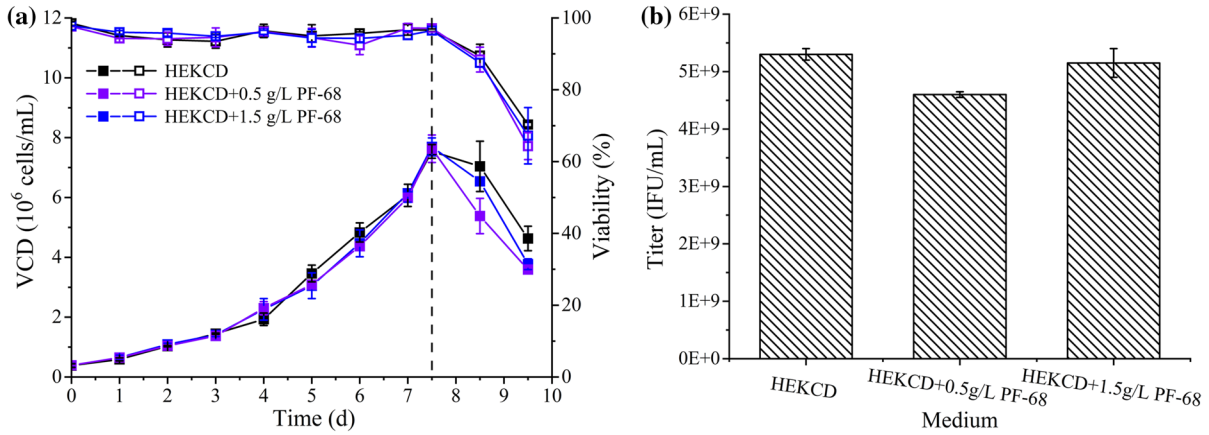

Fig. 4 Effect of PF-68 on HEK293 cells growth (**a**) and Ad-HER production (**b**) in shake flask. Dash line indicates time of infection. Experiments were run in triplicate and error bars represent standard deviations

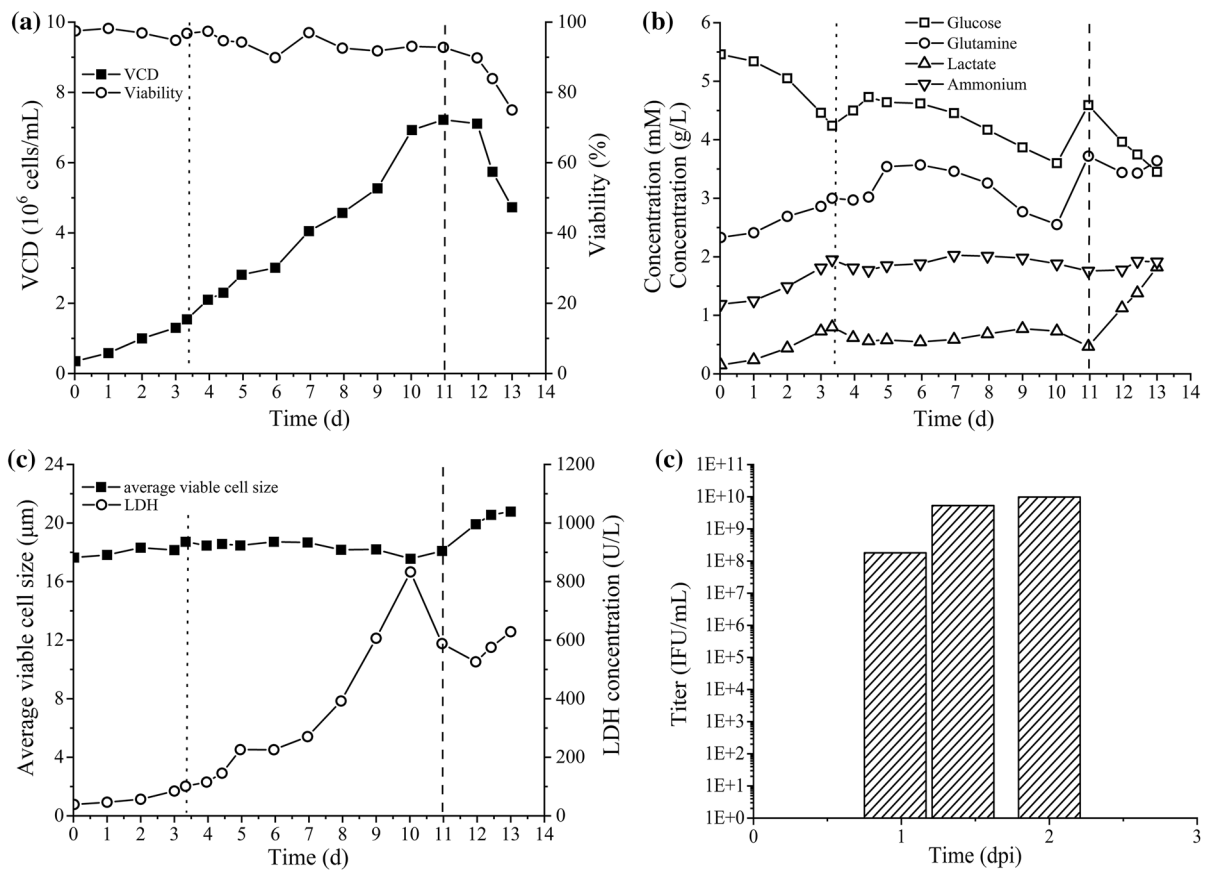

Fig. 5 Effect of PF-68 at concentration of 3.0 g/L on HEK293 cells growth and Ad-HER production in bioreactor with PATFP system. Cell growth curve (**a**), metabolite profiles (**b**), cell size (**c**) and Ad-HER production (**d**). Dot line indicates starting time of perfusion and dash line indicates time of infection

indicating sufficient main nutrition supply and minimal byproducts accumulation during the whole process (Fig. 5b). After infection, specific glucose uptake rate (q_{glu}) and specific lactate production rate (q_{lac}) increased from 1.2–1.8 to 3.3–5.6 pmol/cell/day and from 1.0–1.4 to 3.7–8.4 pmol/cell/day, respectively, implying more glucose requirement for viral replication. Compared with cell cultivation phase, the higher molar ratios of $Y_{lac/glc}$ (increase from 0.7–0.8 to 1.1–1.5) during the virus production process may indicated the increase of oxygen consumption after infection which resulted in anaerobic respiration. The use of L-alanyl-L-glutamine dipeptide in HEKCD medium made it difficult to calculate the amount of glutamine consumed and the molar ratios of $Y_{amm/gln}$. The increase of the cell specific ammonium product rate (q_{amm}) was observed during the infection phase with relative stable ammonium concentration and decrease of viable cell density.

We measured the average viable cell size for this process (Fig. 5c). Cell size remained stable during the growth phase (17.64–18.72 µm) and increased after viral infection, reaching 20.78 µm at 2 dpi, which was similar with that in ATF2 system (growth phase: 17.48–18.91 µm, 20.62 µm at 2 dpi). We also measured extracellular LDH activity as an indicator of cell lysis. LDH activity in the bioreactor with ATF2 system was lower than 400 U/L before infection. However, LDH activity in the culture increased sharply when perfusion started, implying cell lysis caused by shear force of PATFP system. Interestingly, it decreased during 240–264 h (before viral infection), which could be due to switch of perfusion rate from 0.5 RV/day to 2.0 RV/day. Finally, Ad-HER titer reached 9.8×10^9 IFU/mL at 2 dpi in this process (Fig. 5d), close to that using the ATF2 system (1.2×10^{10} IFU/mL), once again confirming the effect of addition of PF-68 in chemically defined medium to alleviate cell lysis in the PATFP system.

Discussion

Several reports indicate the herpes zoster vaccine is effective in preventing herpes zoster and decrease incidence of complications (Harpaz et al. 2008; Adams et al. 2010). Up to now, only two main commercially-available zoster vaccines, ZOSTAVAX® (a live attenuated virus vaccine from Merck & Co., Inc.) and Shingrix® (a recombinant zoster vaccine from GlaxoSmithKline), are recommended by the Advisory Committee on Immunization Practices (ACIP) (Harpaz et al. 2008; Schmader 2018). Since the adenovirus induces both humoral and cellular immune responses in mammalian hosts (Tatsis and Ertl 2004), the recombinant adenovirus expressing herpes zoster antigens has potential to be developed as another promising vaccine.

Use of mL-scale shake flask or tubespin tube (TPP, Switzerland) with a pseudo-perfusion process simulates the real perfusion process in bioreactor, so that process parameters can be optimized in a high through-put manner (Vazquez-Ramirez et al. 2018; Bissinger et al. 2019; Wolf et al. 2019). Several process parameters, such as TOI, MOI and pH, have been optimized to improve the adenovirus titer (Nadeau and Kamen 2003; Kovesdi and Hedley 2010). In this study, we built up the regression models to represent the quantitative relationship between adenovirus titer and the three process parameters by the DoE approach, an excellent tool has been utilized in our previous study for process optimization (Nie et al. 2020). As a part of quality by design (QbD) principle, the DoE approach enables identification of the robust design space of the production process parameters, which actually has been applied in the production of recombinant protein and virus vaccine (Kim et al. 2019; Nie et al. 2020).

The peristaltic pump is an optional circulatory system for perfusion due to its simplicity and scalability (Clincke et al. 2013). Though the PATFP system has obvious higher shear force, it also has advantages over the commercially-available Xcell ATF system by Repligen company system. For example, the vacuum capacity of ATF system usually allows to pull medium with cell density $< 1.3\times10^8$ cells/mL), while thus the peristaltic pump in PATFP system enables to pull medium with high cell densities up to 2×10^8 cells/mL (Clincke et al. 2013). However, compared with ATF system equipped with low shear force patent bulb, the peristaltic pump used for recirculation of cell-containing medium is the major source of shear force, which usually leads to increased cell particles (Wang et al. 2017; Pinto et al. 2020) and reduced lifetime of the hollow fiber membrane. Thus, pump tubing with large inner diameter and low rotation speed are required to operate circulation with a peristaltic pump (Wang et al. 2017).

Replacement of serum-containing medium with serum-free medium could avoid the issues of virus contamination and batch-to-batch variation (van der Valk et al. 2010). Therefore, serum-free media without any animal-derived components have been widely utilized in virus production biotechnology (van der Valk et al. 2010; Nie et al. 2020). Some serum-free media contain soybean protein hydrolysates to supply additional nutrients (such as amino acids, oligopeptides, lipids, and trace elements) to enhance cell growth and productivity (Sung et al. 2004; Chun et al. 2007). However, our study indicates serum-free medium containing plant extracts also raise the variation issue, which results in instability of the cell growth and Ad-HER production (unpublished data). The chemically defined medium could be the alternative solution to avoid all issues above, although it results in impaired cell growth and adenovirus titer (Shen et al. 2016). Moreover, cells cultured in chemically defined medium are more sensitive to shear force (Ritacco et al. 2018) and usually resulted in a decrease in cell growth and volumetric productivity (Shen et al. 2016).

PF-68 is a non-ionic surfactant, widely used as a supplementary of serum-free medium to protect cells from shear stress generated by agitation and gas sparging (Gigout et al. 2008). PF-68 of 1 g/L in culture medium usually provides sufficient protection from shear damage (Xie et al. 2003), however, at some situations it is necessary to increase concentration to 2–5 g/L to reduce cell damage and increase cell viability (Xu et al. 2017). Our data also demonstrates addition of PF-68 restored HEK293 cell growth upon perfusion (Fig. 5), probably owing to its protective effect against shear force generated by peristaltic pump. PF-68 at final concentration of 3 g/L had no negative effect on HEK293 cells growth and Ad-HER production.

Through addition of PF-68 in chemically defined medium, high VCD of 7.2×10^6 cells/mL and Ad-HER titer of 9.8×10^9 IFU/mL were obtained in PATFP system, comparable to the values in ATF2 system, although extra 3 days are required (Fig. 3a). However, PF-68 could offer limited protection on HEK293 cells from lysis, implied from the continuous increased extracellular LDH activity (Fig. 5c). To further reduce cell lysis caused by shear force and hence increase cell growth and virus productivity, the low shear Levitronix® pump (Zurich, Switzerland) driven tangential flow filtration (TFF) system (Wang et al. 2017) could be introduced to replace the peristaltic pump for perfusion cultivation strategy. However, the membrane flux of a TFF system with this pump should be a concern due to the unidirectional flow in membrane surface.

Due to the lack of direct quantitative assays for infectious adenovirus, we measured metabolites and cell morphological parameters post virus infection (Fig. 5). Cell viability decreased upon infection and reached to 75% at 2 dpi. Average cell diameter increased by 2–3 mm within 2 d and reached 20.78 µm at 2 dpi. Glucose consumption, glutamine consumption and lactate accumulation by cells were also increased after infection. These parameters could be used as indicators of adenovirus replication in HEK293 cells. Similarly, increased glucose consumption and cell size during the infection phase were reported in another study (Nadeau and Kamen 2003).

Conclusion

In summary, an adenovirus-based zoster vaccine production process with chemically defined medium and perfusion cultivation strategy was developed efficiently, aided with the DoE approach. In shake flasks, three key process parameters (TOI, MOI, the virus production pH) were optimized and the effect of PF-68 on HEK293 cell growth and Ad-HER production was investigated. We demonstrated addition of PF-68 3 g/L in chemically defined medium reduced the cell lysis in PATFP system and yielded Ad-HER titer of 9.8×10^9 IFU/mL, comparable to that of ATF2 system. This approach described in this study could be used to develop a roust process with stable cell culture performance and adenovirus titer.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by JN, YS and HR. The first draft of the manuscript was written by JN and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Research involving human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

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