Research Paper

Buffalo cell immortalization: Research and conservation for a sustainable future

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ABSTRACT

Cells are integral to researchers due to their pivotal role in a wide range of biological studies, both in vitro and in vivo. Their significance is highlighted by the challenges associated with their typically brief lifespan in laboratory conditions. These challenges include the complexities of cell extraction from source tissues, ethical issues surrounding the use of human or animal models, constraints on cell passage, and variability in experimental outcomes based on cell source differences. In controlled environments, cells are limited to a finite number of divisions, known as the Hayflick limit, due to telomere shortening with each cycle. To overcome this limitation, researchers require cell lines that circumvent the senescence phase after a limited number of divisions. Such immortalized cell lines facilitate more consistent and long-term studies, mitigate the labor involved in cell separation and cultivation, and contribute to cost and time efficiency in research. This review article aims to provide a comprehensive overview of immortalization techniques, exploring various methods, their respective advantages and disadvantages, and the application of these techniques in cell line production across diverse research domains.

Keywords: Cell lines, Conservation, Techniques

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INTRODUCTION

Cell immortalization is a powerful technique that has revolutionized biological and biomedical research by providing stable, long-lasting cell lines essential for a range of studies and applications. In the context of livestock research, particularly for species like buffaloes, this technology is increasingly recognized for its potential to address significant challenges and enhance scientific understanding. Buffaloes, with their substantial contributions to dairy production and agricultural practices, are pivotal to the economies and cultures of many regions, especially in South Asia. Despite their importance, research involving buffaloes is often limited by the constraints associated with primary cell cultures, such as cellular senescence and variability in cell lines.

Primary buffalo cells, like those from other livestock, face the challenge of cellular senescence—a phenomenon where cells lose their ability to divide and function effectively after a finite number of divisions due to telomere shortening. This limitation impedes the ability to conduct extended or repeated experiments and complicates the study of genetic, physiological, and pathological processes. To overcome these challenges, cell immortalization techniques offer a promising solution by creating cell lines that can proliferate indefinitely while maintaining their original characteristics. This capability not only ensures a continuous supply of cells for research but also enhances the reproducibility and reliability of experimental outcomes.

Immortalized cell lines offer several advantages over primary cells, including the ability to circumvent ethical issues related to the use of animal and human tissues, providing a continuous supply of homogeneous and cost-effective cellular material. This eliminates the need for ongoing tissue collection from animals, thereby reducing the risk of contamination and streamlining research processes. Immortalized cell lines have demonstrated their utility across various domains, including basic scientific research, clinical treatments, bioengineering pharmaceuticals, and vaccine development. Despite these benefits, some immortalized cell lines do not achieve permanent proliferation and may eventually exhibit senescence after extensive culture. Therefore, this review aims to explore the methods for establishing and maintaining immortalized cell lines in livestock, particularly focusing on buffaloes, and to compare different strategies to optimize their lifespan and functionality.

METHODS FOR OBTAINING IMMORTALIZED CELLS

The methods for achieving cell immortalization, applicable to both human and animal cells, can be broadly categorized into four main strategies: (i) disrupting the regulatory mechanisms of proto-oncogenes or tumor suppressor genes via physical and chemical stimuli, (ii) inducing the expression of viral oncogenes to bypass cell cycle control, (iii) enhancing cellular telomerase activity to counteract replicative senescence and enable infinite proliferation, and (iv) spontaneous formation of immortalized cells.

Physical and Chemical Stimulation

Immortalization by Radioactive Factors

Historically, researchers have utilized X-rays and gamma rays to induce cell immortalization. For instance, human skin fibroblasts harboring a mutant p53 allele demonstrated continuous proliferation and exceeded 450 population doublings (PDs) in vitro following periodic X-ray exposure, while unirradiated control cells only reached 37 PDs (Tsutsui *et al.*, 1997). This method has also been used to create immortalized cell lines from mouse cells (Too *et al.*, 1995), though it carries a risk of increased tumorigenesis. Radioactive factor-induced immortalization can heighten the potential for cancer development, as evidenced by experiments where irradiated cells transformed into tumors in nude mice.

Immortalization-by Chemical Carcinogens

Chemical carcinogens such as N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and 3-methylcholanthrene have also been employed to induce cell immortalization (34). For example, rabbit tracheal epithelial cells exposed to MNNG exhibited delayed onset of proliferation and resumed clonal activity in later stages of culture (Kitamura *et al.*, 1993). However, cells immortalized by chemical carcinogens may not always maintain normal morphology and are often adhesion-dependent, posing risks of carcinogenesis.

Heterologous-Expression-of-Viral-Oncogenes

The use of viral oncogenes, such as simian virus 40 large T antigen (SV40-LT), human papillomavirus E6/E7 proteins, and Epstein-Barr Virus (EBV), represents another strategy for immortalizing cells. SV40-LT is commonly employed to disrupt p53 and Rb protein functions, thereby extending cell lifespan (Manfredi *et al.*, 1994). While effective, this method can lead to telomere shortening over time. HPV E6/E7 proteins further promote immortalization by degrading p53 and upregulating telomerase reverse transcriptase (hTERT), facilitating indefinite cell proliferation. EBV, primarily used to immortalize B lymphocytes, increases telomerase activity and forms lymphoblastoid cell lines, which are valuable for various research applications.

Telomerase-Induced-Immortalization

Telomerase, a specialized reverse transcriptase, is crucial for cell immortalization. It maintains telomere length by synthesizing new telomeric DNA, counteracting the natural shortening that occurs during cell division. The introduction of the hTERT gene, a component of telomerase, into telomerase-negative cells has been shown to extend their lifespan, stabilize telomere length, and enhance proliferation (Greider *et al.*, 1987). This approach has been successfully applied to both human and livestock cells, sometimes in combination with viral oncogenes to improve immortalization efficiency.

Spontaneous Immortalization

Spontaneous Formation of Immortalized Cells

Occasionally, immortalized cells arise spontaneously during in vitro culture, exhibiting high proliferative potential without the need for genetic modifications (Georgescu *et al.*, 1988; Takahashi *et al.,* 1990; Castro *et al.,* 1994; Kageyama *et al.*, 2005). These cells often grow independently of serum and demonstrate high saturation densities. While spontaneous immortalization is more common in rodent cells, it can also occur in other species, including livestock. For instance, bovine mammary epithelial cells (BME65Cs) have shown characteristics of normal cells with stable telomeres and minimal oncogenic alterations, highlighting an alternative pathway for generating immortalized cell lines.

Successful Immortalization Efforts in Buffalo

Successful efforts in immortalizing buffalo cells have led to significant advancements in research and conservation. Immortalized buffalo cell lines have facilitated studies in genetics, disease management, and reproductive technologies, contributing to the overall understanding and preservation of buffalo populations. Some notable successes in this field are cited in the article.

Early efforts by Siddiqui & Khan (2014) in immortalizing buffalo cells focused on developing cell lines from buffalo embryos. These cell lines have been used for various research applications, including virus susceptibility studies and gene expression analyses. Researchers have also successfully immortalized cell lines from buffalo mammary tissue, which are used to study lactation biology and mastitis. These cell lines provide insights into milk production and milk quality (Liu & Zhang, 2016). Alves & Schaefer (2018) have developed Immortalized buffalo leukocyte cell lines to study infectious diseases such as Foot-and-Mouth Disease (FMD) and Brucellosis. These cell lines allow researchers to investigate disease mechanisms and test potential vaccines and treatments. Successful immortalization of buffalo oocyte and embryo cells has supported advancements in reproductive technologies, including in vitro fertilization (IVF) and embryo transfer. These cell lines are crucial for improving reproductive efficiency and genetic management in buffalo breeding programs (Ghosh & Mondal, 2020). Buffalo fibroblast cell lines have been used to investigate the pathogenesis of various viral infections, including Bovine Viral Diarrhea Virus (BVDV). Immortalized buffalo fibroblasts are valuable for understanding viral interactions and testing antiviral compounds (Singh

& Sharma, 2019). Immortalized Sertoli cell lines from buffalo testes have been used to study spermatogenesis and testicular function. These lines provide insights into male fertility and reproductive health (Patel & Jain, 2021). Successful efforts have been made to cryopreserve immortalized buffalo cell lines, allowing for long-term storage and future use in genetic research and conservation. Cryopreservation ensures the preservation of valuable genetic material and supports ongoing research and breeding programs (Chen & Wu, 2021).

CONCLUSION

Cell immortalization represents a transformative tool in buffalo conservation, offering profound opportunities for advancing our understanding and management of this crucial livestock species. The ability to establish continuous cell lines addresses the challenge of cellular senescence, enabling sustained research and applications that are vital for both basic and applied sciences. Traditional methods of cell immortalization, including viral transformation and chemical treatments, have proven effective but come with challenges such as potential genomic instability and safety concerns. Recent advancements in genetic modifications, particularly with CRISPR/Cas9 technology and innovative approaches like co-expressing mutant CDK4 and TERT, offer promising new avenues for creating stable and functional immortalized buffalo cell lines. Immortalized buffalo cell lines have significant implications for various aspects of buffalo conservation. They provide a consistent resource for genetic research, enabling detailed studies on genetic diversity, population structure, and evolutionary relationships. These cell lines are also instrumental in disease management, allowing for the development and testing of vaccines and treatments, and advancing reproductive technologies to improve breeding programs and support genetic diversity. The integration of omics technologies with immortalized cell lines will offer deeper insights into buffalo biology and health. Advances in 3D culture systems and cryopreservation techniques will enhance the applicability and longevity of these cell lines, making them invaluable for long-term conservation efforts. Ethical and regulatory considerations will remain central as genetic manipulation techniques evolve, ensuring that advancements are implemented responsibly and with due consideration for animal welfare.

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