



## Applications and Advancements of Animal Cell Cultures in Virology: Insights from Lumpy Skin Disease Virus Research

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### ABSTRACT

The article provides a comprehensive review of the current state and future directions for the use of animal cell cultures in virological research. The review critically evaluates the use of animal cell cultures to enhance understanding of viral pathogenesis and improve vaccine development strategies, highlighting promising technologies in this field. It discusses technologies and tools for virus and cell visualization, bioinformatics approaches to data analysis, and the challenges and potential for future research. The review emphasizes the importance of animal cell cultures in studying viruses and developing vaccines, noting the advent of 3D cell culture technologies that enhance the translational significance of *in vitro* models. The adaptation of new culture media mimicking human blood plasma is expected to improve understanding of viral pathogenesis and virus propagation efficiency. The use of animal cell cultures is illustrated through the study of the lumpy skin disease virus. The application of artificial intelligence in cell and tissue cultivation research opens possibilities for modeling and analyzing viral pathogenesis. The article calls for further development in this area, emphasizing the need to establish a National Collection of Cell Cultures.

**Key words:** Animal cell cultures; Virology; Vaccine development; Lumpy skin disease virus

### INTRODUCTION

Animal cell culture is a cornerstone of virological research, offering unparalleled opportunities for the isolation, propagation, and study of viruses under controlled conditions. The adaptability of cell culture systems enables researchers to model viral infections,

test antiviral compounds, and develop vaccines with precision (Alomari et al. 2023; Rehman et al. 2024). With the advent of advanced cell culture techniques, including organoids and 3D cultures, researchers have broadened the scope of applications, making it possible to simulate *in vivo*-like environments (Ozawa et al. 2023).

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Virology research has increasingly relied on cell lines due to their reproducibility, scalability and compatibility with high-throughput screening (Luo et al. 2023; Rajaram et al. 2020). However, challenges remain in mimicking the complexities of *in vivo* systems, particularly for viruses with strict host-specific requirements (Cordeiro et al. 2024; Douglass et al. 2020). Recent advancements, such as microfluidic platforms and computational modeling, have further enhanced the fidelity of these systems (Kheiri et al. 2024; Keshavarz and Nasab 2023).

Despite their widespread use, cell cultures are not without limitations. Issues such as genetic drift, contamination and differences in cellular responses between *in vitro* and *in vivo* systems necessitate careful optimization and validation (Dolskiy et al. 2020; Urazayeva et al. 2024). As the field advances, the integration of artificial intelligence and multi-omics approaches holds promise for addressing these challenges and improving the predictive power of cell culture-based studies (Tzaferis et al. 2023).

This review aims to provide an overview of the critical role of animal cell cultures in virology, highlighting their applications, limitations and future potential. By synthesizing recent advances and practical insights, this work offers a valuable resource for researchers developing novel viral diagnostics, therapeutics, and vaccines.

The importance and timeliness of this research are underscored by the increasing emergence of novel viral threats and the rapid evolution of viral pathogens. In recent years, outbreaks of infectious diseases have underscored the urgent need for reliable *in vitro* models that can accurately recapitulate virus–host interactions (Khan et al. 2022; Zhugunisssov et al. 2023; Eker et al. 2024; Begum et al. 2024; Ashraf et al. 2025). By refining cell culture techniques and incorporating cutting-edge technologies, such as microfluidics, high-resolution imaging, and single-cell analysis, this study addresses critical gaps in our current methodologies (Liu and Zheng 2024; Gupta et al. 2024). These innovations are essential for accelerating the development of effective vaccines and antiviral therapies, ensuring that research keeps pace with evolving global health challenges. As such, this investigation not only advances our understanding of viral pathogenesis but also provides a timely framework for future research endeavors aimed at protecting public health (Eze et al. 2024; Garg et al. 2024; Tursunov et al. 2024; Prajapati et al. 2024).

### Historical development of cell culture

The historical development of cell culture began with the pioneering work of Ross G. Harrison in 1907, who first applied the *in vitro* cell culture method to study the origin of nerve fibers in frogs (Keshavarz and Nasab 2023). This foundational work laid the groundwork for the evolution of cell culture techniques, which have since undergone significant changes and improvements. The progression from embryological techniques to the establishment of tissue culture and the subsequent development of cell culture media further advanced the field (Rogo et al. 2023). The "hanging drop technique" by Harrison and subsequent advancements by Alexis Carel and Charles Lindbergh facilitated the transition from tissue to cell culture, enabling the growth of cells in a monolayer and the cultivation of viruses, which was instrumental in the development of the

polio vaccine (Sahu et al. 2024).

In parallel, plant tissue culture emerged from the ideas of the German scientist Haberlandt in the early 20th century, leading to the development of various *in vitro* techniques and applications in plant biology and biotechnology (Husen and Pant 2024). The historical context of cell culture is also intertwined with the broader understanding of cellular systems biology and the protoplasm concept, which has influenced modern cell biology (Krasnova et al. 2023). The ability to grow and maintain individual cell types *in vitro* has allowed for mechanistic studies of whole-organism physiology and has applications in comparative physiology and conservation biology (Jiménez and Harper 2023).

The field of evolutionary cell biology, which examines the origins and functions of cellular features through an evolutionary lens, has also benefited from the tools and insights provided by cell culture, particularly through experimental laboratory evolution (Helsen et al. 2023; Nuraliyeva et al. 2023). Technological advancements, such as the development of the Advanced TCT<sup>TM</sup> polymer modification, have further refined cell culture techniques, improving the propagation of fastidious cells and enhancing cell-based assays (Almeida-Pinto et al. 2023).

In general, the historical development of cell culture is marked by a series of innovations and refinements that have expanded its applications across various biological fields. As shown in Fig. 1, from its inception in the early 20th century to the present day, cell culture has become an indispensable tool in both basic and applied research, contributing to our understanding of cellular functions and the mechanisms of disease.

### Animal cell cultivation in virology: methods and their application in virus research and vaccine development

The cultivation of animal cells plays a pivotal role in studying viral replication, pathogenesis, and vaccine development. This section explores the key aspects of animal cell cultures in virology, existing knowledge gaps, and current challenges. Animal cell cultures are broadly classified into primary cultures, cell lines and cell strains. Primary cultures originate directly from tissue explants or cell suspensions and have a limited proliferation capacity before growth ceases (Hasan et al. 2023). Cell lines are capable of continuous propagation through multiple passages, often due to transformation, but may exhibit genetic instability and aneuploidy (Piwocka et al. 2024). Cell strains are derived from primary cultures and, despite passaging, retain certain original cellular properties. Table 1 provides a comparative overview of these cell culture types, summarizing their advantages, disadvantages, and primary applications.

There are interesting contrasts between primary cultures and established cell lines. Primary cultures, such as BC160, are closer to the *in vivo* state but are more laborious to maintain, while established cell lines, like KG55T, offer ease of use but may not fully represent the original tissue's biology (Matsuura 1983; Honegger 1999). Additionally, the timing of subculturing can significantly affect the properties of cultured cells, as seen with neural stem cells, where day 7 was optimal for passaging to maintain cell vitality and differentiation potential (Xiong et al. 2011).

Timeline of Animal Cell Culture Development

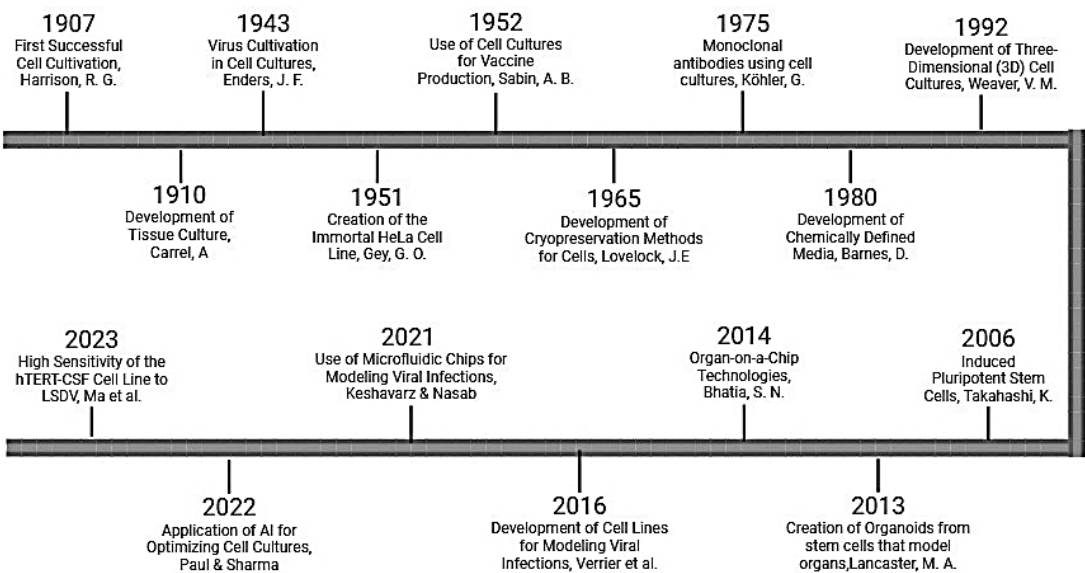


Fig. 1: Timeline of the most significant events in the development of cell cultures.

Table 1: Comparison of Cell Culture Types

| No. | Type of cell culture  | Advantages                                           | Disadvantages                                 | Area of application                     |
|-----|-----------------------|------------------------------------------------------|-----------------------------------------------|-----------------------------------------|
| 1   | Primary cell cultures | High physiological relevance                         | Limited proliferation, difficulty maintaining | Study of viral pathogenesis, toxicology |
| 2   | Cell lines            | Easy to culture, reproducible                        | Risk of genetic instability                   | Vaccinology, drug testing               |
| 3   | Cell strains          | Resistance to passaging                              | Gradual loss of original properties           | Long-term studies of viruses            |
| 4   | 3D cell culture       | Close to <i>in vivo</i> simulation, high sensitivity | High costs, difficult to reproduce            | Modeling of infectious processes        |

Table 2: Examples of Viruses and Suitable Cell Cultures for Their Study

| Virus                                         | Cell Culture Type                                     | Application                                       | References                                          |
|-----------------------------------------------|-------------------------------------------------------|---------------------------------------------------|-----------------------------------------------------|
| Lumpy Skin Disease Virus (LSDV)               | Vero, MDBK, hTERT-CSF, BHK-21                         | Replication study, vaccine production             | Ma et al. 2023; Kumar et al. 2021; Wang et al. 2022 |
| Influenza A Virus (IAV)                       | MDCK, Vero, HEK293                                    | Replication optimization, vaccine production      | Rüdiger et al. 2019                                 |
| Hepatitis C Virus (HCV)                       | JFH-1 cell line                                       | Life cycle study, antiviral strategies            | Wakita 2016                                         |
| Hepatitis B и D Virus (HBV, HDV)              | NTCP-expressing cell lines                            | Infection modeling, therapeutic strategies        | Verrier et al. 2016                                 |
| Dengue Virus (DENV)                           | Primary cultures, mouse models, primate cell cultures | Vaccine evaluation, pathogenesis study            | Kayesh et al. 2022; Zompi and Harris 2012           |
| Crimean-Congo Hemorrhagic Fever Virus (CCHFV) | Primary cultures, mouse models                        | Immune response evaluation, vaccine development   | He et al. 2016                                      |
| Rift Valley Fever Virus (RVFV)                | CelCradleTM-500A Bioreactors                          | Vaccine virus production                          | Rhazi et al. 2021                                   |
| Hepatitis E Virus (HEV)                       | HepG2/C3A cells                                       | Replication and pathogenesis study                | Meister et al. 2019                                 |
| SARS-CoV-2                                    | Vero E6, Calu-3, Caco-2                               | Viral entry mechanism, antiviral testing          | Smith et al. 2022                                   |
| Foot-and-Mouth Disease Virus (FMDV)           | BHK-21 cells                                          | Vaccine testing, neutralizing antibody evaluation | Foglia et al. 2022                                  |

For a more detailed understanding of the cell cultures used for studying various viruses and their respective purposes, Table 2 presents examples of viruses, the corresponding types of cell cultures, and their key research applications.

As shown in Table 2, different viruses require specific cell cultures optimized for replication and pathogenesis studies. This information provides guidance on selecting appropriate cell cultures to achieve accurate and reproducible results in virological research.

Primary cell cultures are valued for their physiological relevance but are limited by short lifespans and labor-intensive maintenance. Subculturing, or passaging, allows continued cell growth but requires precise timing to preserve cell characteristics (Chalak et al. 2024).

Established cell lines offer ease of maintenance and reproducibility but may develop genetic instability and aneuploidy over time (Piwocka et al. 2024). Cell strains balance these qualities, retaining original cellular properties after multiple passages.

Traditional methods, including primary culture establishment and subculturing techniques, laid the groundwork for modern cell culture technology and remain essential tools in viral research (Spier 1992; Zhong et al. 2009; Dirks 2021). These approaches continue to be relevant for applications such as viral pathogenesis studies, toxicology assessments, and vaccine development.

Advances in second- and third-generation sequencing technologies enable precise genomic analysis of cell

cultures (Kazim et al. 2024). New chemically defined and protein-free media formulations offer improved control over cell environments, enhancing growth and productivity (Park et al. 2023). Bioreactor technology has enabled better control over key parameters, such as oxygen supply and redox potential, which are vital for cell physiology and viral protein expression (Ge et al. 2023).

While traditional methods remain indispensable, modern technologies have revolutionized cell culture practices, improving precision, scalability and applicability across virology and biomedical research.

### **The application of animal cell cultures in the study of lumpy skin disease virus**

Lumpy Skin Disease Virus (LSDV), which causes a contagious disease in cattle, belongs to the genus Capripoxvirus (Khalafalla 2022). Despite extensive research, significant gaps remain in understanding the mechanisms of LSDV transmission and the role of vectors, particularly given the virus's ability to adapt to diverse environments and the emergence of recombinant strains (Sprygin et al. 2020; Shumilova et al. 2022; Wilhelm and Ward 2023).

LSDV induces fever, nodular skin lesions, and significant economic losses, especially among susceptible herds (Akther et al. 2023). Interestingly, although the virus is transmitted by blood-feeding insects, studies suggest they act primarily as mechanical vectors, as the virus does not productively replicate in insect cell lines (Tuppurainen et al. 2011; Cook et al. 2019).

Cell cultures play a central role in studying LSDV, providing a controlled environment for investigating viral replication, pathogenesis, and the development of therapeutic interventions (Chervyakova et al. 2019; Orynbayev et al. 2021). For example, the use of MDBK (Madin-Darby Bovine Kidney) cells and chicken chorioallantoic membranes (CAM) enabled researchers to study the role of the viral superoxide dismutase (SOD) homolog in viral growth and histopathological changes (Douglass et al. 2020). In another study, complete inactivation of the Neethling vaccine strain was confirmed following treatment with binary ethylenimine (BEI), inducing a robust immune response in rabbits (Hamdi et al. 2020; Matsiela et al. 2022).

LSDV has also been successfully propagated in lamb testes cell cultures, where pronounced cytopathic effects (CPE) were observed (Khan et al. 2021). In India, the virus was isolated using primary goat cells and later adapted to Vero cells, although CPE was not consistently observed (Kumar et al. 2021). Similarly, successful virus isolation was reported using MDBK cell cultures in China (Wei et al. 2023). However, studies reveal inconsistencies regarding the suitability of Vero cells for primary LSDV isolation, potentially due to differences in viral strains or culture conditions (Kumar et al. 2021; Wang et al. 2022).

Vaccine development for LSDV relies on various cell cultures for virus propagation and the production of live attenuated vaccines. The hTERT-CSF cell line demonstrated the highest viral load, highlighting its potential for vaccine production (Ma et al. 2023). Similarly, the BHK-21 cell line has shown suitability for producing attenuated LSDV vaccines (van Diepen et al. 2021). However, challenges remain concerning genetic stability,

The integration of Process Analytical Technology (PAT) and Quality by Design (QbD) principles ensures reproducibility and product quality in cell-based biopharmaceutical production (Gibbons et al. 2023). Additionally, transient transfection assays have become more refined, streamlining the production of therapeutic proteins (Porosk et al. 2022).

safety and immunogenicity of vaccine strains (Haegeman et al. 2021; Chervyakova et al. 2022). The use of bioreactors has further improved the efficiency of vaccine production (Rhazi et al. 2021).

Serological diagnostic systems for LSDV require substantial viral biomass production. Cell lines such as hTERT-CSF and BHK-21 have shown high susceptibility to LSDV, making them suitable candidates for propagating the virus and producing recombinant proteins (van Diepen et al. 2021; Ma et al. 2023).

Cell cultures remain indispensable tools for studying molecular mechanisms of LSDV infection, evaluating vaccine candidates, and understanding virus-host interactions. These systems are critical for developing effective strategies to combat Lumpy Skin Disease.

### **Challenges and prospects for research development**

The current landscape of cell culture research is marked by both challenges and promising prospects. A major challenge is the standardization of cell cultivation methods, which is crucial for reproducibility and scientific progress (Seisenov et al. 2023; Pamies et al. 2024; Kulpiisova et al. 2024). The lack of standardized protocols and documentation methods can reduce laboratory efficiency and jeopardize the reproducibility of results, a foundational requirement in scientific research (Batista Leite et al. 2024). Moreover, data reproducibility issues are particularly pertinent in certain fields, such as research involving variable genomic integrity (VGI), where the characteristics of the data itself pose difficulties. Another challenge is the need for cell cultivation methods that can accurately predict the biocompatibility of materials, which is essential for research and development in biomaterials (Yadav et al. 2024). Additionally, the development of *in vitro* models that accurately reflect physiological systems and organs remains an ongoing endeavor, as contemporary methods continue to strive to overcome the limitations of standard approaches (Bajek and Tylkowski 2021).

Despite these challenges, significant prospects exist for advancing research. Advances in the cultivation technologies of human pluripotent stem cells (hPSCs) address critical issues related to safety, quality control, and cost management, which are essential for the clinical translation of cell therapy products (Ozawa et al. 2023; Kulpiisova et al. 2024). The evolution of cell cultivation methods, including 3D organoid models, facilitates the development of personalized medicine and translational research (Preksha et al. 2021; Bulekov et al. 2023). Moreover, the validation of "in-field" cell cultivation protocols expands the capabilities of biobehavioral research in local and remote field conditions (McDade et al. 2021). Although this field faces challenges associated with standardization and reproducibility, the continual development of new cell cultivation methods and the integration of innovative technologies open exciting prospects for advancing research and clinical applications

(Kargaeyeva et al. 2023). The future of cell culture research promises more personalized and precise medical interventions, as well as broader accessibility to complex research methodologies beyond traditional laboratory settings (Waldstein et al. 2024). The development of cell lines resistant to common pollutants is a pertinent area of research. Studies have shown that environmental pollutants can induce neurotoxicity and developmental neural toxicity, and human-derived neural cell lines, such as the SH-SY5Y neuroblastoma cell line, are utilized to investigate these effects (Li et al. 2023). Additionally, primary cell lines, like the BC160 breast cancer culture, are valuable for cancer research, although they present challenges in isolation and maintenance, which can lead to a preference for established cell lines (Piwocka et al. 2024).

Among the promising directions for advancing cell cultures in virology is the adoption of three-dimensional (3D) models such as organoids, organ-on-a-chip systems, and spheroids (Li et al. 2023). Organoids mimic entire organs, providing more accurate *in vivo*-like conditions, while organ-on-a-chip devices simulate organ functions, facilitating targeted studies of viral effects. Spheroids offer improved 3D tissue organization, enhancing the understanding of viral growth and pathogenesis. These 3D models are essential complements to traditional approaches and could play a pivotal role in future virological research.

## Conclusion

Animal cell cultures are crucial tools in virological research, offering a controlled environment to study virus growth, replication, and host-pathogen interactions. Advances in cell culture technologies, particularly the development of 3D cultures, have enhanced the relevance of *in vitro* models, making them more reflective of living organisms. This improvement is vital for understanding virus-host dynamics and developing effective therapies. However, challenges such as scalability, reproducibility, and standardization of 3D culture methods persist and require ongoing attention. The integration of AI into cell culture research offers promising avenues for innovation. AI can enhance data analysis, predictive modeling, and the optimization of culture conditions, potentially revolutionizing virological research, although challenges in data integration and model accuracy remain. Additionally, novel media formulations that closely mimic human blood plasma could significantly advance our understanding of viral pathogenesis and improve antiviral drug efficacy. However, these new media require careful validation to ensure they meet the diverse needs of different cell types and experimental setups.

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