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Production of Recombinant Adenovirus Associated COVID-19 Vector by using Suspension HEK293 Cells: Component of SARS CoV-2 Vaccine

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Abstract

Generally, a gene which is inserted directly into a cell does not operate independently. Instead, the transmission of the gene is genetically modified by a biological messenger called a vector, consists of a transgene and a large DNA sequence as a backbone. Since they can deliver the new gene by infecting the cell, such viruses are also used as vectors. The adeno-associated virus (AAV) is a non-enveloped virus that can be designed to deliver DNA to target cells and has attracted considerable interest in the field, especially in experimental therapeutic strategies at the clinical level. For the new age production of COVID-19 vaccine, development of different mammalian cell lines like HEK293 (most reliable growth and prosperity for transfection) and recombinant adenoviral vectors have become the first priority for biopharmaceutical giants and globally approved vaccine manufacturers to scale up their vaccine production. Adenoviruses have a distinctive icosahedral structure, with a protein coat that encapsulates the viral double-stranded DNA genome. The adenovirus genome is relatively compact, making it an attractive option for insertion of foreign DNA. Deletion of the adenovirus E1A gene removes the virus' ability to replicate. This ability can be restored during propagation in cell culture, for example, by using cells that express the E1A protein [1]. Hence, in this mini review, I have shared an overview of the propagation of adenoviral vectors, i.e. recombinant adenovirus SARS CoV-2 vector in HEK-293 cell suspension culture. By evaluating human Ad5 genes (E1A, E1B19K/55K, pTP, DBP, and DNA Pol) and OCT1 for their contributions to adenovirus production, there needs to develop a rapid adenovirus production and amplification (RAPA) line [2].

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Introduction

Long-term gene expression, the inability to propagate autonomously without a helper virus, transduction of dividing and non-dividing cells, and the lack of pathogenicity from wild-type infections are the advantages of using Adeno-Associated Virus (AAV) for gene therapy [3]. The accomplishment of creating an adaptable production technology depends vigorously on understanding the science of AAV to generate reagents like, cell lines, plasmids, or recombinant viral vectors that when utilized together, will intently impersonate wild-type (wt) AAV creation. Multi-plasmid transient transfection of HEK293 cells is mostly considered as the best technique for rAAV production. The motif behind this work was to create a scalable manufacturing technology to deliver high-titer and highly pure rAAV utilizing transient transfection technology and mammalian HEK293 cell lines. Designing an adenoviral vector vaccine is as basic as designing the viral genome to create a foreign antigen for instance, the COVID Spike protein. Whenever that is done, the designed adenovirus can be conveyed as an antibody through infusion. Since the infection is replication-deficient, there's no danger of it causing a disease. Notwithstanding, it can communicate the COVID S protein inside the host cell, which at that point triggers the versatile resistant reaction to ensure against a real COVID infection. The development of this adenoviral vector based vaccine is to target the spike protein present in the coronavirus, i.e. S1 and S2. S1 binds to the ACE2 receptor, while cleavage of S2 by proteases on the host cell surface alters its conformation

and enables the viral envelope to fuse with the cell membrane. Targeting to these two spike subunit proteins of novel coronavirus, rAd5, rAd26, rAd35 have been developed which allows entry into bronchial epithelial cells and some professional antigen presenting, where the ACE 2 receptor allows the fusion of viral cell with the host cell.

Development of Suspension HEK293 Cell Lines

To maintain its ability for high transfection efficiency and rAAV production, selection of an animal component-free and antibiotic-free medium for the development of HEK293 cell line is highly recommended. "Three times a week, cell passaging has to be routinely performed in 125 mL plastic Erlenmeyer flasks (Corning Inc., USA), seeding 15 mL of culture media with 0.5 × 106 cells/mL. Flasks should be shaken at 110 rpm on an orbital shaker in an incubator with a temperature set at 37°C in a humidified atmosphere with 5% of CO2. At each passaging, a sample of culture supernatant should be taken to check the protein expression and viability of cells. Daily cell counting, osmolality check, PH and glucose concentration should be properly performed. All the procedures should be performed in contamination free zone and the deviations should be very low so as to improve the quality. ProS293CDM (Invitrogen, USA), CDM4HEK293 (HyClone, USA), SFM4HEK293 (HyClone, USA) and CPCHO (CIM, Cuba) culture media are the mostly used growth medias used for HEK 293 cell line development" [4] **(Figures 1 and 2)**.

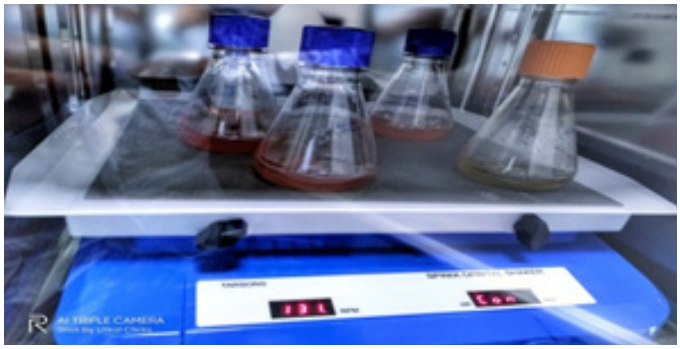

Figure 1: 125 mL plastic Erlenmeyer flasks (Corning Inc., USA).

Figure 2: Harvested cell culture.

Production of rAAV using WAVE bioreactors

To scale up the production of rAAV vectors, WAVE bioreactors are the best choice, as it reduces the shearing stress and have an excellent mixing and gas transfer. Initially for a 5L batch production, 850 ml of media and 1000 ml of cell inoculation has been added to the bag. The parameters are maintained according to GMP. Proper airflow, PH calibration (PH-7), Air flow- 0.12, rocking angle 14 rpm, 7.00, auto CO2 flow have been set. Cell counting, PH, osmolality have been performed within every 12 hours of interval. Media addition was performed in every 24 hr-48 hr accordingly to the growth of cells in the media. After three times addition of media, finally the virus culture was inoculated. Transfection was monitored and after two days continuous same cell count, whole batch has been harvested and the vectors were recovered.

Harvesting suspension HEK293 cells from WAVE bioreactor bags

After Forty eight hours of post-transfection of viral vectors into the HEK293 culture media, the cell suspension has been transferred to conical tubes and was centrifuged at 655 × g for 10 minutes. Then the supernatants were discarded and the cells were resuspended in 1X phosphate buffer saline so as to mention the PH, osmolarity and ion concentration exactly as the human body. Again centrifuged pellet and PBS (phosphate buffer saline) mixer was centrifuged at 655 × g for 10 minutes. Then, the pellets were stored in -80 freezers for further purification.

Purification of rAAV from crude lysate

Pellets present in each tube were adjusted with ddH20 and sonicated for 4 minutes. After sonication DNAse was added and incubated at 37°C. Finally 5M NaCl was added to the pellets to remove the virus bound to the debris and allowed for further centrifugation 9,400 × g. Ion exchange chromatography was performed by using an AKTA FPLC to purify the rAAV vectors.

Conclusion

The increase in the demand of clinical trials emphasizes the need to build up adaptable manufacturing innovations that are widespread for all serotypes, GMP compliant as well as user friendly. This mini review have focused on versatile transient transfection manufacturing technology utilizing a HEK293 cell line that can be cultured in animal component free and antibiotic free medium conditions for the scale up production of adenoviral vector based COVID-19 vaccine. So as to decrease the cost of production and to increase the ease of availability, better chemically defined media should be formulated for the development of HEK293 cells. However, CDM4KEK293 and SFM4HEK293 media have shown significant have shown promising results as culture media for HEK23 cell line growth and development. The outcomes have shown that the transfection of rAd vectors with the HEK293 cells are capable of producing viral spike protein of COVID-19 in the supernatant with conformational and immunogenic characteristics independently of the media in

which they are developed which exhibits the high reproducibility and consistency among protein batches produced by HEK-293 cells even in various culture conditions. It establishes a vital trademark to utilize this cell line in the production process of these viral vectors.

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