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Optimization of suspension culture of adapted baby hamster kidney cells in a bioreactor for maximum cell density

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ABSTRACT: The baby hamster kidney-21 (BHK-21) cell line is a continuous anchorage-dependent cell line. Suspension culture technology enables rapid growth of BHK-21 cells, leading to large-scale and cost-effective production of vaccines. This study aimed to optimize suspension culture of BHK-21 cells to achieve the maximum cell density in a bioreactor. BHK-21 cells were optimized for certain physicochemical factors like seeding ratio, pH, agitation speed, serum concentration, medium composition, and temperature in a bioreactor. Here, this study reports the successful adaptation of adherent BHK-21 cells to grow in suspension to a viable cell density of 7.5×10^6 cells/ml on the 3rd day in self-formulated cell culture medium at pH 7.2 and 37 °C, agitation speed of 50 rpm, seeding density of 4×10^5 cells/ml, and 10% serum concentration in a 5-l bioreactor. The BHK-21 cell suspension culture was expanded to a 5-l bioreactor from a 20-ml flask, which resulted in the maximum cell density. The suspension culture technology improved the maximum cell density of BHK-21 cells in the bioreactor, indicating its potential for foot and mouth disease (FMD) vaccine production.

KEYWORDS: adherent cell culture, baby hamster kidney cell line, bioreactor, foot and mouth disease virus, suspension culture

INTRODUCTION

Suspension culture in bioreactors is a novel and increasingly used process in bio-pharmaceutical product production. Efficiently optimizing the physicochemical aspects of suspension culture in bioreactors is essential to attain the highest possible cell density for specific cell lines such as the baby hamster kidney cell line (BHK-21). Optimization involves a meticulous equilibrium of critical parameters such as medium composition (nutrient supply), pH maintenance, agitation intensity, and temperature control to produce a feasible environment for robust cell proliferation and growth. Researchers can maximize cell density and viability by changing and optimizing these parameters [1]. The BHK-21 cell line was established in 1962 [2]. Later, the cell line was adapted to suspension culture [3]. Telling and Elsworth used the suspension culture-adapted cells to produce foot and mouth disease (FMD) vaccine in a bioreactor [4]. The BHK-21 cells have various applications, mainly in viral veterinary vaccines like FMD vaccine. Suspension cell culture systems have been explored for this cell line when the bio-process scale-up is desired because of adherent culture limitations on large-scale. Suspension culture can be used to overcome this drawback. This will lead to higher cell densities and concomitant volumetric productivity, which could also be achieved, making the scalability of the manufacturing process easier. Suspension culture systems using bioreactors are the most convenient and straightforward to maintain [5]. The optimization of suspension culture in bioreactors leads to high volumetric productivity due to higher viable cell densities (MVCDs) as compared with the adherent cells. Adapting cells from adherent culture to the suspension culture can be performed using a simple strategy: adherent culture cells to the roller bottles, then to the spinner flasks, and ultimately in bioreactors. In bioreactors, adherent culture-adapted cells are converted to suspension culture-adapted cells by interrupting cell-surface protein signaling, like loss of integrin signaling, which occurs after removing cell-adhesive surface proteins [1].

In BHK-21 suspension culture-adapted cells, cell growth and density monitoring are crucial as the first signs of successful adaptation [6]. Feeding media and serum concentrations were added to increase cell density and growth of BHK-21 cells [7]. Seeding density and quality are critical for establishing an efficient bioconversion process and generating large volumes of viable cells in the shortest time. Bovine serum is enriched by biological factors like growth factors, supplemental metabolites, cytokines, hormones, and transport proteins [8]. Cell culture medium is crucial in optimizing suspension culture and achieving the maximum cell density. Nutrients in the cell culture medium utilized and supplementation with carbon source become necessary to sustain cellular metabolism and increase cell density in lesser volumes. In suspension culture, a large volume of cell culture medium is used in a bioreactor [9]. The culture media requirements for suspension culture are distinct from those of adherent BHK-21 cell culture. Dulbecco's modified eagle medium (DMEM) and the Glasgow minimum essential medium

(GMEM) are 2 commonly used media in BHK-21 cell suspension culture. Additional components such as fetal bovine serum are frequently added to enhance cell development and maintenance. DMEM and GMEM are commonly used together as a cell culture medium for BHK-21 cell culture [10]. Self-formulated media are also used [11]. Other factors like temperature, pH, and agitation optimization are vital in maintaining and growing BHK-21 cells in suspension culture. All these sets of physiological factors help in the optimum growth of BHK-21 cells and achieve high cell density in a shorter period of incubation time. Calculation of doubling time and generation number at the highest cell density is used to confirm the optimum cell density of BHK-21 cells adapted in suspension culture. Specific growth rate (/h) is the most important indicator of successful adaptation of cells in suspension culture [1].

There are many studies about the BHK-21 cell metabolism in bioreactors [12–16]. Still, research about optimizing physicochemical factors for BHK-21 cells in suspension cell culture in bioreactors has not been conducted using this set of parameters. In this study, we developed suspension culture-adapted BHK-21 cells capable of growth to a high cell density in a bioreactor. In this suspension, the culture was scaled up to 5 l in the bioreactor. Suspension culture technology for FMD vaccine in bioreactors is new in Pakistan, and this technology has been adopted by the University of Veterinary and Animal Sciences (UVAS) in Lahore. So, optimizing physical and chemical factors for suspension culture in a bioreactor will lead to the maximum cell density in less incubation time and to efficient FMD vaccine production.

MATERIALS AND METHODS

Establishment of BHK-21 cell line

The cryopreserved BHK-21 cells from the Quality Operations Laboratory (QOL), UVAS, Lahore, Pakistan, were revived by placing the vial having cells in a water bath at 37 °C for 5–10 min. Restored liquefied cells were centrifuged at 1,500 rpm for 2 min. Pre-warmed DMEM (Cassian, USA) was added to the vials and centrifuged at 1,500 rpm for 2 min. The BHK-21 cell pellet was collected, and the cell life-to-dead ratio was determined using trypan blue dye exclusion method and a hemocytometer. After calculation of live-to-dead ratio, cells were transferred to a cell culture flask with DMEM and 10% fetal calf serum (FCS; Capricorn Scientific, USA). The seeding ratio was 1–2×10^5 cells/ml, and cells were incubated at 37 °C for 24–48 h [17]. After incubation, cell density, percentage viability, and monolayer development were calculated.

Optimization of suspension culture

Optimization of suspension culture in the bioreactor was done using a 3-step process: adaptation of adherent BHK-21 cell line in the roller cell culture bottles, then in the Spinner cell culture flask, and finally in the bioreactor. This 3-step process helps in the gradual adaptation from adherent to suspension culture and helps ensure the cells can thrive in the more complex bioreactor conditions. This multistep approach allows the cells to acclimate to the changing hydrodynamic environment, resulting in successfully transitioning the BHK-21 cell line to high-density suspension culture suitable for large-scale bioproduction in the bioreactor.

Adaptation of adherent BHK-21 cell line in roller bottles

The adherent BHK-21 cell line was adapted to suspension culture by transferring adherent cells to the Roller cell culture bottles. In this dynamic environment, adherent cells were shifted from the adherent to the suspension phase. For this purpose, cells from the adherent culture were transferred to roller bottles. Firstly, their live-to-dead ratio was determined using trypan blue dye exclusion method. Cells were counted in large boxes of the hemocytometer chamber. Live cells were colorless, and dead cells were stained blue [17, 18].

Number of cells per ml = Average number of cells in a square × dilution factor × correction factor.

Enumerated cells were inoculated in roller bottles with DMEM, placed in the incu-drive (incu-drive; Hybridizer HB-1D by TECHNE, UK), and incubated at 37 °C for 24 h at 25 rpm.

Adaptation of adherent BHK-21 cell line in spinner flasks

The roller bottle-adapted adherent BHK-21 cell line was transferred to the spinner flask. It acted as a transitioning medium for BHK-21 cells. In this process, the availability of a large surface area aids in increased cell density. Adherent culture BHK-21 cells were enumerated using trypan blue and hemocytometer. A seeding ratio of 1–2×10^5 cells/ml was used for inoculation. Spinner flasks were incubated at 37 °C in a controlled environment. The spinner flask speed was set to a level as low as 25 rpm. This speed promoted the gentle mixing of cells and prevented the settling of cells [19].

Optimization of suspension culture

A benchtop bioreactor (Brunswick BioFlo/CelliGen 115; Eppendorf, USA), 14 l total volume capacity with 5 l of working volume, was used. Spinner flask adapter cells were used to maximize suspension culture in bioreactors. For this purpose, one condition was varied at a time, and all other parameters of adherent culture were kept constant. Conditions like temperature, pH, agitation speed, seeding density, and serum concentration (37 °C, 7.2, 50 rpm, 4×10^5 cells/ml, and 10%, respectively) were kept constant, and medium composition was varied for the optimization of cell culture medium composition. Factors like seeding ratio, pH

(6.8, 7.2, 7.5, and 7.8), agitation speed (50, 100, 150, and 200 rpm), serum concentration (7, 10, and 12%), medium composition (DMEM, GMEM, GMEM and DMEM, and self-formulated medium), and temperature (35, 37, and 39 °C) were optimized [1, 20].

Time course of suspension-culture adapted cell growth in the optimized conditions

Time course of suspension culture-adapted cell growth in the optimized conditions was confirmed by calculating cell number, doubling time, generation number, and volumetric productivity of suspension culture-adapted cells in a bioreactor after 0, 24, 48, and 72 h [21–23]. Formulae for percentage viability, doubling time, and generation numbers were as follows.

$$\text{Percentage viability} = \frac{\text{Number of viable cells}}{\text{Total number of cells}} \times 100$$

$$\text{Doubling time} = \frac{\text{Duration of cell culture} \times \log 2}{\log(\text{final cell no.}) - \log(\text{initial cell no.})}$$

$$\text{Generation no.} = \frac{\log(\text{final cell no.}) - \log(\text{initial cell no.})}{\log 2}$$

Statistical analysis

Results of establishment of BHK-21 cell line and effect of incubation time on the percentage viability of the cells were analyzed using CR design, and optimization of physicochemical factors for BHK-21 cells in suspension culture was analyzed by ANOVA and Tukey's pairwise comparison post hoc test with $p = 0.05$ using Minitab 17. All experiments were performed in triplicates [1, 21].

RESULTS

Establishment of the BHK-21 cell line

The cryopreserved BHK-21 cells were revived and counted at 0, 24, 48, and 72 h after incubation. Cells were harvested and counted when approximately 80–90% of the monolayer was formed (Fig. S1A). The total number of cells, total number of viable cells, total number of dead cells, and percentage viability were calculated at different time points (Fig. 1A). Statistical analysis suggested that all incubation time had a considerable impact on the percentage viability of the cells. Cell density at 36 h of incubation has shown significantly high results.

Adaptation of adherent BHK-21 cell line in roller bottles

Cell density and percentage viability of BHK-21 cells in roller cell culture bottles at different incubation times were estimated and statistically analyzed. Adaptation of BHK-21 cells in roller bottles produced a cell density of 6.5×10^6 cells/ml after 72 h of incubation. Results indicated that incubation duration significantly affected the percentage viability of the BHK-21 cells in roller bottles, which increased from 75 to 90% during 0 to 24 h of incubation. The number of cells at different incubation times was significantly higher, and incubation time significantly affected cell density (Fig. 1B and Fig. S1B).

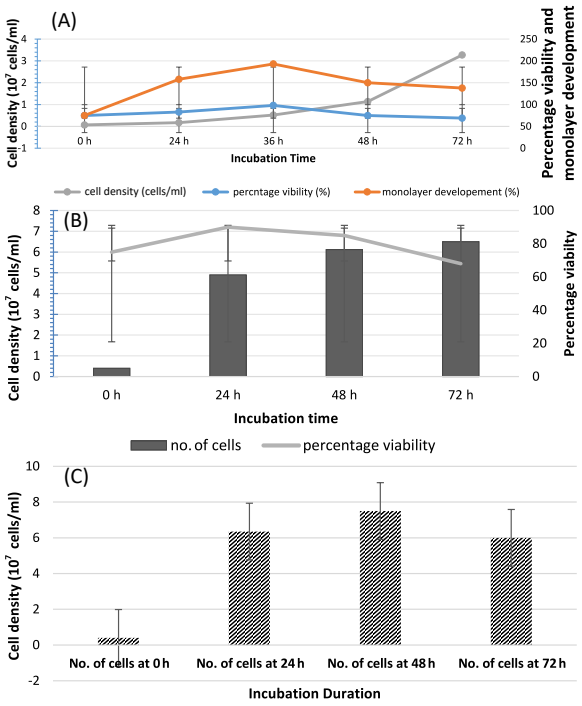

Fig. 1 Effect of incubation time on the growth and adaptation of BHK-21 cells in different cell culture systems. (A) The effect of different incubation times on cell density, percentage viability, and monolayer development in adherent culture, with significant differences at 36 h. (B) The adaptation of BHK-21 cells from adherent dependent to adherence independent growth in the roller bottle culture system with all incubation times, significantly affecting viability and cell density. (C) The cell density of BHK-21 cells at different incubation times in the spinner flask suspension culture where it led to increased growth and cell density of adherence independent cells as compared to the adherent culture.

Adaptation of adherent BHK-21 cell line in spinner flasks

Cell density and percentage viability of BHK-21 cells cultured in spinner flasks were estimated at 24, 48, and 72 h of incubation. Statistical analysis showed that incubation time significantly affected the cell density of BHK-21 cells. The trend of cell density at different incubation times is shown in Fig. 1C and Fig. S1C. The maximum cell density of 7.5×10^6 cells/ml was reported at 48 h of incubation.

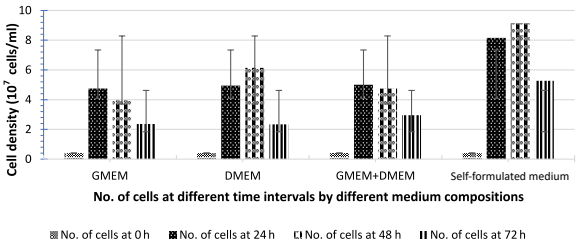

Fig. 2 Effect of different cell culture medium compositions on cell density at different incubation times in a bioreactor. The x-axis represents the incubation times with different combinations of cell culture medium, while the y-axis depicts the cell density. The figure shows the effect of different combinations of cell culture medium on a number of cells in suspension culture-adapted BHK-21 cells. Self-formulated medium has shown a statistically significant effect of the cell density of BHK-21 cells in a bioreactor as compared to the GMEM, DMEM, and DMEM+GMEM.

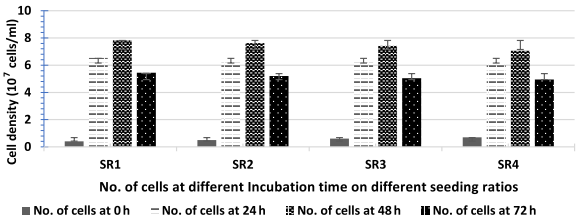

Fig. 3 Effect of different seeding ratios on cell density at different incubation times. The figure shows the effect of different seeding ratios on the suspension culture-adapted BHK-21 cells. The x-axis represents the incubation times with different seeding ratios, while the y-axis depicts the cell density. Data was statistically analyzed which showed that the seeding ratio of 4×10^5 cells/ml significantly affected cell density of BHK-21 cells as compared to the other seeding ratios tested.

Optimization of suspension cell culture medium composition

Cell culture medium composition had a significant effect on achieving desirable cell density. In this study, different cell culture media and their combinations were used, and cell density was estimated at 24, 48, and 72 h at 37 °C, keeping all other conditions the same as the adherent culture conditions. Results showed that self-formulated medium had significantly different results among all medium composition groups, with a cell density of 9.1×10^6 cells/ml at 48 h of incubation. Statistical analysis showed that the composition of self-formulated medium had a significant effect on cell density (Fig. 2).

Seeding ratio

For the optimization of the seeding ratio, seeding densities of 4×10^4, 4×10^5, 4×10^6, and 4×10^7 cells/ml (SR1, SR2, SR3, and SR4, respectively) were adjusted, and cell density was enumerated after 24, 48, and 72 h of incubation at 37 °C, keeping all other conditions the same as the adherent culture conditions. Statistical analysis showed that seeding density of 4×10^5 cells/ml showed significantly different results with the cell density of 7.13×10^6 cells/ml at 48 h of incubation (Fig. 3).

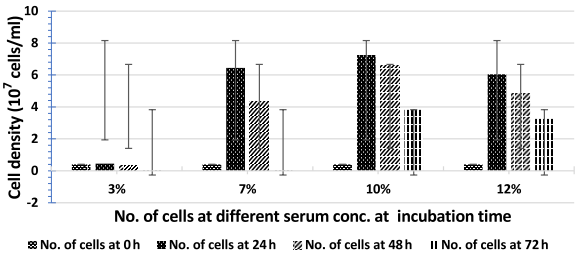

Fig. 4 Effect of different serum concentrations on cell density at different incubation times. The figure shows the effect of different serum concentrations on the suspension culture-adapted BHK-21 cells in a bioreactor. The x-axis represents the incubation times with different serum concentrations, while the y-axis depicts the cell density. Data was statistically analyzed which showed that serum concentration of 10% significantly affected the cell density of BHK-21 cells in a bioreactor as compared to the other serum concentrations tested.

Serum concentration

The following parameter optimized for suspension culture was serum concentration in the cell culture medium. Different concentrations of fetal calf serum (3, 7, 10, and 12%) were used. Cell density was estimated at 24, 48, and 72 h. Results were statistically analyzed and showed that the serum concentration of 10% had a significant effect on cell density, at 48 h of incubation with the highest cell density of 6×10^6 cells/ml (Fig. 4).

Temperature

Temperature was optimized after optimizing medium composition, seeding ratio, and serum concentration. Different temperatures (35, 37, and 39 °C) were used separately. Cell density was calculated at 24, 48, and 72 h of incubation. Statistical analysis showed that the means of cell density of 37 °C were significantly different (Fig. 5). The maximum cell density was 7.15×10^6 cells/ml at 48 h of incubation in the bioreactor.

pH

pH of the suspension culture was optimized to achieve the maximum cell density. Different pH (6.8, 7.2, 7.5, and 7.8) were used, and cell density was calculated at 24, 48, and 72 h of incubation. Analysis has shown that pH 7.2 significantly affected cell density (Fig. 6) with the maximum cell density of 7.5×10^6 cells/ml at 48 h of incubation.

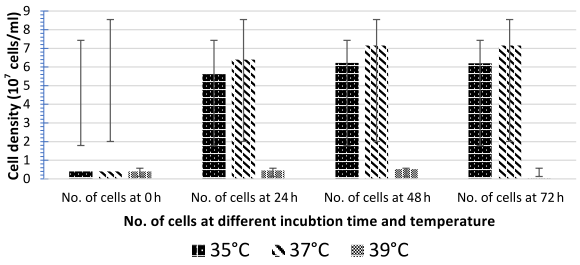

Fig. 5 Effect of different incubation temperatures on cell density at different incubation times. Figure shows the effect of different incubation temperatures on the suspension culture-adapted BHK-21 cells. The x-axis represents the incubation times with different incubation temperatures, while the y-axis depicts the cell density. Data was statistically analyzed which showed that the 37 °C incubation temperature significantly affected the mean cell density of BHK-21 cells in a bioreactor in comparison with the other incubation temperatures.

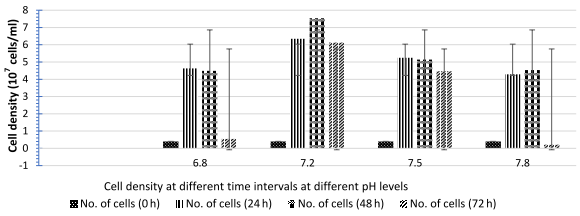

Fig. 6 Effect of different pH levels on cell density at different incubation times. The figure illustrates the effect of different pH levels on a number of cells in suspension culture-adapted BHK-21 cells. The x-axis represents the incubation times with different pH levels, while the y-axis depicts the cell density. Data was statistically analyzed which showed that pH 7.2 significantly affected the cell density of BHK-21 cells in a bioreactor.

Agitation

Optimization of agitation speed in suspension culture plays a vital role in achieving the maximum cell density. Different agitation speeds (50, 100, 150, and 200 rpm) were used, and cell density was calculated at 24, 48, and 72 h of incubation. Results showed that agitation at 50 rpm significantly affected cell density (Fig. 7). The maximum cell density of 8.3×10^6 cells/ml at 48 h of incubation was calculated at 50 rpm in the bioreactor.

Time course of suspension culture-adapted cell growth in the optimized conditions

The time course of suspension culture-adapted BHK-21 cell growth in the optimized conditions was confirmed using cell number, doubling time, and generation number after 0, 24, 48, and 72 h (Fig. S2). Under optimized physicochemical factors in a bioreactor, BHK-21 suspension cells had the maximum cell density of 7.5×10^6 cells/ml with a doubling time of 17 h,

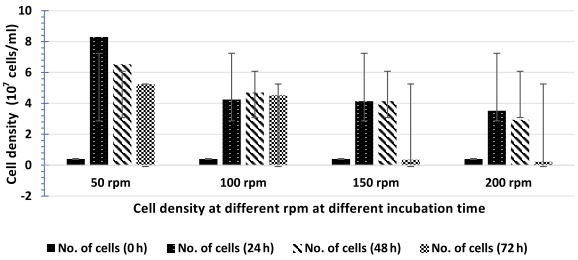

Fig. 7 Effect of different agitation levels on cell density at different incubation times. The figure shows the effect of different agitation levels on the number of cells on suspension culture-adapted BHK-21 cells. The x-axis represents the incubation times with different agitation levels, while the y-axis depicts the cell density. Data was statistically analyzed which showed that the agitation level of 50 rpm significantly affected the cell density of BHK-21 cells in a bioreactor and resulted in higher cell density as compared to the other agitation levels.

generation number of 3.8, volumetric productivity of 0.151×10^6 cells/ml/h, and growth rate of 0.0376/h (Table 1).

DISCUSSION

The suspension culture system in bioreactors enables the cultivation of BHK-21 cells in high volumetric yield and maximum cell densities compared to the adherent cell culture [1, 19, 24]. The unpretentious approach for suspension culture adaptation directly involves conditioning adherent culture cells to the agitation culture conditions. Mass-scale production of suspension culture in bioreactor monitors and controls different parameters for optimum cell growth: cell culture medium, agitation speed, pH, serum concentration, temperature, and seeding ratio. Several reports on BHK-21 cell culture in bioreactors and cellular metabolism are found. However, a whole set of physicochemical factors for optimal BHK-21 cell culture in bioreactors still needs to be studied.

This study optimized suspension culture to achieve the maximum cell density in a bioreactor. The maximum cell density of 6.5×10^6 cells/ml BHK-21 cells in optimized suspension culture in a bioreactor was achieved at pH 7.2, serum concentration of 10%, temperature of 37 °C, agitation speed of 50 rpm, and self-formulated medium with seeding density of 4×10^5 cells/ml in a bench top bioreactor. It is designed to culture large-scale cells under controlled and optimized conditions. The current study used a bioreactor of Eppendorf Beswick bioflo/celligen 115. Optimization of suspension culture was confirmed using cell number, doubling time, and generation number for BHK-21 cells. In the current research, a doubling time of 17 h and generation number of 3.9 was achieved at 48 h of incubation under optimized conditions in a bioreactor.

The BHK-21 cell line was established by Macpher-

Table 1 Different parameters of time course of suspension culture-adapted cell growth in the optimized conditions of BHK-21 cells in the bioreactor.

Parameter	Spinner flask (250 ml)	Bioreactor (5 l)
Cell density (10^6 cells/ml)	7.5×10^6	7.61×10^6
Doubling time (h)	18.42	18.42
Generation number (n)	3.9	3.9
Volumetric productivity (cells/ml/h)	0.151×10^6	0.15×10^6
Specific Growth rate (/h)	0.037	0.0376

son and Stoker [2]. This cell line is extensively used in biological sciences [8]. In this study, BHK-21 cells were viable at maximum after 36 h of incubation with 80–90% monolayer formation. At this time interval, cells were 98% viable. Results were statistically supported by a completely randomized design, $p = 0.000$, interpreted to show that 36 h of incubation significantly affected cell viability. Further incubation to 48 and 72 h decreases the viability of cells. We found these results are inconsistent with the experiment by Ferrari et al [25]. Adaptation of BHK-21 cells in roller bottles produced a cell density of 6.5×10^6 cells/ml after 72 h of incubation. Current study results are inconsistent with those of Muhammad et al, who optimized different physicochemical factors for enhancing biomass production of BHK-21 cells in roller bottles. BHK-21 cells in a roller bottle (480 cm^2) showed more than 4.7×10^7 cell count/bottle under optimized conditions such as 1×10^7 seeding cell density, incubation time of 60 h, incubation temperature of 37 °C, agitation speed of 3 rpm, and 100 ml of growth medium [18].

In roller bottle cell culture, the surface area available for the attachment of BHK-21 cells is increased compared to the conventional adherent cell culture flasks. An increase in surface area, along with the continuous rotation, helps in the constant supply of nutrients to the cells, resulting in increased cell density. The viable cell volume in a spinner flask at a low agitation speed of 25 rpm in 50 ml of total volume was studied, and it was concluded that the maximum volume for a defined surface area in spinner flasks depends mainly on cell concentration and whether the headspace gas is enriched with oxygen. Agitated spinner flask culture after post-inoculation had a cell viability of more than 90% with the maximum cell density of 4.16×10^6 cells/ml [26]. Continuous stirring of the cell culture medium helps in the distribution of nutrients. Constant control of pH helps in the optimal metabolism and growth of BHK-21 cells. Effective pH control throughout the whole batch prevents intracellular acidification. Consequently, normal cell physiology is guaranteed [27].

Various attempts have been made to optimize suspension culture for BHK-21 cells to achieve the maximum cell density. Telling et al described the operation and design of an industrial type of submerged cell culture growth of BHK-21 cells. With controlled pH 7.4 and temperature of 35 °C, seeding density of 0.5×10^6 cells/ml in modified Eagle's medium (MEM) produced 2.5×10^6 viable cells/ml with an agitation speed of 330 and 460 rpm at 50 h of incubation [4]. Compared with the study of Telling et al [4], we have achieved a high cell density of 7.5×10^6 cells/ml in 48 h of incubation using self-formulated medium at pH 7.2, 37 °C with a seeding density of 4×10^5 cells/ml at 50 rpm. Temperature is one of the crucial factors for the optimum growth of cells in cell culture [3]. Sartori et al conducted a study to evaluate the kinetic performance in bench top mode of BHK-21 cells cultured in different configurations of pH and dissolved oxygen concentration control strategies. Other parameters like dead and viable cells and various concentrations of ammonia, glucose, glutamate, and lactate were monitored. The maximum cell density of 1.85×10^9 cell/l after 50–90 h was estimated [16]. Inoculum preparation is critical in achieving high viable cell concentration of BHK-21 cells for suspension cultures in bench top bioreactors. Nunez et al have reported a schedule to produce more than 4×10^9 BHK-21 cells from 4×10^6 cells in 13 days in a culture medium. Another study was conducted to determine hydrodynamic characteristics for BHK-21 cell cultivation in bioreactors of different capacities. The optimal growth hydrodynamic environment for BHK-21 cell culture was determined in a 5-l bioreactor with other impellers at different agitation speeds. The maximum cell density of 4.8×10^6 cells/ml was achieved in a scaled-up 5-l bioreactor [28].

Optimization of suspension culture medium plays a vital role in achieving the maximum cell density in a bioreactor. Nutrients in the cell culture medium utilized with the growth of cells and supplementation with carbon source become necessary to sustain cellular metabolism and achieve higher cell density. In suspension culture, a large volume of cell culture medium is used in a bioreactor [9]. The culture medium requirements of suspension culture differ from adherent BHK-21 cell culture. DMEM is a widely used cell culture medium for BHK-21 cells that provides essential nutrients, vitamins, amino acids, and inorganic salts required for cell growth. GMEM is another basal cell culture medium suitable for BHK-21 cell culture. It is often augmented with additional components, i.e. fetal bovine serum, to support cell growth and maintenance. DMEM and GMEM in combination are

also used in cell culture medium suitable for BHK-21 cell culture [10, 29]. In this study, self-formulated medium for suspension culture is used, and it is the combination of basal media with balanced salt solution, phosphate buffer saline, lactalbumin hydrolysate, tryptose phosphate broth, fetal calf serum, sodium bicarbonate antibiotic mixture of neutral red, and deionized water [11]. Ubertini et al concluded that the supplementation of lactalbumin hydrolysate in DMEM results in higher cell density in spinner flask suspension culture [14]. Lactalbumin hydrolysate and DMEM showed statistically significant results when BHK-21 cells were cultivated in suspension culture compared to other experimental media containing tryptose soya broth, which Zamecnik and Stephenson used to obtain higher cell yields [30]. Park and colleagues have reported the successful adaptation of adherent BHK-21 cells to grow in suspension to a viable cell density of 7.65×10^6 cells/ml on day 3 in serum-free culture, and we found our results inconsistent by using self-formulated cell culture medium [1]. Wang et al compared 6 media having optimal concentrations of bovine serum albumin (BSA), calcium ions, tyrosine, and glutamine and achieved the maximum viable cell density of BHK cells in cell suspension culture reaching 140.21×10^5 cells/ml, which was 1.95 times higher than in basal medium [19]. Azka et al concluded that when basal medium is supplemented with lactalbumin hydrolysate, it resulted in high cell density in spinner flask suspension culture. It may be due to the high availability of carbohydrates (glucose) and proteins (amino acids) provided by the lactalbumin hydrolysate required for cellular metabolism, ultimately resulting in high cell density [9].

Various cell culture systems can get the maximum cell density at a large scale; however, cell density is reduced to some extent due to the cell-density effect [31]. This resulted in limiting essential nutrients, cell cycle arrest, and by-product inhibitions at high density, but the exact mechanisms involved are still unknown [1, 32]. The time course of suspension culture-adapted cell growth in the optimized conditions of BHK-21 was performed by doubling time, generation number, volumetric productivity, and specific growth rate determination of suspension culture-adapted BHK-21 cells. The standard of doubling time for BHK-21 cells is 13–17 h, and we found our results consistent with the reported data. Although there is significant variation across cell lines and culture conditions, the typical doubling period for suspension culture-adapted BHK-21 cells is between 12 and 24 h.

During the adaptation process, it is critical to monitor the cell growth rate because its increase is the first indication of successful adaptation. To ensure successful adaptation with increased cell growth, it is typical to use cell growth-promoting supplements such as commercial serum replacements or defined feeding media. The specific growth rate of suspension culture-adapted cells in a bioreactor was reported as 0.04 per h which is comparable with the growth rate calculated in this study.

CONCLUSION

The maximum cell density of 7.5×10^6 cells/ml BHK-21 cells in optimized suspension culture in the bioreactor was achieved. The time course of suspension culture-adapted cell growth in the optimized conditions was confirmed by calculating cell number, doubling time, generation number, volumetric productivity, and specific growth rate for BHK-21 cells. In the current research, a doubling time of 17 h and generation number of 3.9 were achieved at 48 h of incubation with the growth rate of 0.0376 under optimized conditions in a bioreactor, laying a foundation for the industrial production of the FMD vaccine in bioreactor.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2025.013.

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Appendix A. Supplementary data

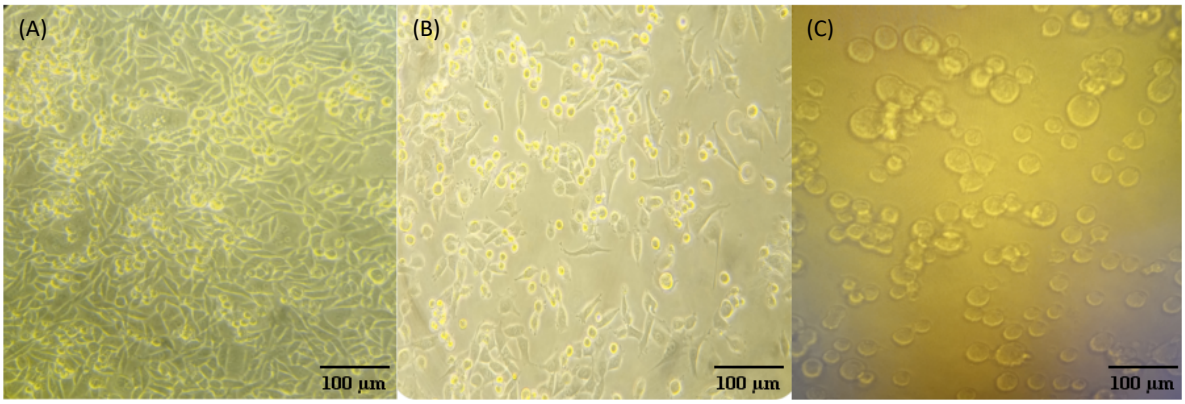

Fig. S1 Development and adaptation of BHK-21 cells from adherent monolayer to suspension culture in a bioreactor. (A) The BHK-21 cell monolayer development in adherent culture at 4 × magnification. (B) The adaptation of adherence dependent cells to the adherence independent cells in roller flasks at 4 × magnification. (C) The complete adaptation of BHK-21 cells in suspension culture system in a bioreactor.

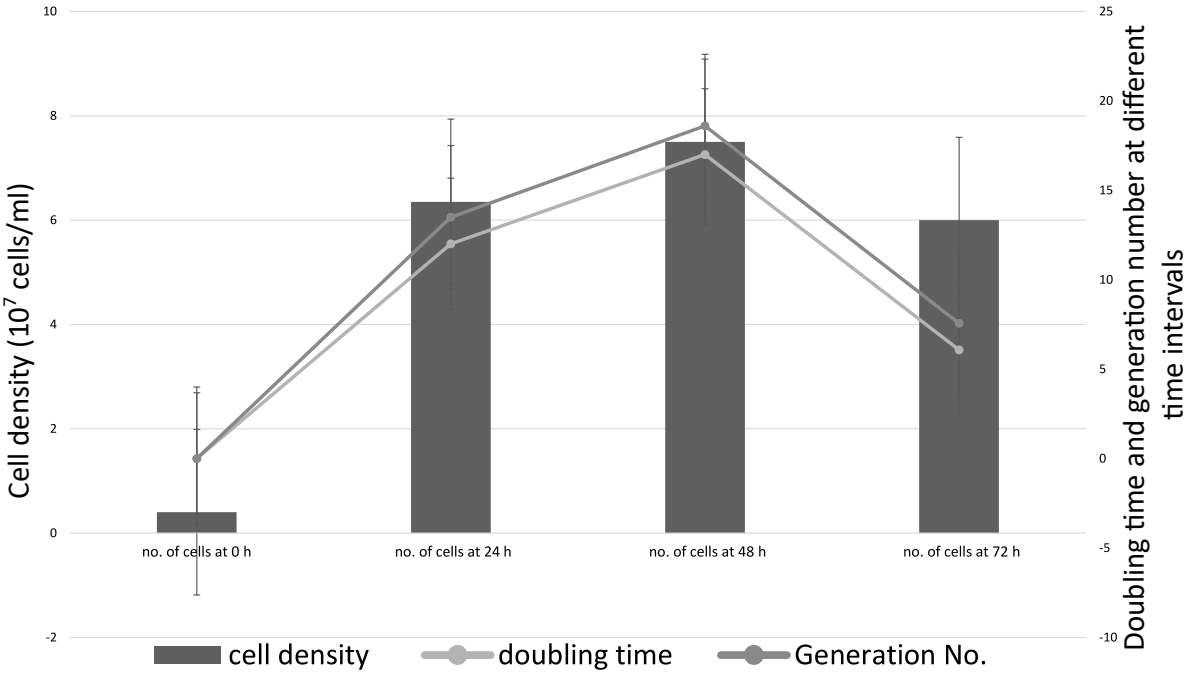

Fig. S2 The time course of suspension culture-adapted cell growth in the optimized conditions of BHK-21 cells in a bioreactor. Time course of suspension culture-adapted cell growth in the optimized conditions using a bioreactor was performed based on cell count, doubling number, and generation number at different incubation times. The x-axis represents the incubation time, while the y-axis depicts the cell density and secondary axis shows doubling time and generation number of BHK-21 cells. The maximum cell density with optimal generation number and doubling time was observed at 48 h of incubation in a bioreactor.