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Human Adenovirus Serotypes Efficiently Transducing HEK293 Cells: An *In Vitro* Propagation of HAdv

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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 adenovirus is a non-enveloped virus that can be tailored to transfer DNA to target cells, and it has sparked a lot of interest in the field, particularly in clinical trial therapy techniques. 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 an icosahedral shape, with a protein coat encasing the viral double-stranded DNA genome. Because the adenovirus genome is relatively small, it's a good candidate for insertion of foreign DNA. The adenovirus E1A gene is deleted, and the virus loses its capacity to replicate. This ability can be restored during cell culture propagation by employing cells that produce the E1A protein, for example. Hence, in this mini research, 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.

Keywords- Cells, Culture media, COVID-19, Adenovirus vector

I. INTRODUCTION

The advantages of utilising adenovirus for gene therapy include long-term gene expression, inability to proliferate without a helper virus, transduction of dividing and non-dividing cells, and absence of toxicity from wild-type infections. Understanding the science of adenovirus is critical to developing reagents such as cell lines, plasmids, or recombinant viral vectors that, when combined, closely resemble wild-type (wt) adenovirus production. Multi-plasmid transient transfection of HEK293 cells is mostly considered as the best technique for recombinant adenovirus production. Using transient transfection technology and mammalian HEK293 cell lines, the goal of this research was to develop a scalable production technology for delivering high-titer and extremely pure recombinant adenovirus. Creating an adenoviral vector vaccine is as simple as creating a foreign antigen, such as the COVID Spike protein, using the viral genome. The customized adenovirus can then be delivered as an antibody by infusion at any time. There is no risk of the infection generating disease because it is replication-deficient. It can, however, communicate the COVID Spike protein inside the host cell, which then activates the versatile resistance response, which protects against a true COVID infection. The goal of developing this adenoviral vector-based vaccine is to target the coronavirus's spike proteins, S1 and S2. S1 binds to the ACE2 receptor, whereas proteases on the host cell surface cleave S2 and change its conformation, allowing the viral envelope to merge 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 cells, where the ACE 2 receptor allows the fusion of viral cell with the host cell. The adenovirus vector system has seen a lot of progress in the last two decades, from its use as a vector for transgene delivery and vaccine supplements to its use as an oncolytic agent. The adenovirus is currently one of the three principal viral vector groups in the gene therapy "toolbox," alongside adeno-associated viruses and lentivirus/retrovirus vectors. We will compare adenovirus to other viral and non-viral vectors, discuss the common methods for producing adenovirus, discuss the current clinical and preclinical use of adenovirus as gene transfer tools, and discuss the current challenges and opportunities in using adenovirus vectors in gene and cell-based medicine in this review.

II. HUMAN ADENOVIRUSES

Human adenoviruses (Ads) are non-enveloped DNA viruses with linear, double-stranded genomes of roughly 35 kbp that have been linked to a number of disorders, but are most commonly linked to upper respiratory and gastrointestinal tract infections. Human adenoviruses are classified into six groups (A–F) based on hemagglutination, restriction enzyme analyses, and DNA homology differences. Adenoviruses have been connected to a number of different disease patterns.

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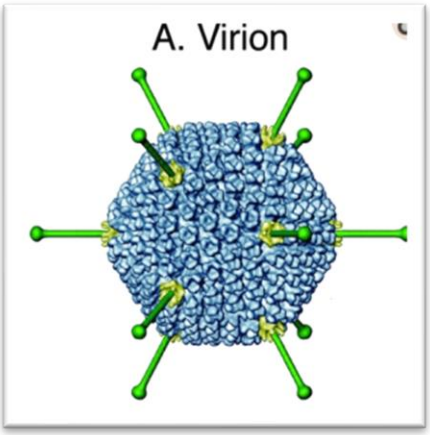
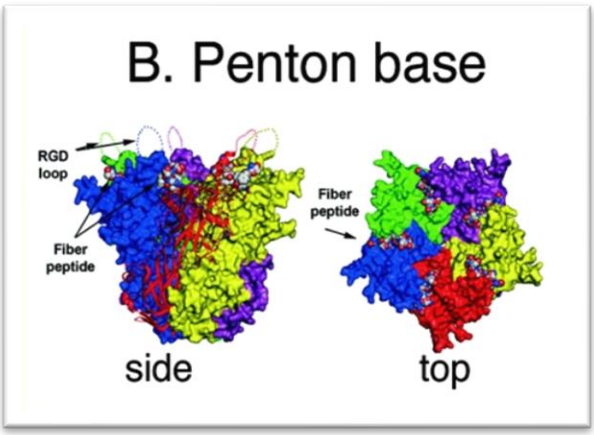
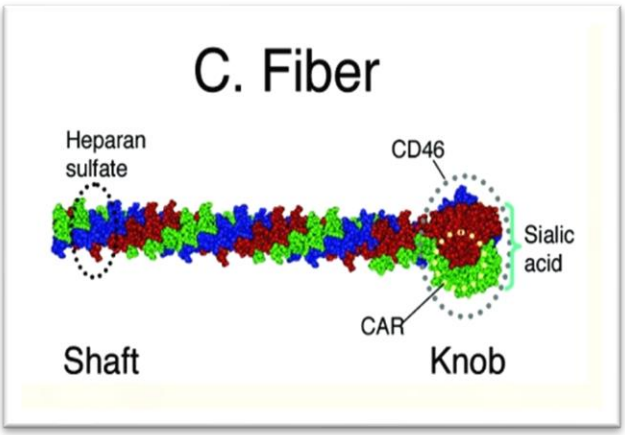
Having a linear, double-stranded DNA genome, adenoviruses have been coated with different viral proteins constructing an icosahedral shell; hence electron microscopic study has confirmed its structure as an icosahedral virus. The adenovirus shell is made up of almost around 252 structural capsomeres, which are the sub units of the capsid protein with diameters ranging from 70 to 100 nm. Multiple copies of three main capsid proteins (hexon, penton base, and fibre) and four minor/cement proteins (IIIa, VI, VIII, and IX) are structured with icosahedral symmetry in the capsid shell of an adenovirus. Adenovirus has received a lot of attention as an effective gene delivery vector due to its well-defined biology, minimum infection rate in animals, greater genetic stability, high gene transmission efficiency, and ease of large-scale production, and it was the first DNA virus to enter rigorous therapeutic development. There are 57 different human serotypes of adenovirus, which are non-enveloped, double-stranded DNA virus. Adenovirus serotypes have different tropisms, and they are further divided into six subgroups and those serotypes have been classified under category A to G. Differences in viral capsids define tropisms between serotypes. The viral capsid is made up of capsid proteins, core proteins, and cement proteins. These different serotypes have the ability to produce a wide range of symptoms. As a result, it's no surprise that adenovirus is still at the forefront of gene therapy.

Sub Groups (Adenovirus)	Serotypes
A	12, 18, 31
B	3, 7, 11, 14, 16, 21, 34, 35, 50, 55
C	1, 2, 5, 6, 57
D	8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 36, 37, 38, 39, 42, 43, 44, 45, 46, 47, 48, 49, 51, 53, 54, 56, 58, 59, 60, 62, 63, 64, 65, 67, 69, 70, 71, 72, 73, 74, 75
E	4
F	40, 41
G	52







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Adenoviral vectors have a number of advantages over other methods for delivering viral genes. Adenovirus is the most effective way of delivering genes in vivo because most human cells have the primary adenovirus receptor known as CAR receptor and secondary integrin receptors. Normally adenovirus entry into the host cell requires two receptors, i.e. CAR, which generally binds to the viral fiber protein and the pentons, engages with cellular integrins receptor, which helps in the internalization of virus for further entry into the host cell. Using other viruses might not be effective because of the lack of receptor recognition in the host cell. As a result, they are susceptible to adenovirus vector infection and produce large amounts of transgenic expression. Second, the development of gutless adenoviral vectors allows us to avoid the immune response to anti-adenoviral vectors. Third, despite concerns about their safety, adenovirus vectors have been used extensively in a variety of therapeutic applications, and the safest doses and routes of delivery are now well established. Adenovirus vectors are the most often utilized vector in clinical trials around the world, accounting for more than 20% of all gene therapy trials. Finally, adenovirus vectors provide a flexible framework for developing techniques to change viral capsids to improve therapeutic qualities and virus targeting specificity. Some of adenovirus's inherited flaws, such as immunity elicited against the adenovirus capsid and low-level expression of adenovirus genes, may now prove useful in the development of anticancer immunotherapies, in which the goal is to induce immunity against the cancer or to kill the cancer cell directly. Furthermore, the adenovirus's combination immunity and short expression time make it a suitable platform for vaccine development.

From its use as a vector for transgene delivery and vaccine supplements to its use as an oncolytic agent, the adenovirus vector system has advanced significantly in the last two decades. Along with adeno-associated viruses and lenti/retrovirus vectors, the adenovirus is one of the three main viral vector groups in the gene therapy "toolbox." We will compare adenovirus to other viral and non-viral vectors, discuss the common methods for producing adenovirus, discuss the current clinical and preclinical use of adenovirus as gene transfer tools, and discuss the current challenges and opportunities in using adenovirus vectors in gene and cell-based medicine in this review.

III. ADENOVIRUS-MEDIATED GENE DELIVERY

One of the most key characteristics of the adenovirus is its high incidence and stability among healthy people. As a result, the general population has a broad pre-existing immunity to Adenovirus like common cold and cough infection mostly caused by the human friendly adenoviruses. Adenoviruses produced from the most common adenovirus serotypes are severely limited by this protection. As a result, researchers have concentrated on developing adenoviruses for gene therapy using rare serotypes 26 and 5. Furthermore, the proteins of all adenoviruses have significant immunogenicity towards the infection as well as good stability till long period of time. As a result, repeated Adenoviral injections to a single patient are strictly prohibited to avoid anaphylactic shock or death. Adenoviruses, surprisingly, have high immunogenicity, making them attractive candidates for oncolysis and vaccination. Adenoviruses have been researched in the creation of vaccines against HIV and influenza, and now COVID19 causing deadly virus SARS CoV2 as well as other pandemic diseases, and can be utilized to trigger antitumoral immunity within the tumor microenvironment. These oncolyitc adenoviral vectors can be potentially replicated multiple times to destroy the cancerous cells. Adenoviral vectors may infect both replicating and dormant cell populations, making them a useful tool for delivering transgenes in vivo and inside mature tissues. AdVs can transport larger transgenes up to 8 kbp in size than lentiviral vectors; however, their DNA does not integrate into the host genome and instead dwells episomally in the host nucleus. Without direct incorporation into the host genome, episomal transduction eliminates the possibility of insertional mutagenesis; yet, transgenic expression is temporary, subject to cell silencing mechanisms, and destined for dilution among daughter cells should cell division occur.

IV. REPLICATION OF HUMAN ADENOVIRUS

Human adenovirus has a 36-kb linear double-stranded genome with 100-bp inverted terminal repeats called ITR repeats. A 55-kDa terminal protein (TP) is covalently linked to both 5′ termini (TP). The replication of Adenovirus DNA is an extremely efficient process. Infected cells create around one million copies of viral DNA in just 40 to 48 hours. An in vitro system that replicates HAdV-5 and -2 DNA with purified proteins provided a wealth of knowledge on the replication mechanism. Within the inverted terminal repeats, two identical replication origins span 1–50 bp. The minimal origin is formed by the final 18 bp, while the auxiliary origin is formed by the remaining basepairs. The covalent attachment of a dCMP residue to an 80-kDa TP precursor kicks off protein-primed DNA synthesis (pTP). Three viral proteins expressed by E2 genes are required for replication: precursor terminal protein (pTP), AdV DNA polymerase (AdV Pol), and the DNA-binding protein (DBP). Two cellular transcription factor called nuclear factor I and Octamer binding protein I, bind to the auxiliary origin and boost initiation of replication and formation of thousands of copies. A viral protease like endopeptidases cleaves the specific peptides bonds in between pTP to TP in the last stages of

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replication, resulting in offspring DNA, which is then packed in virions.

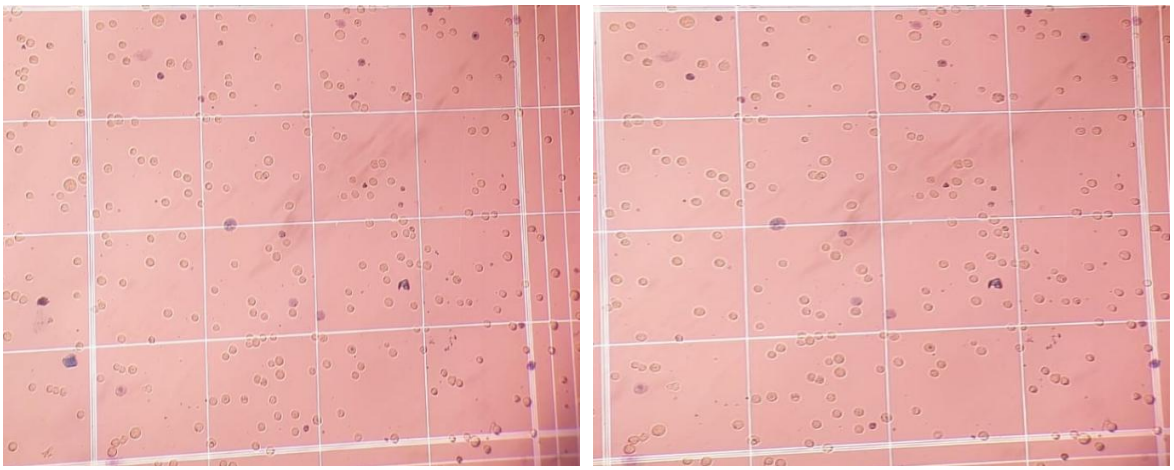

Figure 1: HEK 293 cell culture

V. GENETICALLY ENGINEERED HEK293 CELLS

Our laboratory purchased and delivered HEK293 cell lines to carry out the research methodology and protocols. The HEK293 cell lines were chosen for a variety of reasons; including good repeatability, ease of growth and maintenance, and high transfection capability, among others. Earlier studies using human adenoviral vectors to transfect HEK293 cells yielded promising findings in the production of recombinant proteins and virus-like particles in cells.

Selection of an animal component-free and antibiotic-free media for the development of the HEK293 cell line is highly advised to retain its potential for high transfection efficiency and recombinant adenovirus production. “Cell passaging was done three times a week in Erlenmeyer flasks (Corning Inc., USA), with 15 mL of culture fluid seeded with 1 106 cells/ml. Flasks were shaken at 110 rpm on an orbital shaker in an incubator set at 37 °C in a humidified environment with 5% CO2 in a humidified atmosphere. A sample of culture supernatant was obtained at each passaging to assess cell count, pathogenic particle expression using a bioassay approach termed TCID50, and cell viability. The daily cell count, osmolality check, PH, and glucose concentration were all done correctly. To increase the quality, all procedures were carried out in a contamination-free zone with very minimal variations. ProS293CDM (Invitrogen, USA), CDM4HEK293 (HyClone, USA), SFM4HEK293 (HyClone, USA), and CPCHO (CIM, Cuba) culture mediums were utilised to determine the growth scale of the cell lines (4). To preserve the cell lines from contamination, an aseptic environment was constructed, including the use of disinfectants such as trillium, 70% IPA, LAFU, and others.

VI. PRODUCTION OF rAD VECTORS USING SINGLE USE WAVES BIOREACTORS

WAVE bioreactors are the ideal solution for scaling up the production of rAd vectors because they minimise shearing stress and provide good mixing and gas transmission. 850 mL of medium and 1000 mL of cell inoculation were initially added to the bag for a 5L batch production. The parameters are kept in line with GMP. The correct airflow, PH calibration (PH-7), air flow-0.05, rocking angle 14 rpm, 7.00, and auto CO2 flow have all been established. Within a 12-hour period, cell counting, PH, and osmolality were done. According to the proliferation of cells in the medium, media addition was performed every 24hr-48hr.Finally, the virus culture was injected after three additions of medium. The vectors were retrieved after the full batch was collected and the transfection was monitored for two days with the same cell count.

VII. HARVESTING SUSPENSION HEK293 CELLS FROM WAVE BIOREACTOR BAGS

The cell suspension was transferred to conical tubes and centrifuged at 655 g for 10 minutes after 72 hours of post-transfection of viral vectors into the HEK293 culture media. The supernatants were then discarded, and the cells were resuspended in 1X phosphate buffer saline to mimic the human body's PH and ion concentration. The pellet and PBS (phosphate buffer saline) mixer were centrifuged for 10 minutes at 655 g. The pellets were then placed in -80 freezers to be purified further.

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VIII. PURIFICATION OF RECOMBINANT ADENOVIRAL VECTOR

Each tube's pellets were adjusted with ddH20 and sonicated for four minutes. Following sonication, DNAse was added and incubated at 370C for 30 minutes. Finally, 5M NaCl was added to the pellets to eliminate any virus associated to debris, allowing for additional centrifugation. The rAd vectors were purified using an AKTA FPLC and 9,400 g for 20 minutes followed by HPLC. Tangent flow filtration (TFF) was used immediately after purification to identify the concentrated VLPs (Virus like particles) produced by the cells.

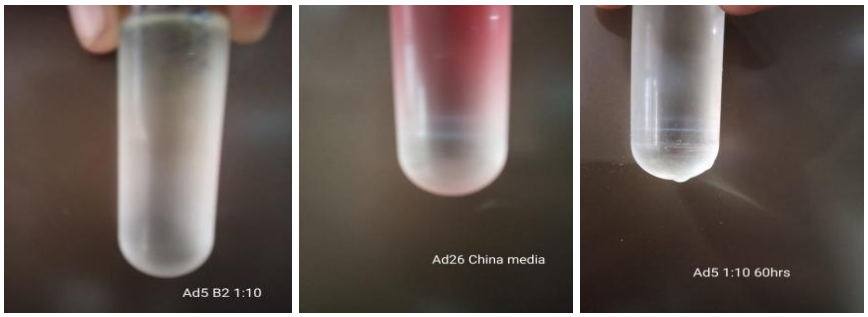

Figure 2: Analytical ultracentrifugation analysis

IX. DISCUSSIONS

The growing need for clinical trials highlights the importance of developing flexible manufacturing innovations that are applicable to all serotypes, GMP compliant, and user friendly. This brief review focused on a versatile transient transfection manufacturing technology that uses a HEK293 cell line that can be maintained in animal component-free and antibiotic-free media conditions to scale up the production of an adenoviral vector-based COVID-19 vaccine. Better chemically defined media for the development of HEK293 cells should be formulated to reduce the cost of production and increase the ease of availability. CDM4KEK293 and SFM4HEK293 media, on the other hand, have showed great promise as culture media for the HEK23 cell line's growth and development. The results show that transfection of rAd vectors with HEK293 cells can produce COVID-19 viral spike protein in the supernatant with conformational and immunogenic properties regardless of the media in which they are produced, demonstrating high reproducibility and consistency among protein batches produced by HEK-293 cells even in various culture conditions. It establishes a crucial hallmark for using this cell line in the viral vector generation process. Finally, for packaging, the viral genome must be transfected into HEK293 cells. The E3 and E4 genes of recombinant adenovirus have been altered, which are required for virus propagation. These genes are expressed constitutively in HEK293 cells, and adenovirus can be reproduced and packaged in this cell type. Adenovirus-producing cells appear as fluorescent comet-like foci under fluorescence microscopy. Recombinant adenoviruses have traditionally been created using two methods. The first involves ligating adenoviral genome DNA fragments to restriction endonuclease fragments containing a transgene directly. However, due to the low efficacy of big fragment ligation, this approach is useless. Homologous recombination in mammalian cells, which can supplement faulty adenoviruses, is the second and more extensively utilized approach (packaging lines). When homologous recombination occurs, a faulty adenovirus is produced, which can multiply in the packaging line (e.g., 293 or 911 cells) and deliver the missing gene products. This procedure, however, is difficult due of the low effectiveness of homologous recombination, which necessitates multiple rounds of plaque purification from the mammalian cell line. As a result, the procedure takes a long time and requires a lot of effort.

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