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How to choose the correct cell line for producing your viral vaccine: what is important?

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Keywords: animal cell technology • expression system • host cell line • human viral vaccine • virus replication

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Background

Human viral vaccine manufacturing formed the basis of using animal cell technology for biopharmaceuticals in the 1960–1970s, replacing products derived from animals or human blood [1]. The majority of recombinant protein products, such as hormones and blood factors, made this transition from mammalian to a recombinant source, and later, being relatively well-characterized products, adopted stringent regulatory guidelines [2] based on scientific understanding. This also led to the use of a limited number of standard target expression systems to generate a product with specific predefined product characteristics and quality (CHO, *Escherichia coli*, *Saccharomyces* or *Picchia*).

In contrast to recombinant biopharmaceutical proteins, the present situation for viral vaccines is characterized by a lack of standardization and diversity in expression systems. This diversity is further enhanced by the various approaches followed in viral vaccine development. Although recombinant subunit products to generate viral vaccines, such as virus-like particles (hepatitis B and human papillomavirus) and virosomes (Inflexal® V; Crucell, The Netherlands), have reached the market, the majority of viral vaccines, as discussed in this paper, still takes the production of viruses (split, inactivated or live attenuated) as a starting point.

Since most vaccines are given to healthy children, the introduction of new cell lines in viral vaccine production has been a low priority compared with product safety. Therefore, manufacturers may have selected cell lines based on conservative approaches, while tolerating potential inefficiencies. However, recent endeavors in modernization of classical (e.g., influenza and polio) and new (e.g., respiratory syncytial virus) viral vaccines have initiated exploration of exciting new viral expression systems [3].

Overview of feasibility criteria

Firstly, a host cell candidate should be able to propagate the virus. The highest potential for success is to use cells originating from the natural viral pathogen host organism. The replication cycle of viruses has common events (binding, internalization, transport, disassembly, multiplication of viral building blocks, transport and assembly), but also specific viruses have particular deviating events. Nonenveloped viruses need to lyse the cells to reach other cells or form syncytia first. Enveloped viruses can show a nonlytic replication; however, most often, a standard batch production-culture harvest contains lysed and nonviable cells, creating cell debris, which is a challenge for the purification process.

The propagation of a virus is scored by a titration assay and monitoring of cytopathological effect or by quantitative PCR. When working with common viruses, additional assays are available to determine a specific immunogenic component of the virus, such as hemagglutinin for influenza or D-antigen for polio. It is important to not just monitor cytopathological effect only, but include specific viral components from the start. To assess intermediate product quality and in-process yields, this component can preferably

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be measured in each individual unit operation, independent of the matrix [4].

Virus culture conditions

For efficient replication of most viruses, it is important that host cells are actively growing and therefore to choose the appropriate cell concentration and time of infection as a specific point of the batch curve for cells in a particular culture medium. Initial testing of any viral replication, and monitoring of replication events, can be carried out at the end of the growth phase using a high (≥10) multiplicity of infection (MOI). For process optimization and efficient use of virus seedlots, including testing of lower MOIs (<0.01), it is important that the cell culture continues to grow during viral replication to support multiple rounds of virus replication.

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For some viruses, it has been suggested [5] that virus replication is better in adherent cells with a specific orientation. This is related to the replication of a virus in the epithelial layer of cells with strict orientation, mimicking natural conditions that would favor correct virus replication. However, in recent years, no clear general advice has emerged from various studies. MDCK suspension cells and insect suspension cells have been approved for influenza manufacturing, and BHK-21 cells have been used for nearly half a century for veterinary foot and mouth disease vaccine [6]. Mimicking the more natural situation of cells growing in organic tissue (e.g., in hollow-fiber modules) is not an option for uniform and scale-able production of biopharmaceuticals. In addition, another issue is the culture of a virus in diploid cells or in nondiploid immortal cancer cells. Is the virus able to take over control and replicate in cells that have lost control over their own replication? So far, it is clear that influenza replication to an acceptable yield is possible in stable cancer cell lines such as MDCK suspension cells, PER.C6 and HEK-293 suspension cells [7–9].

Cells can have a defense response to virus replication, such as interferon signaling to surrounding viable cells that can prevent entry of the virus. Usually, low MOI feasibility testing indicates whether this mechanism plays a role for a certain virus–host cell combinations. For example, even though MDCK cells show a high influenza virus titer at low MOI, it was recently reported that knockdown of interferon regulatory factor 7 significantly increased virus yield [10].

Furthermore, the host cell has an effect on virus glycosylation and therefore the immunogenicity of viral products [11]. In addition, the host cell can also determine the structure of the viral product; for example, it has been demonstrated that influenza vaccine production in eggs produces spherical particles; however, in mammalian cells – or whole animals – a more filamentous virus is produced [12]. Such so-called 'harvest quality' aspects may also be dependent on the type of virus strain produced, and will impact on the purification and inactivation of the virus.

If there is initial replication of the virus at a low level, the adapted virus taken from the supernatant can generate higher yields during subsequent passages with fresh host cell cultures. The resulting changes of the virus should be checked if the genome stability and immunogenicity of the viral product is affected.

Cell growth properties

With respect to cells as hosts for viruses, standard growth targets can be applied: at least three doublings per batch passage should be feasible and the doubling time at exponential growth should be within 1 day. Death rate should be negligible during the growth phase and increase very slowly after this growth phase.

To ensure safety and support optimization and control for the upstream process, the preferred culture medium is chemically defined and free of components from mammalian source. However, available, defined, animal component-free media often require improvement to provide sufficient robustness for a large-scale manufacturing process. Besides influencing cell growth and virus yield, the culture medium can contain components such as cholesterol for the budding of enveloped virus [13] and trypsin to activate hemagglutinin [9].

To enable efficient high cell density cultures single-cell suspension is preferred, which also facilitates the use of established techniques for recombinant protein production processes (well-mixed, stirred-tank bioreactors; sparged aeration; perfusion; nutrient feeding strategies during both cell and virus culture) for scale-able industrial virus manufacturing.

Regulatory aspects & intellectual property

In both regulatory and intellectual property, there is a tradeoff between the benefit in yield and quality, and the effort to get a cell line approved [14]. A stable human suspension cell line is the most desired universal host cell, but gives a considerable regulatory burden with regards to demonstrating safety. To achieve fast-track vaccine development towards market launch, the only feasible option is to select a host that is approved for manufacturing.

Important items for the expression system are the absence of retrovirus, mycoplasma and adventitious viruses, and a well-documented overview of the cell history, including culture and storage conditions. Furthermore, cell stability over the required number of generations to produce a product has to be demonstrated [15,16].

For the use of a commercial host cell, such as PER.C6, CAP, EB66 or AGE1.CR, the expected benefit should be weighed against the costs of a license agreement. If the costs are reasonable, and license conditions do not present upfront roadblocks, commercial cells can be included for initial feasibility testing.

Future perspective

A future perspective for viral vaccine production could be recombinant viral antigen expression in standard expression systems, as for other recombinant biopharmaceuticals. These antigens may be combined to form a virus pathogen-like structure, evoking both humoral and innate response. However, in the near future, it is anticipated that wild-type, attenuated or newly engineered viruses [17] will continue to provide the basis for viral vaccine development. No standard expression system for the majority of viral vaccine products dominates, although Vero takes a prominent position. Vero has a long history in viral vaccine production, and is used for at least five approved viral vaccines [18,19]. In addition, Vero processes are used at industrial scale [20].

To conclude, as illustrated above, selection of a suitable viral vaccine expression system requires substantial desk research. This should lead to the description of boundary conditions and a target product profile that governs the choice of a few candidate host cells. For these cells, evaluation of appropriate growth and – after repeated passaging – virus yield at low MOI can be tested to determine initial feasibility. Subsequently, cell selection is confirmed in a laboratory-scale model of the target production process.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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