

Comparative evaluation of macroscopic carriers for Vero cell expansion: Influence of surface properties, incubation parameters, and nutrient media

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Abstract

The aim of this study was to identify optimal conditions for maximising biomass yield and preserving the viability of Vero cells cultured on different macroscopic carriers. The macroscopic carriers, including cellulose, composite, polystyrene (standard and modified), and polyethylene, were analysed in terms of adhesion, cell mass accumulation, doubling time, and morphological stability as well as the effects of incubation parameters and nutrient medium composition. After 48 h of cultivation, cellulose and composite carriers showed the highest adhesion levels (90-94%), followed by modified and standard polystyrene carriers (76-92%), whereas polyethylene carriers were the least effective (50-55%). Optimal growth was observed at 37°C, 21% O₂, and pH 7.2, resulting in a 3.5-fold increase in cell density (4.2×10^5 /cm²) and viability of 95-96%. Moderate deviations from these conditions led to reduced cell growth (2.6-3.2-fold; $3.3-4.0 \times 10^5$ /cm²) and viability (89-93%). However, extreme deviations (35 °C, 15% O₂, pH 6.8-7.0) significantly reduced growth to 2.1-2.4-fold, with cell densities of $2.6-2.8 \times 10^5$ /cm² and viability of 83-85%. The nutrient medium composition significantly influenced culture performance; the addition of L-glutamine (4 mmol/l) resulted in the highest cell biomass (4.6-fold) with final cell density of 9.2×10^5 /cm² and viability of 95% after 48 h. In contrast, glucose deficiency or serum deprivation reduced cell growth (2.0-2.5-fold) and cell layer uniformity. A comparison of cultivation systems showed that macroscopic carriers provided 20-25% greater adhesion levels and effective surface coverage compared to suspension culture. The study demonstrated that cellulose and composite carriers provided the highest adhesion and cell viability, while optimal growth was achieved at 37 °C, 21% O₂, and pH 7.2. Nutrient medium composition, particularly L-glutamine supplementation, further enhanced cell proliferation and final cell density.

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1. Introduction

Improving the efficiency of cell culture techniques in biotechnology and biomedical applications remains a primary challenge. Because of their higher proliferation rate, stable morphology, and versatility, Vero cells are extensively used for the production of vaccines, viral diagnostics, and development of other therapeutic preparations. Owing to their high sensitivity and suitability for antiviral testing, Vero cell lines have also been widely used as a model system in antiviral research, including studies on SARS-CoV-2 (Saliev et al. 2024; Khaidarov et al. 2024). However, modern industrial demands not only require sufficient cell mass but also stable quality, high viability, and reproducibility (Sene et al. 2022). Therefore, optimisation of cultivation strategies, including carrier selection and nutrient medium composition, is essential for the further development of applied biotechnological processes. A key limitation of current approaches is the use of traditional suspension cultivation methods, in which cells often form aggregates with reduced viability, unstable morphology, limited surface area for adhesion, uneven cell distribution, all of which limit productivity and process scalability. Macroscopic carriers may potentially overcome these limitations; however, their effectiveness depends on optimised incubation conditions and nutrient media, which remain insufficiently characterised for Vero cell systems. Previous reports have emphasised the use of three-dimensional cultures for reproducible viral propagation in Vero cell systems. For example, the growth of sheep pox virus on a three-dimensional Vero cell system ensured improved productivity and consistency of cell mass growth

(Nakhanov et al. 2024). Advances in carrier-based cell culture systems have been reported, including surface modification of microspheres (e.g. ultraviolet and ozone treatment), which improves cell attachment and distribution (Sia et al. 2023). Huang et al. (2024) further developed a scalable three-litre Vero cell culture system based on microspheres, which was appropriate for vaccine production.

In the applied studies, vaccine production in Vero cells has been shown to depend on the choice of culture media and bioreactor configuration (Sha 2021). These results demonstrate the importance of identifying conditions associated with high yields and stable cultures. The dependence of yield on medium composition and multiplicity of infection was also demonstrated in the production of oncolytic measles virus in Vero cells (Eckhardt et al. 2024). The results indicate that the adjustment of these parameters directly affected the stability of the viral process. Based on the experiments of Shen et al. (2023), suspension cultivation of Vero cells was successfully scaled up to obtain high titres of oncolytic herpes simplex virus type 1. The study showed that the transition to a suspension system enabled industrial application. In the review by Sene et al. (2022), genomic characterisation and engineering modification of Vero cells were considered a strategic approach to increasing vaccine process productivity. It was further emphasised that knowledge of the genetic features of the line facilitated the design of new bioprocesses. According to Fang et al. (2022), the introduction of bioreactor technologies in cell cultivation improved scalability and controllability of vaccine production processes. The study indicated that such platforms provide a basis for industrial application.

As shown by Hesley et al. (2025), accelerated methods for adjusting bioreactor parameters ensured effective scaling of the production of a vaccine candidate against Coronavirus Disease (COVID)-19 based on the measles virus. The results confirmed that the correction of medium and mixing conditions provided a high level of stability. As shown by Luo et al. (2021), the use of microcarriers in stirred bioreactors for fish cells demonstrated the potential of the method on an industrial scale. This finding indicates the versatility of microcarriers and their applicability to the Vero model. However, the long-term stability of Vero cells on macroscopic carriers and the comparative efficiency of various nutrient medium modifications on an industrial scale remain insufficiently studied. The aim of the study was to assess adhesion, growth dynamics, and productivity of Vero cells on various macroscopic carriers under different incubation conditions and nutrient media, followed by comparison with suspension culture. The objectives were: to determine the adhesion efficiency of Vero cells on various types of macroscopic carriers; to analyse the influence of incubation conditions and nutrient medium composition on the growth dynamics and accumulation of cell mass; and to compare the cultivation efficiency on macroscopic carriers and in suspension culture.

2. Materials and Methods

2.1. Cell culture conditions

Experiments involving Vero cell cultivation (epithelial kidney cells derived from the African green monkey) were carried out in 2024 (April-July) at the "Cell Biotechnology" Laboratory of the Research and Production Enterprise "Antigen". These cells were chosen because they are widely used in biotechnology and virology owing to their high growth rate, morphological stability, and sensitivity to culture conditions. All procedures were performed under strict aseptic conditions using Class II biological safety cabinets (ESCO Airstream, Singapore). For stable cultivation, incubators with automatic temperature, CO₂, and O₂ control (Binder CB 170, Germany), allowing controlled variation of conditions within the permitted range. Standard incubation conditions were set at 37 °C, 21% O₂, and pH 7.2. Experimental variations included reduced temperature (35 °C), elevated temperature (38 °C), reduced O₂ concentrations (18% and 15%), and altered pH (6.8 and 7.4). Oxygen concentration (21%, 18%, and 15%) was controlled using the built-in gas regulation system of the incubator and maintained throughout the cultivation period. The CO₂ level was set at 5% in all experiments to ensure stable pH. The culture medium was buffered using a standard bicarbonate buffering system.

2.2. Cell adhesion assessment

To assess the adhesion efficiency of Vero cells, five types of macroscopic carriers were used: modified cellulose, composite carriers with silicate inclusions, standard polystyrene, modified polystyrene, and hydrophobic polyethylene. Composite carriers consisted of a cellulose-based matrix with embedded silicate components, providing a combined porous and structured surface. Cell were seeded at a density of 2.0×10^5 /cm² and incubated at standard conditions. Cell adhesion rates, final cell density, and morphology were evaluated at 2, 6, 12, and 24 h. Cell counts were performed in a Goryaev chamber and validated by Countess II FL, an automatic cell counter (Thermo Fisher Scientific, USA). Viability was assessed using trypan blue staining (Sigma-Aldrich, USA), and the percentage of cell attachment was calculated as follows:

$$P = (N_{\text{adherent}} / N_{\text{seeded}}) \times 100 (\%)$$

where: P – percentage of attached cells (%), N_{adherent} – number of attached cells, N_{seeded} – number of seeded cells.

Cell morphology was recorded under an inverted phase-contrast microscope (Axio Vert.A1, Carl Zeiss, Germany) equipped with a digital camera (Axiocam 503 mono, Carl Zeiss, Germany), allowing assessment of distribution uniformity and attachment characteristics. Surface properties of the carriers (e.g. roughness, porosity, and wettability) were considered based on manufacturer specifications; no additional surface characterisation (SEM or contact angle analysis) was performed.

2.3. Growth dynamics of cells

The cells were evaluated in a CO₂ incubator Binder CB 170 (Binder, Germany) under different conditions: standard (37 °C, 21% O₂, pH 7.2), reduced temperature (35 °C, 21% O₂, pH 7.2), increased temperature (38 °C, 21% O₂, pH 7.2), hypoxic conditions (37 °C, 15 and 18% O₂, pH 7.2), and pH variation (37 °C, 21% O₂, pH 6.8-7.4). In each regime, the cells were analysed after 12, 24, and 48 h. The growth parameters measured were cell density increase over 48 h, average doubling time, final cell density, and cell viability. Cell density was determined using the automatic counter Countess II FL (Thermo Fisher Scientific, USA). The doubling time was calculated as follows:

$$T_d = t \times \ln 2 / (\ln N_t - \ln N_0)$$

where: T_d – population doubling time (h), t – incubation interval (h), $\ln$ – natural logarithm, N_t – number of cells at time t (units), N_0 – number of cells at seeding (units).

Cell viability was assessed using trypan blue staining and confirmed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test (Petty et al. 1995) (Sigma-Aldrich, USA), with optical density measured using a Multiskan GO spectrophotometer (Thermo Fisher Scientific, Finland). Cell morphology was analysed using an inverted phase-contrast microscope (Axio Vert.A1, Carl Zeiss, Germany), while the uniformity of distribution and aggregation zones were analysed using the ImageJ software (NIH, USA).

2.4. Effect of nutrient medium composition

Five types of media (Gibco, USA) were used for the experiment: standard Dulbecco's Modified Eagle Medium (DMEM) (1.0 g/L glucose), DMEM with low glucose content (0.5 g/L), DMEM with high glucose content (4.5 g/L), DMEM supplemented with L-glutamine (4 mmol/L), and DMEM without serum. Cells were cultured under standard incubation conditions (37 °C, 21% O₂, pH 7.2) and analysed at 2, 6, 24, and 48 h of incubation. The evaluated parameters included cell mass yield, final cell density, mean doubling time, and cell viability. Cell density was determined using a Goryaev chamber and an automatic cell counter. Viability was determined using trypan blue staining and a fluorescent method using acridine orange and propidium iodide (Sigma-Aldrich, USA). The fold increase in cell number was calculated as the ratio N_t/N_0 . Cell morphology and surface coverage area were assessed under the inverted microscope (Axio Vert.A1, Carl Zeiss, Germany) and digital image analysis was performed using the ImageJ software.

2.5. Comparison between carrier-based and suspension culture systems

To compare cultivation efficiency, Vero cells were simultaneously grown on macroscopic carriers and in a suspension system in Spinner

flasks (Corning, USA). In all cases, standard incubation conditions (37 °C, 21% O₂, and pH 7.2) were used and evaluation was performed after 2, 6, 24, and 48 h. The parameters assessed were cell density, growth rate, cell viability, doubling time, and morphology. Cell counts were performed using the Countess II FL, and viability was determined using trypan blue staining and confirmed by the MTT test (Petty et al. 1995). Morphological characteristics were analysed under the phase-contrast microscope (Axio Vert.A1, Carl Zeiss, Germany). For macroscopic carriers, surface coverage area and uniformity of cell distribution were assessed, whereas, in suspension culture, aggregate diameter was measured and the average value was calculated in ImageJ software based on micrographs.

2.6. Statistical analysis

All experiments were performed in three independent biological replicates, each with three technical replicates. The results are presented as mean $\pm$ standard error (SE). Statistical significance was assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Efficiency of Vero cell attachment

Significant differences were observed in cell attachment efficiencies across the carriers as early as 2 h of incubation. After 2 h, cellulose (72%) and composite (70%) carriers showed the highest attachment efficiencies, followed by modified polystyrene (65%) and standard polystyrene (45%), whereas polyethylene showed the lowest adhesion (42%). After 6 h of cultivation, the differences between carrier groups

persisted and intensified, with attachment efficiency increasing to 85% and 82% on cellulose and composite carriers, respectively, while the attachment on modified polystyrene carriers amounted to 80%, which was significantly higher than that observed for standard polystyrene carriers (60%). Again, the cell attachment efficiency was lowest on polyethylene carriers (37%). These findings suggest that carrier surface properties influence cell attachment at early stages of cultivation, with cellulose-based materials supporting faster initial attachment than hydrophobic surfaces. After 24 h of cultivation, the values reflected the final stable attachment efficiencies across the carrier groups. Cellulose, composite, and modified polystyrene showed the highest attachment efficiencies (90-94%), whereas standard polystyrene and polyethylene remained lower (~55-79%). A similar trend persisted at 48 h of cultivation, indicating consistent carrier-dependent effects on cell attachment. Differences between carriers remained statistically significant, indicating the influence of surface properties on cell attachment. Cell viability after 48 h followed the trend of cell attachment. The highest viability was observed on cellulose and composite carriers (95-96%), followed by modified and standard polystyrene (91-93%), whereas polyethylene showed the lowest viability (82%). Thus, cellulose and composite carriers maintained the highest cell viability, whereas polyethylene showed the lowest values. These results indicate that higher initial attachment is associated with improved subsequent proliferation and viability.

Gradual surface coverage and preservation of the elongated morphology of the cells under adhesion conditions of culture leads to the formation of a structurally organised monolayer, which is consistent with the observed improvements in cell attachment, viability and growth stability (Fig. 1).

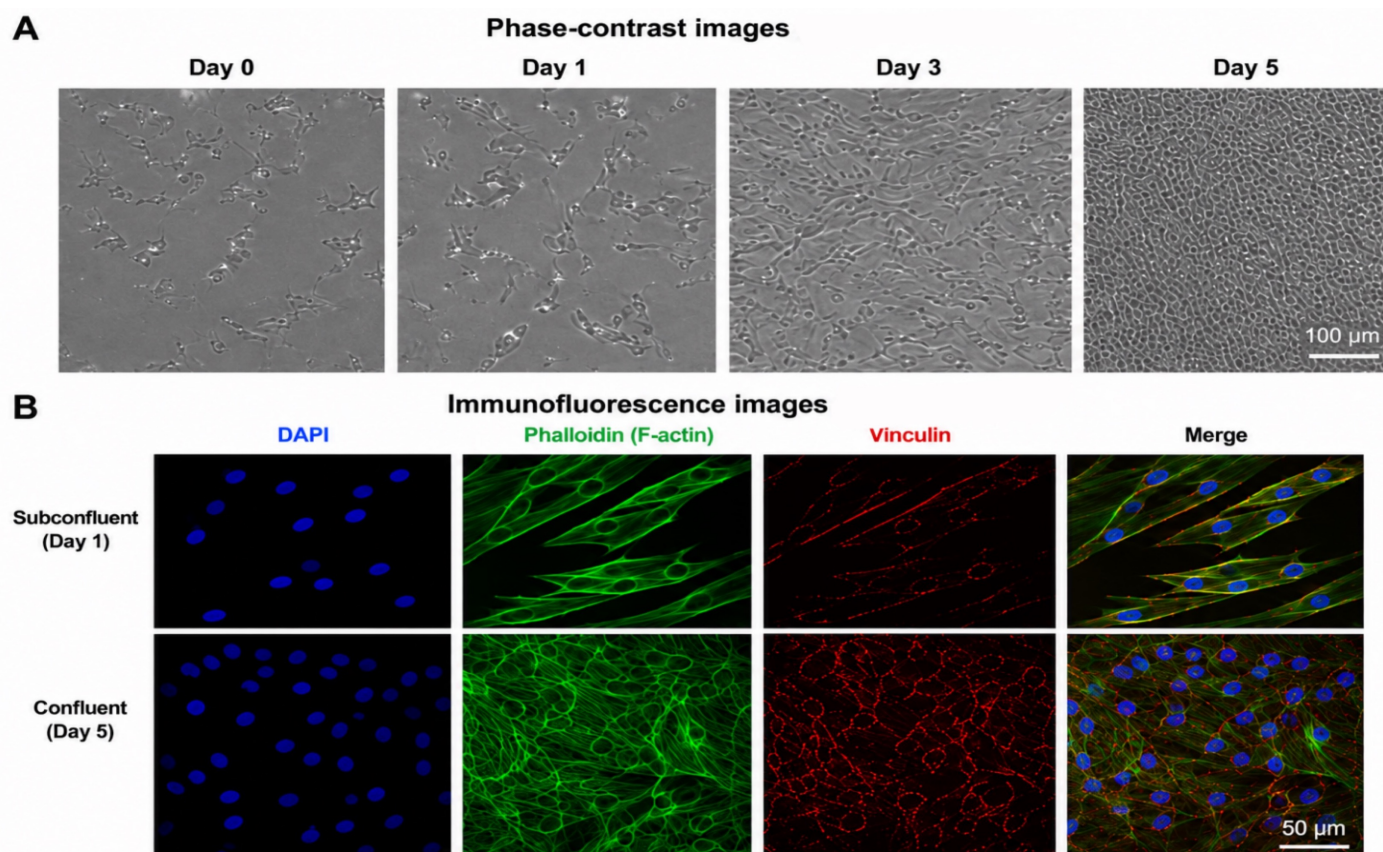


Fig. 1. Phase-contrast and immunofluorescence images of adherent Vero cell culture during monolayer formation

Table 1. Viability of Vero cells on various macroscopic carriers

Type of macroscopic carrier	2 h	6 h	24 h	48 h	Proportion of viable cells after 48 h (%)
Cellulose (modified)	72 ± 2 ^a	85 ± 2 ^a	94 ± 1 ^a	93 ± 1 ^a	96 ± 1 ^a
Composite (with silicate inclusions)	70 ± 2 ^a	82 ± 2 ^a	90 ± 2 ^a	91 ± 1 ^a	95 ± 1 ^a
Polystyrene (standard)	45 ± 3 ^c	60 ± 3 ^c	79 ± 2 ^b	81 ± 2 ^b	91 ± 2 ^b
Polystyrene (modified)	65 ± 2 ^b	80 ± 2 ^b	92 ± 1 ^a	91 ± 1 ^a	93 ± 1 ^b
Polyethylene (hydrophobic)	42 ± 3 ^c	37 ± 3 ^c	55 ± 3 ^c	53 ± 3 ^c	82 ± 2 ^c

Values are presented as mean ± SE (n = 3). Different superscripts within a column indicate statistically significant differences (p < 0.05)

Qualitative observations revealed the formation of uniform and dense monolayers supported by the cellulose and composite carriers. The modified polystyrene displayed a uniform cell distribution but the density was slightly lower. Standard polystyrene revealed partial cell aggregation with reduced effective adhesion area, while rounded and weakly attached cells with limited spreading was displayed by polyethylene. After 48 h, three categories of carriers could be distinguished based on efficiency: high-efficiency (cellulose and composite, 90-94%), moderate-efficiency (modified and standard polystyrene, 76-92%), and low-efficiency (polyethylene, 50-55%). Attachment efficiency and viability across different carrier types are summarized in Table 1.

3.2. Dynamics of cellular growth under different incubation conditions

The key identified factors affecting Vero cell growth dynamics were temperature, oxygen level, and pH. The features of optimal growth, high viability, and formation of a uniform dense monolayer were demonstrated by Vero cells under standard incubation conditions of 37 °C, 21% O₂, and pH 7.2. Slow proliferation, an increase in doubling time, and less uniform cell layer formation were observed at reduced temperature (35 °C), whereas initial acceleration of growth followed by a decline in proliferation stability and reduced viability over time was observed at higher temperature (38 °C). This was paralleled by increased rounding of cells, suggesting reduced growth stability. Growth and viability were not significantly affected by a moderate reduction in oxygen concentration (18% O₂). However, more severe hypoxic and acidic conditions (15% O₂, pH 7.0) significantly decreased

proliferation efficiency, increased doubling time, and resulted in heterogeneous cell populations with a higher proportion of rounded cells. pH deviations towards either acidic and alkaline conditions reduced growth stability, with lower cell proliferation and increased morphological heterogeneity. The suppression of growth and formation of sparse and poorly organised cell colonies were most prominent under combined deviations in temperature, oxygen, and pH. A short-term acceleration in growth followed by reduced stability and viability was observed at elevated temperature combined with alkaline conditions. The results show that the best growth is achieved under standard incubation conditions, and that deviations in temperature, oxygen and pH lead to lower proliferation efficiency and stability (Table 2).

3.3. Formation of Vero cell mass under the influence of various medium components

In the standard DMEM medium supplemented with 10% serum, cells demonstrated stable growth dynamics, forming a dense and uniform monolayer by 48 h. Increased glucose concentration (4.5 g/L) accelerated proliferation at later stages but was accompanied by reduced viability and morphological instability, including the appearance of rounded cells and aggregation zones. Reduced glucose concentration (1.0 g/L) resulted in slower growth, lower cell density, and less uniform cell distribution. Increasing L-glutamine concentration to 4 mM significantly enhanced cell proliferation, resulting in the highest cell density and formation of a stable homogeneous monolayer. In contrast, reduced glutamine levels led to slower growth, lower viability, and decreased structural uniformity of the cell layer.

Table 2. Dynamics of Vero cell growth under various incubation conditions

Incubation conditions	Density increase over 48 h (fold)	Doubling time (h)	Final density (cells/ cm ²)	Viability (%)	Morphological features
37°C, 21% O ₂ , pH 7.2	3.5 ± 0.2 ^a	22 ± 1 ^a	4.2 × 10 ⁵ ± 0.15 ^a	96 ± 1 ^a	Uniform dense monolayer
35°C, 21% O ₂ , pH 7.2	2.6 ± 0.2 ^b	28 ± 2 ^b	3.1 × 10 ⁵ ± 0.12 ^b	91 ± 1 ^b	Loose colonies, slowed growth
38°C, 21% O ₂ , pH 7.2	3.0 ± 0.2 ^b	20-26	3.9 × 10 ⁵ ± 0.15 ^a	88 ± 2 ^c	Rapid growth in first 24 h, subsequent cell rounding
37°C, 18% O ₂ , pH 7.2	3.2 ± 0.2 ^a	24 ± 1 ^a	4.0 × 10 ⁵ ± 0.15 ^a	93 ± 1 ^a	Shape preservation, slight heterogeneity
37°C, 15% O ₂ , pH 7.0	2.4 ± 0.2 ^c	30-32	2.8 × 10 ⁵ ± 0.10 ^c	85 ± 2 ^c	Increased number of rounded cells
35°C, 15% O ₂ , pH 7.0	2.1 ± 0.2 ^c	>32	2.6 × 10 ⁵ ± 0.10 ^c	83 ± 2 ^c	Loose small colonies, mosaic distribution
38°C, 21% O ₂ , pH 7.4	2.9 ± 0.2 ^b	20-26	3.6 × 10 ⁵ ± 0.12 ^b	87 ± 2 ^c	Aggregation, local densifications
37°C, 21% O ₂ , pH 6.8	2.7 ± 0.2 ^b	27 ± 2 ^b	3.3 × 10 ⁵ ± 0.12 ^b	90 ± 1 ^b	Slower growth, stable morphology
37°C, 21% O ₂ , pH 7.4	3.1 ± 0.2 ^a	24-25	3.7 × 10 ⁵ ± 0.12 ^b	89 ± 1 ^b	Local aggregation, moderate growth fluctuations

Values are presented as mean ± SE (n = 3). Different superscripts within a column indicate statistically significant differences (p < 0.05)

Reduction of serum content decreased overall productivity and resulted in looser and less uniform cell layers. Serum-free conditions showed the weakest growth performance and were associated with pronounced aggregation and unstable morphology. Comparative analysis showed that cell growth is primarily determined by nutrient composition, with L-glutamine supplementation providing the most pronounced effect (Table 3). Therefore, the nutrient medium formulation directly impacted cell yield productivity. Cell yield productivity was generally maximised by optimising medium amino acid content via L-glutamine supplementation, while deficiency in either an energy source (glucose) or a growth factor (serum) detrimentally impacted proliferation, and gave rise to less uniform growth. The best indicators were recorded under increased L-glutamine content (4 mmol/L): after 48 h, the cell mass increase reached 4.6-fold, final density was $9.2 \times 10^5/\text{cm}^2$, and viability remained at 95%, significantly exceeding all other medium variants.

3.4. Evaluation of differences in the growth of Vero cells on carriers and in suspension

At the early stage of cultivation (2-6 h), macroscopic carriers provided faster cell attachment and more uniform distribution compared to

suspension culture. Cells in the adherent system formed stable adhesion zones, whereas in suspension, growth initiation was slower and characterised by the predominance of rounded cells. As cultivation progressed (up to 24 h), these differences became more pronounced. Macroscopic carriers supported higher growth rates, more uniform surface coverage, and better preservation of cell morphology. In contrast, suspension culture was associated with lower proliferation, increased doubling time, and the formation of local aggregates, which limited efficient nutrient and oxygen exchange. After 48 h of incubation, the superiority of macroscopic carriers became more obvious in terms of higher cell density, greater viability, and formation of a stable homogeneous monolayer. Cell growth was lower in suspension culture and was associated with marked aggregation and structural heterogeneity. At each time point, the comparison showed consistently higher cell density and growth rate in macroscopic carriers. Macroscopic carriers revealed higher cell viability and specific adhesion area than suspension culture. Cells retained an elongated morphology and formed homogeneous monolayers whereas morphology in suspension culture was variable and associated with aggregation (Table 4). These results confirm that cell growth is more efficient and stable with macroscopic carriers than with suspension culture.

Table 3. Dynamics of Vero cell accumulation in various media

Medium	Time (h)	Increase (fold)	Density ($\times 10^5/\text{cm}^2$)	Doubling time (h)	Viability (%)	Morphological features
DMEM + 10% serum	2	1.1	1.9	–	96	Uniform attachment
	6	1.3	2.2	–	95	Denser contacts
	24	2.0	4.0	22	95	Uniform monolayer
	48	3.6 ± 0.2^b	7.8 ± 0.3^b	22 ± 1^a	95 ± 1^a	Dense homogeneous layer
DMEM + glucose 4.5 g/l	2	1.1	1.9	–	95	No difference from control
	6	1.4	2.3	–	94	Less uniform layer
	24	2.4	4.8	21	92	Appearance of rounded cells
	48	4.2 ± 0.2^a	8.4 ± 0.3^a	21 ± 1^a	91 ± 1^b	Aggregation zones
DMEM + glucose 1.0 g/l	2	1.0	1.8	–	95	Minimal changes
	6	1.1	1.9	–	94	Sparse cell distribution
	24	1.7	3.4	27	93	Less dense layer
	48	2.9 ± 0.2^c	5.9 ± 0.3^c	28 ± 2^c	92 ± 1^b	Mosaic distribution
DMEM + L-glutamine 4 mM	2	1.1	1.9	–	96	Normal
	6	1.5	2.5	–	95	Accelerated growth
	24	2.5	5.0	20	94	Dense monolayer
	48	4.5 ± 0.2^a	9.0 ± 0.3^a	20 ± 1^a	94 ± 1^a	Homogeneous stable layer
DMEM + L-glutamine 1 mM	2	1.0	1.8	–	95	No deviations
	6	1.2	2.0	–	93	Limited growth
	24	1.8	3.6	25	91	Less uniform layer
	48	3.1 ± 0.2^b	6.7 ± 0.3^b	26 ± 2^b	90 ± 1^c	Unstable morphology
DMEM + 2% serum	2	1.0	1.8	–	94	Attachment preserved
	6	1.2	2.0	–	93	Looser layer
	24	1.9	3.8	24	91	Slower growth
	48	3.4 ± 0.2^b	7.1 ± 0.3^b	25 ± 2^b	90 ± 1^c	Loose monolayer
Serum-free + growth factors	2	1.0	1.8	–	93	Sparse cell distribution
	6	1.1	1.9	–	92	Minimal activity
	24	1.7	3.4	27	90	Loose colonies
	48	3.2 ± 0.2^b	6.9 ± 0.3^b	28 ± 2^c	89 ± 1^c	Aggregation, unstable morphology

Values are presented as mean $\pm$ SE ($n = 3$). Different superscripts within a column indicate statistically significant differences ($p < 0.05$)

Table 4. Comparison of Vero cell growth on macroscopic carriers and in suspension culture			
Time	Indicator	Macroscopic carriers	Suspension culture
2 h	Density (×10 ⁵ /cm ²)	1.9 ± 0.1	1.4 ± 0.1
	Increase (fold)	1.2 ± 0.1	1.1 ± 0.1
	Viability (%)	96 ± 1	94 ± 1
	Doubling time (h)	-	-
	Morphology	Adhesion islands	Single rounded cells
6 h	Density (×10 ⁵ /cm ²)	2.6 ± 0.1	1.9 ± 0.1
	Increase (fold)	1.5 ± 0.1	1.3 ± 0.1
	Viability (%)	95 ± 1	93 ± 1
	Doubling time (h)	27 ± 2	31 ± 2
	Morphology	~45% surface coverage	Aggregates up to 50 μm
24 h	Density (×10 ⁵ /cm ²)	4.9 ± 0.2	3.2 ± 0.2
	Increase (fold)	2.5 ± 0.2	1.9 ± 0.1
	Viability (%)	94 ± 1	91 ± 1
	Doubling time (h)	24 ± 1	29 ± 2
	Morphology	Monolayer, 70-75% coverage, ≤6% rounded cells	Aggregation 12-14%, form variability
48 h	Density (×10 ⁵ /cm ²)	9.0 ± 0.3	6.1 ± 0.3
	Increase (fold)	4.5 ± 0.2	3.0 ± 0.2
	Viability (%)	93 ± 1	89 ± 1
	Doubling time (h)	22 ± 1	27 ± 2
	Morphology	Uniform monolayer, 90-95 % coverage, ≤8% aggregation	Aggregates up to 120 μm, heterogeneity
Values are presented as mean ± SE (n = 3)			

4. Discussion

The present study shows that Vero cell behaviour is controlled by the interaction between surface physicochemistry, culture conditions and nutrient availability, rather than by a single parameter. Superior cell adhesion, viability, and morphological stability were demonstrated by cellulose and composite macroscopic carriers over other tested materials. This superiority is potentially associated with increased surface energy and hydrophilicity of cellulose-based carriers that facilitate rapid adsorption of extracellular matrix (ECM) proteins from the medium. These proteins create an intermediate layer that allows for integrin-mediated cell adhesion and the stabilisation of cell-surface interactions, thus promoting the formation of a monolayer. On the other hand, the low surface energy of polyethylene limits protein adsorption, reducing integrin binding and resulting in unstable adhesion and lower cell viability. These results indicate that Vero cells function in a relatively narrow physiological window, where disruption of metabolic balance and reduced proliferation efficiency are observed following small changes in temperature, oxygen concentration, and pH. At low temperature and hypoxic conditions, slower growth, longer doubling times, and disrupted morphological stability were observed, possibly due to lower metabolic activity. However, accelerated initial growth was observed at elevated temperature and a shift in pH to the alkaline side with subsequent reduction in cell viability and morphological stability, and higher aggregation of cells, suggesting stress-induced cell responses.

The observed differences in cell adhesion and growth can be explained by the physicochemical properties of the carrier surfaces. Cellulose and other hydrophilic materials enhance protein adsorption and cell-surface interactions, resulting in more stable attachment and

uniform monolayer formation (Swan et al. 2024; Rauer et al. 2024). In addition, increased surface roughness and porosity enhance the effective contact area, supporting more uniform cell distribution and monolayer formation (Swan et al. 2024). In contrast, hydrophobic materials such as polyethylene limit protein adsorption and weaken cell-substrate interactions, resulting in reduced adhesion efficiency and lower cell viability. The role of carrier material properties has been increasingly recognised in recent studies. The advances in bioreactor systems with improved environmental control (Ge et al. 2023) and biofunctionalised materials (Rauer et al. 2024) can regulate cell adhesion and local microenvironmental interactions. These studies are consistent with the observations of the present study that cell attachment, proliferation, and morphological stability are directly influenced by the surface physicochemical properties, including hydrophilicity and structure. Previous studies on Vero cell cultivation have primarily focused on bioreactor-based production systems and carrier technologies. For example, Rhazi et al. (2021) and Guo et al. (2024) demonstrated that advanced bioreactor platforms can enhance viral yield and process efficiency, while Swan et al. (2024) summarised developments in carrier materials and surface modifications. However, these studies largely emphasised virological or engineering outcomes, with limited attention to cell attachment, morphology, and viability. In contrast, the present study integrates both quantitative and qualitative parameters of cell growth, providing a more comprehensive evaluation of cultivation efficiency.

Adaptation strategies of Vero cells under varying culture conditions have also been explored. Mohammed et al. (2021) showed that gradual adaptation to serum-free media supports viral production, while Zinnecker et al. (2024) highlighted the potential of high-density systems for vaccine development. Nevertheless, these studies did not

systematically assess how changes in cultivation conditions affect cell attachment, morphology, and population stability. The present work addresses this gap by linking incubation parameters and nutrient composition with both growth dynamics and structural characteristics of the cell population. From a transcriptomic perspective, differences in gene expression related to the cell cycle and stress response have been reported between suspension and adherent Vero cell cultures, with adherent systems generally demonstrating more stable proliferation and reduced stress-associated signalling (Sene et al. 2023). Furthermore, Bastin et al. (2021) demonstrated that variations in metabolic flux influence energy availability and biosynthetic activity, directly affecting proliferation rate, doubling time, and overall growth efficiency under different culture conditions. However, unlike the present study, these studies did not directly assess cell attachment, density, viability, or morphology under controlled experimental conditions. Research on alternative culture systems and process scaling has highlighted the potential of suspension-based approaches and advanced bioreactor configurations (Sharma 2021; Chen et al. 2024; Robitaille et al. 2024; Gobel et al. 2022). While these systems can increase productivity and support large-scale cultivation, they often do not capture differences between adhesion and suspension growth in terms of cell behaviour and structural organisation. In the present study, direct comparison demonstrated that adherent systems provide more stable attachment, more uniform cell distribution, and overall more consistent growth under controlled conditions. Thus, these findings confirm that optimisation of carrier surface properties, in combination with controlled cultivation conditions, is critical for achieving stable and efficient Vero cell growth.

5. Conclusions

The present study revealed that the Vero cell expansion is primarily affected by the properties of the carrier surface, the stability of the incubation conditions and the composition of the medium. The most efficient substrates were cellulose and composite macroscopic carriers, which gave the highest attachment rates (90–94%), viability (95–96%) and formation of a uniform monolayer, whereas the hydrophobic polyethylene displayed poor adhesion qualities. The optimal growth conditions were 37 °C, 21% O₂ and pH 7.2, where the cell density increased 3.5 fold and the viability was 95–96% after 48 h. Moderate deviations reduced proliferation, while combined low temperature, hypoxia and pH imbalance strongly suppressed growth. The highest biomass accumulation (4.6-fold and 9.2×10^5 cells/cm²) was obtained with L-glutamine supplementation at 4 mmol/L. The macroscopic carriers provided more stable adhesion, higher surface coverage and better morphological preservation compared to suspension culture. These findings support the use of optimized adherent carrier systems for Vero-cell-based biotechnological production.

Declarations

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