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The role of pH in growth of BHK-21

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Abstract
Over the past century, the study of cell culture has proliferated, leading to the development of several viral vaccine production. Since cell culture technology was first developed, the primary media GMEM is used for BHK 21 cells for vaccine production. BHK 21 cells are anchorage-dependent cells that can be grown or cultured on the polystyrene-based surface and mainly depend on the environment of the media required. Along with serum, the pH of the medium is generally essential for cell cultures. The balance between sodium bicarbonate ($NaHCO_3$) in the culture medium and CO_2 in the incubator typically maintains the pH of the medium for cell cultures. In this study, the different pH media namely acidic (pH-6.5), normal (pH 7.2-7.4), and alkali (pH-8) were examined for their effect on cell growth.

Keywords: Cell culture, BHK-21, media, pH

1. Introduction
The BHK-21 cells are an anchorage-dependent continuous line of kidney cells of baby hamster developed by Macpherson and Stoker in 1962 [1]. At the industrial level, there are restrictions on the usual tissue culture practice of regulating pH by altering the carbon dioxide content in connection with the bicarbonate buffer present in the medium [2]. The required CO_2 concentration changes as cells develop, and at large cell densities, efficient control is challenging [3]. Since metabolism inside cells naturally tends to create an acid drift, the cell culture's pH (7.2- 7.4) may be controlled by the administration of either acid or alkali in media [4]. The color of the media changes from red to orange, as cell growth increases, because the pH changes against buffers occur due to the cell growth.

2. Materials and Methods
A line of Baby hamster kidney cells (clone 13) is sub-cultured (MP19) and propagated at the Author's research laboratory, which was used in this study. The growth media used for the proliferation of cells was Glasgow Modified Essential Medium (GMEM, Himedia, India) with 10% foetal bovine serum (M P Biomedicals, South America).

2.1 Preparation of media
Three different type of media was prepared namely acidic (pH-6.6), normal (pH-7.2) and alkali (pH- 8) (Fig. 1). In accordance with the requirements, all necessary reagents such as GMEM (Himedia, India), peptone (Himedia, India), $NaHCO_3$ (Himedia, India), and antibiotics like penicillin G (Himedia, India), Streptomycin sulphate (Sigma, USA) and Kanamycin sulphate (Sigma, USA) were weighed and dissolved in autoclaved double distilled water. Sterile-prepared phenol red (Amresco, US) was mixed in media as a pH indicator. The pH was checked before filtration, which should be 7.2-7.4, and adjusted by filtered 1N HCl (Himedia, India). The media were sterilized by filtration using a filter assembly with 0.22 µM filter paper. A aliquote of the prepared media was kept for sterility testing and incubated for 24 h at 37 ℃. Sterility-tested Media was used for cell subculture work.

Table 1: Three different type of media was prepared namely acidic

S. No.	Growth media color	pH
1	Yellow to orange	Acidic (Below 6.7)
2	Pink to red	Appropriate (Near 7.2-7.4)
3	Dark pink to purple (fuchsia)	Alkaline (Above 8)

2.2 Revival of cells
BHK21 cells were recovered from frozen storage cryovials by rapid thawing vials in a water bath at 37 ℃.

The cryovials were wiped with ethanol and cells were resuspended in GMEM media and transferred to a 15 mL centrifuge tube. Centrifugation was done at 800 rpm for 10 min. The supernatant was removed, and the pellet was resuspended in prewarmed growth media, transferred to a new cell culture T-25 cc flask (Thermo Scientific, USA), and incubated in a 5% CO_2 incubator at 37 ℃.

2.3 Sub-culture of BHK21 cells

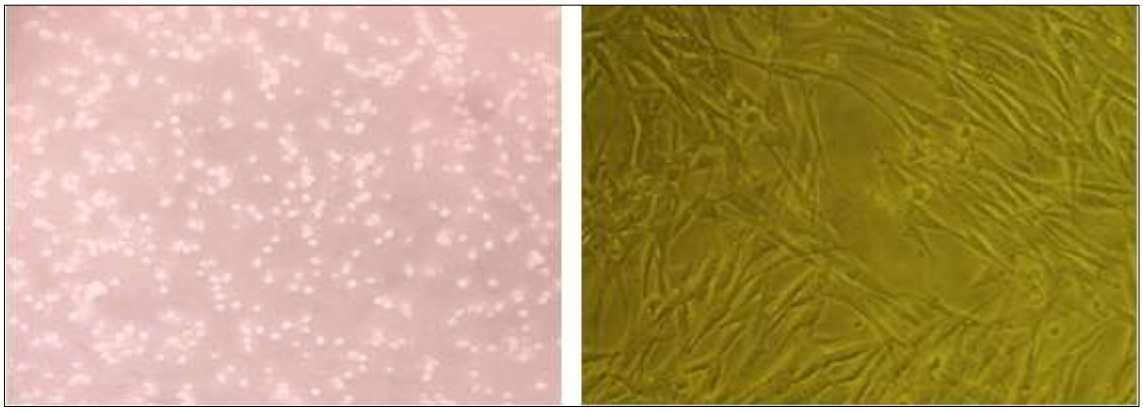
A fully confluent monolayer cell culture flask was sub-cultured in a BSL II laminar airflow as discussed below. First, spent media was discarded from the flask by decanting. The cell monolayer was washed by the plain media first followed by a wash with 1X TVG. The cells are trypsinized by treating with 1X TVG solution and incubating for 1 min at 37 ℃. After incubation, the flask is tapped gently to shred the cells from the surface. The cells were dissociated by gentle pipetting by a serological glass pipette fitted with a rubber bulb to make a single-cell suspension. The dissociation of cells was examined under an inverted microscope (Nikon, Japan). Cells suspension was split into the desired flask and growth media were added and incubated at 37 ℃ with 5% CO_2. For the scaling-up of cells, one T-75 cc flask was made from one T-25 cc flask and will be used for making one T-175 cc flask and the roller flask was made up of three such T-175 cc flasks. Roller flasks (Greiner Bio-One, Germany) were incubated with 150-200 mL growth media using roller incubator at 37 ℃. Different roller flask was added with three different media namely acidic (pH-6.5), appropriate cell growth pH (pH-7.2) and alkali (pH-8). For each media triplicates of roller flask were prepared. Roller flasks were used finally for bulk production of virus antigen.

3. Results and discussion

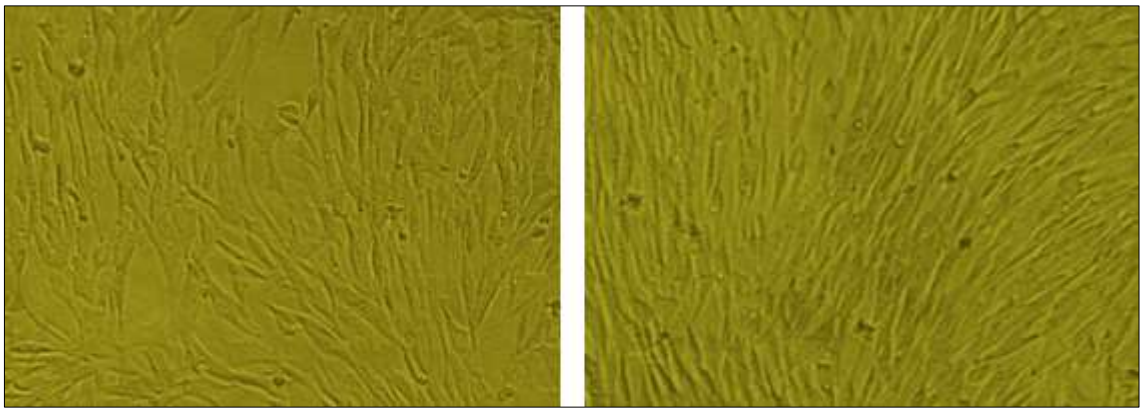
In the present study, cell growth was found virtuous in the presence of normal cell growth media (pH-7.2) in vented or non-vented T-25 cc flask at 37 ℃ in a 5% CO_2 incubator (fig 2). In the case of acidic media (pH-6.6) cell growth was found not satisfactory, only some cells were attached followed by detachment from the surface of the flask whereas alkaline media (pH-8) was not supported the attachment and growth of cells. This study focuses on the relevance of the two vital factors carbon dioxide (CO_2), pH, and their aligned effects in the BHK-21 cell culture. In the presence of atmospheric 5% CO_2 cells are used to grow using buffered media with $NaHCO_3$ (sodium bicarbonate) and maintain a pH of 7.2-7.4 [5]. As the T-25 cc flask, roller flasks were also observed for cell growth in different pH of culture media. Under normal pH (7.2-7.4) the growth of the cells was poor because the absence of CO_2 in the roller flask resulted in an alkali environment (fuchsia color) (figure 3) and the reduction of pH (6.9 -7) supports the growth of cells when roller flasks were inoculated with CO_2 directly and kept on rotator at 37 ℃. Dissolved CO_2 hydration originates bicarbonate (HCO_3-) and H+ equilibriums which play a crucial role in preserving homeostasis with bicarbonate buffer.

A B C

Fig 1: Different pH of media a) Acidic (pH- 6.5), b) Normal (pH-7.2-7.4), and c) alkaline (pH-8).

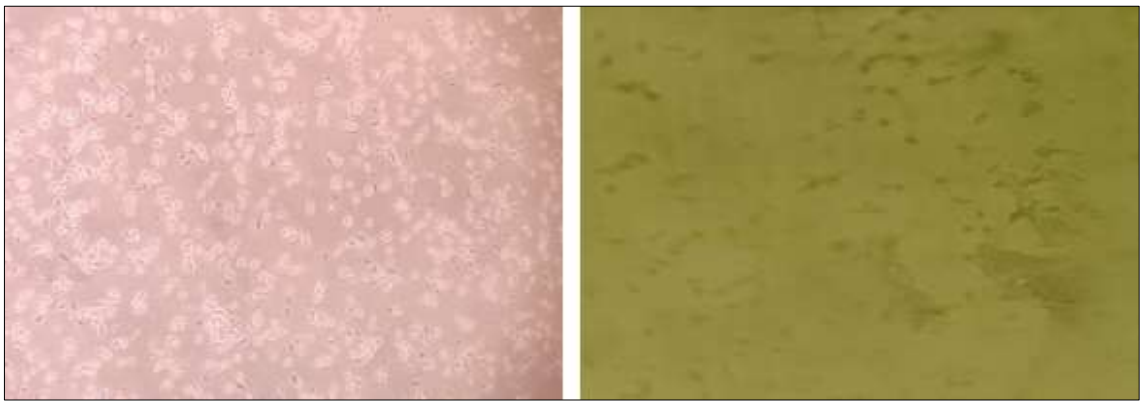


A B



C D

Fig 2: Microscopic pictures representing growth of BHK-21 cells in presence of normal media (pH-7.2-7.4) in T-25cc flask at a) 0 h b) 6 h c) 12 h d) 48 h



A B

Fig 3: Microscopic picture representing attachment of BHK-21 cells in presence of normal media (pH-7.2-7.4) in roller flask. a) On first day BHK-21 cells attached on surface b) On second day monolayer was detached and floating in media because of pH imbalance in absence of CO_2.

4. Conclusion

The present study focuses on growth of BHK-21 cells growth in acidic, alkaline, and normal media in presence or absence of CO_2 in the T-25 cc flask and roller flask at 37 ℃. For the growth of cell culture, Carbon dioxide and pH are essential components. A trial was attempted to the effective comparison of cell growth in different pH media. Cells were grown at normal pH in T-25 cc flask, whereas in roller flask cell were grown at a slightly acidic pH 6.8-6.9 without CO_2. Since there is limited study on the efficiency of carbon dioxide and the data acquired on cell growth with the balanced pH so it is important to comprehend the process. The emphasised areas need to be thought about and tested in order to reveal various aspects that are still unexplored in order to understand cell physiology and develop cell culture processes.

5. Acknowledgment

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6. References

1. Park S, Kim JY, Ryu KH, Kim AY, Kim J, Ko YJ, *et al*. Production of a foot-and-mouth disease vaccine antigen using suspension-adapted BHK-21 cells in a bioreactor. Vaccines. 2021 May 13;9(5):505.
2. Dubey AK, Lavanya L, Sadananda D, Gouthami K, Elfansu K, Singh A. Inferences of Carbon Dioxide in Present-Day Cell Culture Systems: An Unacknowledged Problem and Perspectives. Austin Therapeutics. 2021;6(1):1033.
3. Kumar A, Ergas S, Yuan X, Sahu A, Zhang Q, Dewulf J, *et al*. Enhanced CO_2 fixation and biofuel production via microalgae: recent developments and future directions. Trends in biotechnology. 2010 Jul 1;28(7):371-80.
4. Radlett PJ. The use of BHK suspension cells for the production of foot and mouth disease vaccines. Vertrebrate Cell Culture I. 2005 Sep 24:129-46.
5. Nema R, Khare S. An animal cell culture: Advance technology for modern research; c2012.
6. Bell CL, Quinton PM. Effects of media buffer systems on growth and electrophysiologic characteristics of cultured sweat duct cells. In Vitro Cellular & Developmental Biology-Animal. 1991 Jan;27(1):47-54.