

A Review on Serum in Culture Medium to Proliferate Mammalian Cell Lines for Human Rabies Vaccine Production

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Abstract

Serum is the liquid form blood component prepared by centrifuging the whole collected blood from animals. In culture medium serum used to enhance the nutritional content of the medium for cell lines proliferation. However; it has some effects such as: serum is animal product therefore; its chemical composition is unknown to maintain consistence of the products, source of contaminant agents, based on the age; sex; geographic existence; climate and breeds of source animals serum components cause batch to batch variation of the products, the shortage of serum that didn't meet the global need makes it very expensive medium components. There different animals those used to collect the serum are bovine, goat, sheep, horse, fish, porcine and other animals also serve as source of serum used for biopharmaceutical and biomedical researches. The most commonly used serum in culture medium to proliferate mammalian cell lines for rabies vaccine production is fetal bovine serum; due to its low immunoglobulin and other proteins than other sources. The proteins concentration in serum also hinders the downstream process of the products like in vaccine. Considering these effect of serum; there are several alternatives were developed to reduce, refine and replace the serum in medium by serum free chemically defined medium. Therefore; this reviews paper indicates the basic approaches to develop the alternative sources to replace the serum with other serum free medium and fetal bovine serum with other non-expensive serum sources.

Keywords: Growth medium; Mammalian cells; Serum; Rabies viruses; Vaccines

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Introduction

Rabies is neglected viral zoonotic disease caused by negative stranded RNA virus belongs to genus *Lyssavirus*. It is vaccine preventable disease that is responsible for death of more than 59,000 human in the world every year as per WHO, in 2018. Rabies can be prevented and control before the onset of clinical signs however; once the sign appear there is no any medication or treatment to cure the disease. Vaccination occurs for prevention includes pre-exposure immunizing individuals resident in rabies endemic area, veterinarians and animal laboratory technicians to boost their immune response or post-exposure immunization for individuals bitten by suspected animals immediately based on the size and site of bite. Cell culture based modern vaccine used in many countries to prevent and control rabies disease; the cells

proliferate and virus propagate in serum supplemented medium is used as substrate. Serum is the fluid and solute component of blood collected from animals and used in culture medium as source of different macromolecules, hormones, nutrients, binding proteins, growth factors, protect against mechanical damage and facilitate attachment those support the cells proliferation. Serum collected from different animals; however Fetal bovine serum is the most widely used serum supplement in culture media of many mammalian cell lines; due to it contains low level of antibodies, more growth factors and flexibility to be used in many different cell cultures. Even though it is a source of different nutritional components serum used in culture medium has its own side effects in vaccine productions as per WHO in 2010. It is expensive medium component, contain toxic components for cell lines, can be a source of contaminant agents that can hinder

downstream process and affects batch to batch consistence of cells proliferation and products. In addition to this, considerable ethical concerns were raised in serum harvest and collection due to animal cruelty [1]. Therefore in order to avoid the drawbacks of using serum a number of chemically defined serum free media formulations that replace the serum has been developed as alternative to serum containing media with known chemical composition that support the viral replication equivalent to serum supplemented grown cells to avoid the side effects of serum [2].

Literature Review

The aim of this review was to provide basic information on the advantages and disadvantages of using serum in culture medium of mammalian cell lines for production rabies vaccines.

Mammalian cell lines

Mammalian cell lines are those cells derived from mammals and proliferated in artificial medium under physically controlled environment. Mammalian cell culture is the process of growing animal cells in vitro aseptic conditions generally outside of their natural environment such as: in flask or petri-dish. There are many mammalian cell lines with five sub-categories Dogs cell lines (e.g. MDCK-11), Human cell lines (e.g. HeLa-S3 cells, MDBK cells, PANC-1, Caco₂, BT-20, HEK293 cell lines, HL-60 cell lines) Marsupials (e.g. Ptk2 cell lines), Monkey cells (e.g. Vero cell lines) and rodents (e.g. AHL-1 cell lines, CHO cell lines, BHK-21 cell lines, GL-261 cell lines) are used as substrates for different vaccine productions and biomedical researches such as. Different continuous cell lines were developed and used as substrates for biopharmaceutical products. Among those continuous cell lines Vero and MDCK were recommended by regulatory authorities and improved screening technologies remove fears regarding their problems as per WHO in 2010. Vero cells are isolated from the kidney of African green monkey (*Cercopithecus aethiops*) on 27 March 1962, at the Chiba University in Chiba, Japan. The medium for novel cell lines determined experimentally the suspension and adherent cell lines require different growth medium to proliferate [3]. The Vero cell lines are characterized as continuous and absence of normal chromosome numbers in the cells. This continuous cell lines can be replicated many cycles of division without biological aging. They are interferon-deficient and do not secrete interferon alpha or beta when infected by viruses. However, they have the interferon-alpha or beta receptor that responds normally when recombinant interferon is added to their culture media. Vero cells are used for different vaccine production, screening for Shiga-like toxin, as host cells for virus propagations for vaccines production, testing for the presence of rabies antigens for research and diagnostic purposes [4]. The verorab vaccines produced by Sonafi pastuer from vero cells lines don't have any adverse event and immunogenic to humans. The cells yield obtained from cell lines proliferated in serum free and serum supplemented medium is similar; whereas the viral productivity higher in serum free grown cells to produce rabies vaccine [5]. Economic aspects of using serum in culture medium.

The serum is the most expensive components than all other

medium compositions in culture medium to grow mammalian cell line for vaccine production. Its composition and cost are varying in geographical existence, types, age, sex of source animals from which it is collected and different manufacturing batches. The fetal bovine serum harvested from byproduct of the beef packing industry slaughtered cows; therefore as the consumption change beef to other alternatives it is difficult to control the serum market [6]. Increase the demand for serum across different areas of the biotechnology industry, biomedical researches, different vaccine productions put a pressure on supply and resulted in an exponential price increase [7]. Besides to this vaccine production companies those dependent on animal serum will be affected by the availability and fluctuate the serum supplement market. Serum adulteration and fraud of the serum industry will affect the quality and products. The exact amount of global production of serum and demand are not known yet that may cause abuse, mislabeling and adulteration of serum [8]. Fetal Bovine serum is extremely versatile has significant issues with both a lack of reproducibility due to variation of composition as well as reliability of supply.

Ethical aspects of using serum

Concerning the ethical perspectives in terms of animal protection arguments regarding the harvest and collection of serum from bovine fetuses and other animals carried without anesthetizing the animals that resulted in pain, discomfort these conditions encourage unethical practices and these animals suffering is misconduct or inhumane [9]. The world organization for animal health established the standard for transporting and slaughtering of pregnant cattle during the last 10% of their gestation periods considering the animal welfare [10].

Rabies vaccine production in serum supplemented media

The rabies virus vaccines produced in both bioreactors and in roller bottles with serum-free medium. The most disadvantages of using serum are due to its chemically undefined, the risk of contaminant agents and composition variation based on animal source of serum. There are also other side effects of serum in culture medium is high ratio of proteins content which affects downstream processing. Infectivity of virus seems to be hindered by the presence of serum in culture medium this is due to the penetration of virus into the cells is higher in serum free medium than serum supplemented media. The rabies vaccines produced from Vero cell line substrate in our laboratory using roller bottle with 10% serum without considering the side effect of the serum mentioned before [11]. However; Rabivax-S is produced in a CellCube system that is closed system with limited human intervention, reduces the risk of any cross contamination. The in process quality controls were stringently followed at every step of vaccine production that make this technique differ from others those used roller bottle and different sized flasks [12]. There are several rabies vaccines that are both effective and safe available that are recommended world health organization to prevent rabies before the onset clinical sign as per WHO in 2018.

Rabies vaccines production in serum free media

There are several serum free medium developed and used to grow mammalian cell lines for rabies vaccine production. Serum free media such as V-SFM and EX-CELLTM Vero are regulatory complaint media that are free of animal components. They produce Vero cell lines and virus productivity comparable to serum supplemented medium grown cells [13]. The use of serum-free medium to propagate the cells for vaccine production facilitates the downstream process to minimize residuals those affects the purification process. This alternative technique and low cost technology for rabies vaccine production could be suitable for developing countries where rabies is an important health problem [14]. There are several commercial serum free medium developed by various biological product companies; but they are specific and suitable to grow the cells; since each cell lines need specific medium composition to proliferate. For instance OptiVERO medium proliferate Vero cells with lower doubling time and vigorous viral replication than V-SFM. Additionally OptiVero serum free medium is proliferate the cell lines similar with 10%-EMEM serum supplemented medium [15]. The disadvantages of commercial serum free medium are: they are available in only liquid form, expensive with unknown chemical composition due to serious commercial security, are only suitable for small scale investigation, there is no universal serum free medium that can grow applicable to all mammalian cell lines/ they are specific for cell lines and high cost in industrial mass production [16]. Serum free media are devoid of potential presence of adventitious agents of animal derived components that enhance the quality of vaccine produced with serum free media [17]. The serum free medium developed to grow many anchorage dependent mammalian cell lines, including MDCK and BHK-21 and Vero in stationary and agitated micro-carrier cultures. Serum free medium developed from home-made rapeseed proteins are alternate medium supplement to grow the CHO cells [18]. Serum free and serum supplemented cells growth profile and cells yield was not significantly different from serum supplemented cultures; this suggest that increasing the serum concentration will not further improve cell proliferation and virus production [19]. The cells density of Serum free developed medium from combination of plant hydrolyses proliferate the cell lines are comparable with 10% serum supplemented medium. The viable cells density of culture for monoclonal antibody production of serum free and serum supplemented medium proliferated mammalian cells similar [20].

Reducing serum concentration in culture media

The methods of reducing the undesired effects of serum such as: contamination and it's in cell culture medium is to use reduced serum media. This is performed by enriching basal media formulations with nutrients and animal derived components that reduce the amount of serum required in culture medium. Sequential adaptation is process reducing the serum in culture medium through decreasing the from the standard serum concentration contained medium at which cells grown to adapt in serum free conditions without any change in growth kinetics in both serum supplemented and serum free media As the

serum in culture medium reduced; the glucose consumption and lactate production of the cells also decreased. The mixture of serum supplemented and serum free medium is recommended to minimize the cells stress due to new environment until passage three before complete switch to serum free medium. The serum free adapted anchorage dependent cells has long lag phase periods to reach the maximum cells density due to acclams to relatively new environment. Direct adaptation of the cells in to limited serum concentration is also another method of reducing the serum; but this technique is harsher to the cell lines and may cause culture loss. Adaptation of mammalian cell lines to serum free condition is important methods to because of serum contained culture medium presence of animal derived components may introduce batch to batch variability and adventitious pathogens to the process. However; mammalian cell lines adaption to serum free medium is challenging and time consuming techniques .Sources of serum

The serum may collect from different animals and used for different areas of biomedical researches and vaccine productions both for human and animals such as: cows, horses, pigs, goats, sheep, fish and others animals However; not all serum supports the growth the cell lines for vaccine production. The most common serum used for proliferation of the cells and rabies vaccine production fetal bovine serum. The use of serum from another species other than bovine; the NRA/NCL should be endorsed regarding acceptable testing methods as per WHO in 2010.

Bovine serum: It is a byproduct of the beef industry that is produced from blood collected aseptically at commercial abattoir from cows slaughtered for supply meat intended for human consumption. The serums collected from bovine are fetal bovine serum (FBS) and newborn calf serum (NBCS). FBS is a blood product collected from fetal cows and centrifuged with high speed to separate the serum. It is the most commonly used serum supplement for cell culture based vaccine production and research to proliferate cell lines. The different between FBS and NBCS is that the FBS is rich in growth factors and contains low levels of immunoglobulin that inhibit the cell growth.

Fish serum: It is collected from different species of adult fish aseptically; filtered and heat treated. It is used in culture medium to enhance attachment and nutritional content of the medium for various mammalian cells. For instance; fish serum supports the growth Vero cells in limited concentration as compared to fetal calf serum. This alternative supplement also saves the cost of fetal calf serum required for proliferation cell lines for viral vaccine production. Even though fish serum still requires improvement to replace fetal bovine serum; surimi wash water processing line fish serum used as an alternative substitute for serum in mammalian cell lines. Fish serum also substitutes fetal calf serum in hybridoma cell culture for production monoclonal antibody. The blood components of salmon fish contains easily digested proteins and a high concentration of poly-unsaturated compounds that makes it favorable medium fortification.

Horse serum: Horse serums promote growth of the cells monolayer with higher protein content of the cells than fetal

bovine serum grown cells. The passage number and consistent cells morphology produced in fetal bovine serum proliferated cells. However; cells doubling time to reach the confluence stage is longer in horse serum supplemented grown cells.

Goat serum: It is used as alternative to NBCS in different cells without showing any toxicity effects. It can also be used instead fetal bovine serum in biomedical research since it is suitable for most of the cell lines and primary cultures.

Discussion and Conclusion

There are several mammalian cells lines were developed for rabies vaccines productions and researches. However; not all cell lines allowed producing the vaccine because they may have an oncogenic property that affects the final product and causing health problem to human beings. Therefore; the cell lines used for biopharmaceuticals including rabies vaccine must be get license from National regulatory authorities. Cell lines were requiring

the growth medium fortified with nutritious components like serum to proliferate to its maximum viable cells density. Modern cell culture based anti-rabies vaccines were developed by using mammalian cells as substrate such as Imovax (MRC-5), Verorab and Chirorab (Vero cells lines) vaccines were used for rabies pre-exposure and post-exposure prophylaxis to against the disease outbreaks. The challenge faced to cover the demand of rabies vaccines were its production cost unfortunate from the expensive medium component specifically serum and its adverse effects since its chemically undefined substance. Therefore; to avoid those risks there are several serum free media developed as alternative to serum supplemented medium to produce safe, effective and affordable rabies vaccines to control and prevent the rabies disease.

Conflict of Interest

All authors declared that they have no any conflict of interests.

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