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Aasif Durrani
Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola (M.S.) India – 444001.

Arshiya Mirza
Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola (M.S.) India – 444001.

Zia H Khan
Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola (M.S.) India – 444001.

Naziya Khan
Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola (M.S.) India – 444001.

Surekha S Kulkarni
Department of Biochemistry, Dr. Panjabrao Deshmukh Memorial Medical College, Amravati. (M.S.) India.

Yusuf A Ali
Department of Microbiology, Jazan Medical College, Jazan (K.S.A.).

Correspondence
Aasif Durrani
Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola (M.S.) India – 444001.

Adaptation of mammalian cell from 10% serum medium to serum free or low serum media

Aasif Durrani, Arshiya Mirza, Zia H Khan, Naziya Khan, Surekha S Kulkarni, Yusuf A Ali

Abstract
A study was conducted to adaptation of mammalian cell from 10% serum medium to to serum free or low serum media, to subcultured cell line Baby hamster Kidney-21 (BHK-21) to optimize the *In vitro* culture requirement and conditions for maintenance and longtime cryopreservation. BHK-21 cells multiply fast during first 48 hrs and make a complete layer and got a confluence within 72 hrs post incubation, followed by a decline phase. Fetal bovine serum has a growth stimulating effect 10% to 3% serum level is satisfactory for the maintenance of the cell line. While harvesting the cells from a flask, Trypsin (0.25%) with neutralization by fetal bovine serum was found suitable. For cell storage 10% Dimethyl sulfoxide (DMSO) used to maintain maximum recovery of viable cells during cyropreservation.

Keywords: BHK-21, Fetal Bovine Serum, Dimethyl sulfoxide, Trypsin, Artificial growth medium.

1. Introduction
Culturing is a process of growing animal cells artificially. The most important and essential selection of the medium depends on the type of cells to be cultured. The purpose of animal cell culture can be growth differentiation, or even production of desired products like pharmaceutical compounds. Animal cells selected for culture are maintained as independent units. Cultures normally contain cells of one type (e.g. fibroblasts). Cell culture is widely used technique and is the main purpose of producing a variety of recombinant protein and vaccines (R. Stephen Sennott, 2003). Mammalian cell culture can be described as *in vitro* maintenance and propagation of animal cells using a suitable nutrient media.

1.1 Adaptation of cells to serum free medium
Some cell lines can be subcultured directly from a serum based to a serum free medium without loss of growth performance. However, in other cases cells may be adapted slowly into the new serum free medium. The process of adaptation may involve changes in cellular metabolism or the induction of specific cellular growth factors (Paul J., 1975). The cell line which are used for adaptation from different serum to serum free media are baby hamster kidney cells (BHK)
The BHK cell line was derived from baby serian hamster (Mesocricetus auratus) kidney (Macpheron and Stoker, 1962)

2. Material and Methods
2.1 Cell line
BHK cells were obtained from the American culture collection type, HiMedia Loboratories Pvt. Ltd.-23., Vadhani, Ind. Est., LBS Marg, Mumbai.

2.2 Cultures
DMEM, 1x: was [DULBECOS MODIFIED EAGLES] medium (gibco) with 4.5 g/l glucose 4.0 MM-l- glutamine and sodium pyruvate. (Storage at 2 °C to 8 °C) obtained from HiMedia Laboratory, Mumbai, India.

2.3 Adaptation of BHK Cells from Different Serum Concentration

All materials required for experiment such as serum, media, phosphate buffer, trypsin, DMSO (Di-Methyl Sulfo Oxide) were kept in water bath at 37 °C for 5 to 10 minutes. Above reagents are then kept under U.V. light of laminar air flow for 10 to 15 mins. BHK cells were obtained from the American type culture collection (ATCC) in T_{24} flask. Now media from BHK cells was discarded in sterile flask. Cells remained in T_{24} flask were then washed with phosphate buffer having pH 7.4 and discarded after few minutes. Now 0.5 ml of trypsin was added and incubate for 5 to 10 minutes at room temperature. In above trypsinized sample, 3 ml of fetal bovin serum was added and cell were passage for 2 to 3 miunutes. From above 1 ml of sample was incubated in new flask containing 9 ml of media. In remaining 2 ml of trypsinized FBS flask 0.2 ml of DMSO was added. 1 ml of above sample was used for cell counting and 1 ml was preserved for prepation of cell bank.

2.4 Cell count

1 ml of sample (passaged cell + DMSO) was taken in an ependroff tube and then used haemocytometer to count the cells.

3. Results and Discussion

After the cell counting initial concentration of cells with 10% serum (1ml serum + 9ml media) was 78.75 x 10^4 cells per ml with 3ml of original suspension was added that became 0.78 x 10^6 with main value of 2.34 x 10 cells per ml.

After 24 hours incubation with 8% serum (0.8ml serum + 9.2ml media) an increase in life cell population i.e, 79.5 x 10^6 when added 3ml original cell suspension that became 0.79 x 10^6 with main value of 2.37 x 10^6 cells per ml. During next 24 hours (48 hours post incubation) the life cell number was increased with again 8% serum i.e, 89.64 x 10^6 that became 0.89 x 10^6 after multiplied by 3ml of original cell suspension with main value 2.67 x 10^6 cells per ml however a slight increase in life cell population was observed in the next 24 (72 hours post incubation) again with 8% serum that is 92.78 x 10^4 (0.92 x 10^6) with main value 2.76 x 10^6 a complete monolayer was observed on the glass surface. There was a declined in the cell number with 4% serum (0.4ml serum + 9.6ml media) in the 96 hour post incubation (24 hours) i.e, is 46.25 x 10^4 (0.46 x 10^6) with main value 1.38 x 10^6 after 72 hours again subcultured with 4% serum there was increase in a live cell population slightly than again 4% serum 2 to 3 times then 3% serum was subcultured 2 to 3 times then 2% serum was subcultured 2 to 3 times then 1% serum was subcultured 2 to 3 times.

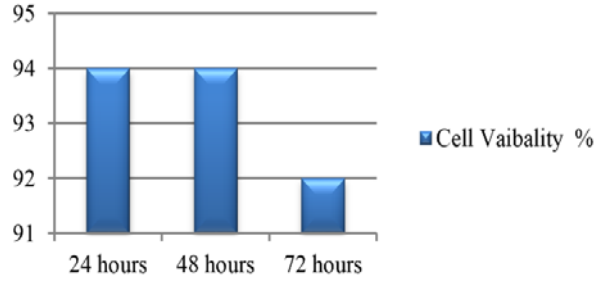

Fig 1: Different subculturing of cells in 8% of serum against Cell viability %

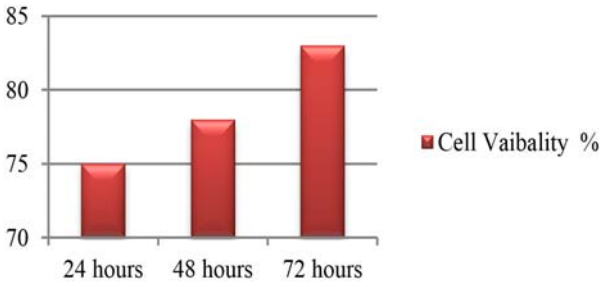

Fig 2: Different subculturing of cells in 4% of serum against Cell viability %

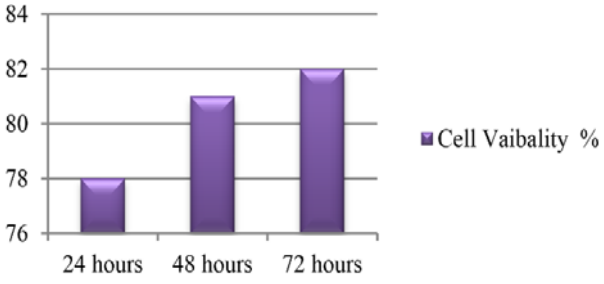

Fig 3: Different subculturing of cells in 3% of serum against Cell viability %

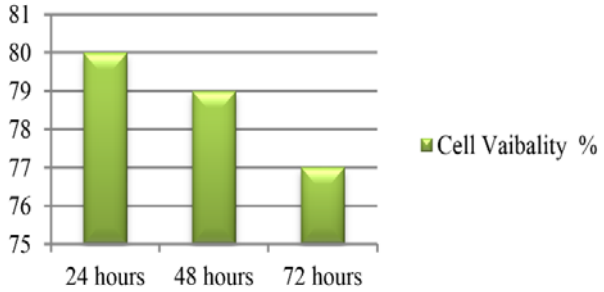

Fig 4: Different subculturing of cells in 2% of serum against Cell viability %

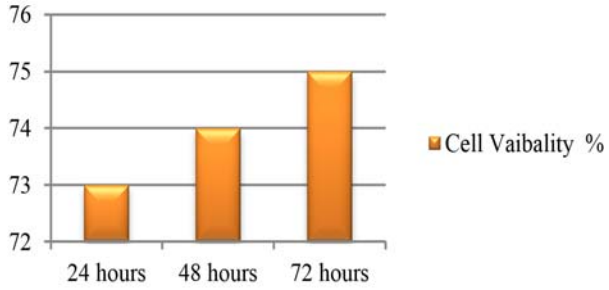

Fig 5: Different subculturing of cells in 1% of serum against Cell viability %

4. Discussion

Sequential serum free or low serum adaptation to the BHK-21cells was successfully studied, it was observed that cells shows no difference with respect to viability when shifted from 10% serum to 8% serum. Cell viability with 10% serum showed 95% viable cells and 5% dead cells. Cell viability with 8% serum showed 94% viable cells and 6% dead cells when the percentage of serum reduced in the further steps, the number of viable cells decreased as expected. The cells show shocking effects due to sudden shifting from 8% serum to 4% serum and the total number of cells/ml reduced from

2.76 x 10^6 cells/ml (inoculum of 4% serum) to 1.38 x 10^6 cells/ml after 24hrs with sharp reduction in cells viability i.e 92% (8% serum) to 75% (4% serum 24hrs), moreover the cells adopted new serum concentration (4%) after 48hrs of incubation and cell growth increased with not able increase in the viable cell count i.e 78%, by considering the performance of the cells after 48hrs, it was decided to keep some flask with 4% serum for 72hrs. As expected the cells recovered the growth pattern with increase in cell number from 1.86 x 10^6 cells/ml (48hrs) to 2.1 x 10^6 cells/ml (72hrs) with 83% cell viability in 4% serum, moreover little morphological changes in the cell structure is observed. Cells shows similar adaptation pattern when shifted from 4% serum to 3% serum with 78% cell viability after 24hrs that finally increased to 81% cell viability, moreover morphological changes in the cells structure is notable. Serum reduction to 2% increased the number of cells with irregular shapes with slight change in the cell viability after 72hrs i.e 77%. The cells adapted to 1% serum shows large number of cells with irregular shape and reduced the cell viability 73% after 24hrs, adaptation for 48hrs and 72hrs increased the number of cells 2.49 x 10^6 cells/ml but the morphological changes in cells was not appeared appreciable to continue the further reduction of serum.

5. Conclusion

The following conclusion has been drawn on the basis of present piece of work.

The BHK-21 cells successfully adapted to the 1% serum, but due the unaccepted morphological changes in the cell structure is not advisable to consider the cells adapted with 1% serum for research and commercial purpose. Because it is known that BHK-21 cells with irregular shape can't produce the protein of interest and can reduce the growth pattern if exposed with low serum for long period. Cells adapted to 2% serum carries the probability of increasing the number dead cells during the long period of cell growth and further passaging. Cells adapted with 3% and 4% serum shows little difference with respect of cell viability and growth pattern, moreover by considering the disadvantages of serum, it is preferable to proceed with 3% serum and to give cells more passages to have the consistence in the cell growth and viability. All the cells adapted at different percentage of serum preserved in the cryo-vials and further studies can be planned with the cells adopted with 3% serum.

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