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Devlopment and commercialization of cell based viral vaccines for animal health in national immunization in india

Abstract

Cell Biology is dynamic in synthesizing enzymes, proteins, carbohydrates, polysaccharides helping the living man and animals for continuous growth in mammalian system. Single cell protein as customized and designer cells are being produced for cloning, stem cell research in biology even artificially growing organs for transplantation in man and animals is of great importance in cell biology. National Immunization programme have helped to eradicate small pox and rinderpest from man and animals in this planet both are viral infectious disease which is a great threat to life. Therefore BHK 21, Vero cell line and many more are being exploited for production of vaccine for Polio and Foot and Mouth Disease (FMD) as a next level cost effective vaccine production to control Polio and FMD globally including Rabies in man and animals. In order to develop vaccines which need to be produced in large scale one has to first grow the viruses with which they want to develop the vaccines in large quantities and with great consistency. Compared with bacteria, which can be grown in a laboratory environment when placed in a suitable growth medium, viruses cannot reproduce on their own and require living cells to infect. After a virus infects a cell, it uses the cell's own components to produce more copies of itself. The cells can be used as a substrate for development of the vaccines and making the cell banks and virus bank. The Master Cell Bank of the cells can be used years together for the cell maintenance and can be used to expand the cells from T-175 to 3000 L in Bioreactor after that they will be infected with the virus against which one need to produce the vaccine, observation for development of cytopathic effect is carried out, then Harvested, Clarified and stored with labeling at proper temperature, which can be used for further formulation and filling and downstream processing. The type of media which is going to be used for normal culture of cells and infection varies significantly. In case of media which need to be used for growing the cells will be normal growth medium with defined percentage of serum but in case of the media which need to be used for infection will be serum free and called as virus infection media (VIM). The cells being used for production of the vaccine will be subjected to characterization in the context of identity, adventitious agent and other type of testing etc. The different types of cell lines will be used for the quality control testing and production as per the type of the vaccine to be produced. The BHK21, Vero, CHO, HeLa etc are some of the examples of the them, the monolayer and suspension characteristics of the cell line can be used differently depending upon the characteristics of the virus to be grown. As some viruses is having the characteristics to grow in monolayer whereas some in suspension and also some viruses in both monolayer and suspension.

Keywords: cell, bioreactor, vaccine, monolayer, suspension, virus infection media

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Abbreviations: FMD, foot and mouth disease; VIM, virus infection media; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; BHK2, baby hamster kidney; HEP, human epithelial cell; MDCK, madin darby canine kidney; MRC, medical research council; 3 day transfer; MA, microbiological associates; NCCS, national centre for cell science; ATCC, american type culture collection; IBN, infectious bursal disease; RD, ranikhet disease; MD, mareke's disease

Introduction

The cell in Latin is called as cell a which means "small room"[1] is the basic structural, functional, and biological unit of all every living organisms. Cells are the smallest and basic unit of every known living entity that can replicate independently, and are often called the "building blocks of life".

Cells constitute cytoplasm enclosed within a membrane, which contains many biomolecules like proteins and nucleic acids.[2] The number of cells in plants and animals varies from species to species, humans contain more than 10trillion (10^{13}) cells.[3]

The term cell is coined by Robert Hooke in 1665, Cell theory, first developed by Matthias Jakob Schleiden and Theodor Schwann in 1839, states that all organisms are composed of one or more of cells and that cells are the fundamental unit of structure and function in living organisms, that all cells came from preexisting cells, and that all cells contain the hereditary information necessary for regulating normal cell functions and for transmitting information to the next generation of cells.[4] It is stated that cells emerged on Earth at least 3.5billion years ago.[5,6]

Types of cells: prokaryotic and eukaryotic

Cells are of two types one is called as eukaryotic, which contain a nucleus, and another is prokaryotic, which do not contain the nucleus. Prokaryotes are single-celled organisms, while eukaryotes can be either single-celled or multicellular. Prokaryotic cells are simpler in structure and lack membrane bound organelles like nucleus and their size ranging from 0.5 to 2.0µm in diameter. The cell consists of cell envelope comprising of plasma membrane and covered by cell wall. Inside the cell cytoplasmic region which consists of genome,

ribosomes, and various sorts of inclusions, the Extra chromosomal DNA present in the prokaryotic called as plasmids which acts as a genetic material. Flagella and pili project out from the cell surface for the locomotion of the cells. The Bacteria and Archea consists of the prokaryotic type of cells in their structure.[7]

In contrast Plants, Animals, Fungi, Moulds, Protozoa etc consists of Eukaryotic cells which are more than 10-15times bigger than prokaryotic type of cells. Presence of membrane bound organelles with compartment inside the cell cytoplasm is the peculiar structure of the cell.

Components of the cell

The different primary components of both prokaryotic and eukaryotic cells are lies in the plasma membrane. Specifically Cell Membrane, Cytoskeleton, Genetic component, Cell organelles, Cell wall, Capsule, Flagella, Fimbria. The main function of these components is like in case of cell membrane which surrounds the cells and protects the cell from outer shock and protection from surrounding environment. The membrane works as semi permeable membrane which selectively permits the ions across the cell cytoplasm and surrounding environment. Cell surface receptor present on cell surface drives the signaling pathways across the cells.[9]

The cytoskeleton is structural organization of cell components like Microfilaments, intermediate filaments and microtubules organized which maintain the shape of cell and keep them in place. Endocytosis, cell division and cytokinesis are some key function of it. Actin, Tubulin, Vimentin, Desmin, Lamin and Keratin proteins are another constituent of cell skeleton. Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are two different types of genetic material present in the cells for storage of information. Prokaryotic genetic material is organized in circular DNA molecule in the nucleoid region of cytoplasm and Eukaryotic material is divided in to different linear molecule called as chromosomes and inside genetic material additionally discrete nucleus. Cell organelles are part of the cells responsible for carrying specialized vital functions. Both eukaryotic and prokaryotic cells have organelles, in case of prokaryotic organelles which are simpler and not membrane bound. In case of eukaryotic cells it consists of cell nucleus, Mitochondria and Chloroplast, Endoplasmic Reticulum, Golgi apparatus, Lysosomes and Peroxisomes, Centrosomes, and Vacuoles. The structure outside the cell consists of cell wall, Capsule, Flagella and Fimbria. All the components of the cell plays vital roles in the cellular processes.[10]

Cellular processes

Growth and metabolism of cell, protein synthesis, cell movement, cell specialization etc are some of the cellular processes. Cell metabolism is the process in which individual cells process the nutrient molecules for its growth and metabolism. The cell metabolism consists of catabolism and anabolism in which complex molecules will break down in to simpler ones and simple molecules will be constructed as complex molecules. Cell division involves replication of genetic materials and dividing into daughter cells. Binary fission in prokaryotic cell and mitosis in eukaryotic cells is the characteristics type of cell division observed in different cells. The proteins which are responsible for modulation and maintenance of cellular activities will be synthesized by the cells. The structure outside the cells called as flagella and cilia will be helpful for the cells for the movement for the purpose of finding food, processes during wound healing, immune responses and cancer metastasis.[11,12]

Wide applications of the cells

The cells have wide applications in different sectors of the life sciences and allied areas. Mainly Vaccines and Biologicals, Research and Development, Pharma & Biotech, Health care, consumer and life science research, cells for medical breakthrough, cancer research, drug development, gene therapy and more.

Applications of cells at industry level

The cells as a basic unit of every living organism, in context of the industry level also it plays a pivotal role in the development of the vaccines for human and veterinary use. The role of the cells is divided in to following context:

a. Development of Cell Bank

b. Development of seed viruses

c. Bulk Production

d. Quality Control Testing

e. Monolayer to suspension type of cell culture

Development of cell bank

The different cell lines which are being used in production of vaccines for human and veterinary use need to be stored properly in cryogenic temperature i.e. Liquid Nitrogen by developing the cell banks. The Master Cell Bank and Working Cell Bank are the two which every organization needs to maintain and which can be used for years together as their stock. These banks needs characterization in context of its sterility, Mycoplasma free status, Identity of the cell line, Adventitious agent status etc are the crucial testing one has to do after making the cell bank. The common cell lines used for the vaccines production lies in between Vero cells, Baby Hamster Kidney (BHK21) cells, Human Epithelial Cell line (HEP2), Madin Darby Canine Kidney cells (MDCK), Medical Research Council (MRC-5), 3Day Transfer (3T3) and Microbiological Associates (MA104) cell line etc. Depending upon the type of vaccine to be produced the cell lines are used. The firms which are engaged in the manufacture of the vaccine should have well characterized cell banks with proper label and storage. The primary source for getting the cells includes national and reference laboratories like National Centre for Cell Science (NCCS), American Type Culture Collection (ATCC), In vitrogen, Thermo Fisher Scientific etc.

Development of seed viruses

The seed viruses are also one of crucial commodity which the manufacture needs to be prepared and stored properly. The development of the seed virus according to type of the virus with proper characterization and labeling is mandatory. The development of the virus banks involves growing of the cells at a confluent level and infecting with desired type of virus and observing for cytopathic effect and then harvesting it and making the virus bank. The test which involves in the characterization of the virus and preparation of its bank includes its Sterility, Identity, Mycoplasma status, Titer etc. the common viruses and vaccines which are produced in human and veterinary uses with cell as a substrate includes Hepatitis A, B,E, Human Papilloma, Influenza, Japanese Encephalitis, Measles, Mumps, Polio, Rabies, Rota, Rubella etc and in case of veterinary vaccines includes conventional live and inactivates vaccines like Foot and Mouth Disease, Black Quarter, Haemorrgic Septicemia, Enterotoxaemia, Johne's Disease, Blue Tongue, Classical Swine Fever, Goat Pox, Anthrax, Sheep Pox, Peste Des Petits Ruminitis

and Poultry vaccines like Infectious Bursal Disease (IBD), Ranikhet Disease (RD), Mareke's Disease (MD), Fowl Pox, Fowl Cholera etc.

Bulk production

The master cell bank and master virus bank can be used for the bulk production of the antigen of the desired type of the virus and can be subjected with further downstream processing. The antigen production involves production of the small volume of the culture with the help of cells and virus and scaling it up to 3000 L in case of veterinary vaccines. The small scale starts with 1 L bottle culture to 125 L Bioreactor culture which continuously scaled up to 3000 L. The full grown bioreactor culture will be infected with desired type of virus on the basis of Multiplicity of Infection (MOI) and observed for cytopathic effect. Upon complete cytopathic effect it will be harvested and clarified and subjected for further processing. The bulk production of the vaccines depends upon characteristics of the virus type like in which cell type and cell line it will be grown. The monolayer and suspension are two types of the cells in which bulk antigen need to be produced. The large quantity of the bulk in case of suspension type of culture will be produced in bioreactor with suspension culture and cell stack or cell factory in case of monolayer cells.

Quality control testing

The vaccine as a finished product or in process bulk will be subjected to testing to release it for further usage. The following types of the testing will be carried out so as to release the product.

a. Virus Inactivation Kinetics

b. Virus Titration

c. Serum Neutralization Test

d. Virus Amplification Test

e. Virus Typing Test/Virus Indentity Test

The above tests are routinely used for the testing of the vaccines using cells as material for the testing; the brief description for the same is as follows.

Virus inactivation kinetics: Most of the vaccines are divided in to the live vaccines; Live attenuated and inactivated or killed vaccine, depending upon the type of vaccines the tests will be carried out. The virus Inactivation Tests is required for the inactivated or killed type of vaccine in which the inactivation of the particular virus will be assessed briefly the multiple samples will be collected during inactivation from start of the inactivation to the end of the inactivation with different intervals of time and they will be subjected to the testing in cell culture. The inactivated samples should not show any cytopathic effect in the cells which confirms its inactivation, the different time interval will provide the inactivation kinetics of that particular viral sample.

Virus titration: The infectivity potential of any viral harvest called as virus titre is the common test applies on all types of the viral vaccines. Briefly the viral sample will be harvested and will be diluted in different dilutions and will be incubated with monolayer cells and incubated for the defined time and observed for the cytopathic effect. The result will be calculated with Reed and Muench formula and expressed as TCID50/ml. The result of the virus titration will be helpful for the further formulation and filling.

Serum neutralization test: The Serum Neutralization Test abbreviated as SNT is the key test to know the titre of the antibody in the paired serum samples of the vaccinated animals or humans. Briefly the sera samples will be diluted in different dilutions as per the desired specification and will be incubated with fixed quantity of the virus and allowed to neutralize the virus by antibody present and then added a substrate like cells to show the effect on them by the virus sample which has not been neutralized. After the fixed duration of the incubation the plate may be stained and interpreted the result by stasticial calculation and expressed the titre.

Virus amplification test: The virus amplification test abbreviated as VAT is meant for assessment of the inactivation of the viral harvest. Briefly the sample will be incubated with monolayer flask for a defined time and the media after the incubation will again be sub cultured in another flask and will be given in the animal to check the effect of the samples as the virus if not inactivated will grow in the culture and again after the second passage it will grow and will show the effect in the animals. The inactivated sample will not grow in the culture and will not show any reactions in the animals injected.

Virus typing test/virus identity test: The virus Typing Test or Virus Identity test will be used for the identification of the particular virus against which the vaccines being produced. This will be done with a particular strain of the virus. The hyper immune sera raised against the particular serotypes will be used for the test. The test sample and standard sera sample will be incubated with the cells and compared the result with that of standard samples. The direct inoculation of the test samples in the defined laboratory animal model is also one of the criteria for the identification. Again the samples which need to be tested for its identification will be incubated with the sera raised against it and the mixture will be tested in laboratory animals. The signs and symptoms in the animal will help to assess the identification of the virus.

Summary

The cell is the basic and functional unit of living organism has many fold applications at the industry level. It can be used widely as a model like development of vaccine, Research and Development, Drug Development, Biotech and Pharma industry etc. The preliminary applications of the cells includes Development of Cell Bank , Development of seed viruses, Bulk Production, Quality Control Testing, Monolayer to suspension type of cell culture and Virus Inactivation Kinetics and also Virus Titration, Serum Neutralization Test, Virus Amplification Test and Virus Typing Test/Virus Indentity Test etc.

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Conflicts of interest

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References

1. http://www.etymonline.com/index.php?term=cell
2. Cell Movements and the Shaping of the Vertebrate Body in Chapter 21 of Molecular Biology of the Cell. In: Bruce Alberts (Eds.), Garland Science, (4th edn), USA. 2002.
3. Maton A. Cells Building Blocks of Life. New Jersey, USA. 1997.

4. Schopf JW, Kudryavtsev AB, Czaja AD, et al. Evidence of Archean life: Stromatolites and microfossils. *Precambrian Research.* 2007;158(3–4):141–155.
5. Schopf JW. Fossil evidence of Archaean life. *Philos Trans R Soc Lond B Biol Sci*. 2006;361(1470):869–885.
6. Peter HR. Biology. George Brooks Johnson, McGraw-Hill Education, USA. 2002. p. 68.
7. Karyn's Genomes: Borrelia burgdorferi. *European Bioinformatics Institute*, UK. 2012.
8. Ménétret JF, Schaletzky J, Clemons WM, et al. Ribosome binding of a single copy of the SecY complex: implications for protein translocation. *Mol Cell*. 2007;28(6):1083–1092.
9. Prokaryotes. Earth and Environmental Sciences. Elsevier, USA. 1996.
10. Revathi A, Allen E. The Forces Behind Cell Movement. *Biol sci*. 2009;3(5):303–317.
11. Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. Garland Science, New Jersey, USA. 2002.
12. Ananthakrishnan R, Ehrlicher A. The Forces Behind Cell Movement. *Int J Biol Sci* . 2007;3(5):303–317.

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