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Concepts for the Production of Viruses and Viral Vectors in Cell Cultures

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<http://dx.doi.org/10.5772/66903>

Abstract

The industrial-scale manufacturing of viruses or virus-like particles in cell culture is necessary for gene therapy and the treatment of cancer with oncolytic viruses. Complex multistep processes are required in both cases, but the low virus titers in batch cultures and the temperature sensitivity of the virus particles limit the production scale. To meet commercial and regulatory requirements, each process must be scalable and reproducible and must yield high virus titers. These requirements are met by establishing a cell culture process that matches the properties of the virus/host-cell system and by using serum-free cell culture medium. This chapter focuses on two case studies to consider the different aspects of process design, such as the reactor configuration and operational mode: the continuous production of retroviral pseudotype vectors in a retroviral packaging cell line and the production of oncolytic measles virus vectors for cancer therapy.

Keywords: virus production, cultivation systems, online monitoring, measles, retrovirus

1. Introduction

Virus production techniques were originally developed for the manufacture of vaccines, but they are now becoming more important in other areas of the biopharmaceutical industry [1]. The scope for therapeutic strategies has broadened and now encompasses vectors for replacement protein expression, gene therapy and the treatment of cancer [2, 3]. Viruses for *in vivo* applications show a limited affinity for their target cells, they are generally unstable and large doses of infective virus particles (up to 10^{12} active virus particles per dose) are needed to achieve a therapeutic effect. Effective upstream production must therefore be combined with

optimized downstream processing. The production of viruses with clinical applications also raises important product safety requirements [4]. Bioprocess design for virus or virus vector production therefore depends strongly on the virus/host cell interactions and the kinetics of virus particle synthesis and virus release. There is no overall optimal process for virus production, and each system must be optimized on a case-by-case basis.

In this chapter, we introduce two virus production processes as case studies, emphasizing the differences in process design. The first case involves the murine leukemia virus (MLV) carrying the human immunodeficiency virus (HIV-1) envelope protein, produced using the retroviral packaging cell line TELCeB6/pTr712-K52S (K52S). The second case involves the production of oncolytic measles virus and considers process design options for lytic viruses.

1.1. Vectors for gene therapy

Somatic gene therapy involves the transfer genes into an organism with the aim of replacing and repairing genes in somatic cells, thus curing the disease without affecting healthy cells rather than providing symptomatic relief [5, 6]. Efficient, non-toxic vectors have to be developed for delivering foreign genetic materials into specific cells. Ideally, viral vectors harness the viral infection pathway without the expression of viral genes, which leads to replication, triggering the disease [7]. Stable vectors are required because of the long residence times *in vivo*. These treatments typically involve doses of 100–1000 mL with a virus density of at least 10^6 – 10^7 infective units per mL.

Gene therapy is currently used in ongoing clinical trials for the treatment of cancer [8], hereditary diseases [9], infectious diseases such as HIV infection [10, 11] and tissue engineering [12]. The replication of the virus particle must be inactivated to ensure that the vector itself does not cause a disease. Furthermore, all pathogenic virus genes must also be removed. The most effective way to do this is to generate virus-like particles (VLPs) that do not contain any viral genetic material. However, pseudotyping is an alternative approach in which the normal virus envelope proteins are replaced with those from another virus, either to improve stability or to favor interactions with particular host cells.

Non-replicating viral vectors for gene therapy are typically expressed in packaging cell lines containing only the essential structural virus genes [13]. This results in a virus construct that contains therapeutic RNA and regulatory elements to activate gene expression. The packaging cell lines thus continuously release RNA-containing vector particles into the medium. Established packaging cell lines can produce retroviral particles over a long period depending on their growth characteristics and are suitable for pilot-scale processes with a bioreactor volume of up to 50 L [14, 15].

This chapter describes the cultivation of the retroviral packaging cell line TELCeB6/pTr712-K52S (K52S). The cell line in this case study was derived from the *env*-negative MLV packaging cell line TELCeB6 by transfecting the HIV-1 *env* gene using plasmid pTr712 [16–18]. This results in the production of MLV(HIV-1) vector particles containing the transfer vector MFGlnslacZ. MLV/HIV-pseudotyped retroviral vectors can transduce human primary T lymphocytes [19] and are therefore suitable for the treatment of cutaneous T-cell lymphomas

[20], making them an effective tool for gene therapy. An efficient production process is necessary because one therapeutic dose typically requires 10^{10} – 10^{11} colony-forming units (cfu) per dose [17].

1.2. Oncolytic viruses

Over 60% of all ongoing clinical gene therapy trials worldwide deal with cancer. For this reason, cancer is by far the most common disease treated by viral vectors [21]. A promising approach is the inactivation of oncogenes or the activation of tumor suppressor genes. While oncogenes enhance cell proliferation, tumor suppressor genes induce apoptosis. The combination of chemotherapy and gene therapy will especially result in an effective suicide gene strategy [22]. Oncolytic viruses have a special status in the field of therapeutic viruses. Such viral particles kill tumor cells via two major mechanisms: (a) killing selective “degenerated” cells and (b) inducing a systemic anti-tumor immunity response [23]. Several research groups have attempted to increase the natural oncolytic effect of these viruses by genetic modification. For example, the immune response can be increased by modifying the expression of proteins such as granulocyte-macrophage colony-stimulating factor (GM-CSF) [24, 25] or by displaying the human carcinoembryonic antigen (CEA) [26–28]. Another strategy is the insertion of a tumor-specific promoter, which restricts viral expression to tumor cells [29]. Viral tropism can be engineered by displaying ligands that bind to cell surface receptors unique to cancer cells [10, 30–32]. Several genetically modified viruses have been adapted as potent oncolytic agents for clinical trials, including herpesvirus [33], adenovirus [34], poxvirus [35], coxsackievirus [36], polyovirus [37], Newcastle disease virus [38] and reovirus [39]. In October 2015, the US Food and Drug Administration (FDA) granted approval for the first oncolytic virus (talimogene laherparepvec or T-VEC, brand name IMLYGIC) indicated for cutaneous and nodal melanoma. In January 2016, this product was also approved in the European Union.

Another oncolytic virus candidate is the measles virus. Attenuated measles virus vaccines have been used for several decades [40] with an excellent safety profile following the administration of millions of doses. Measles virus particles have a high natural affinity for tumor cells because they favorably interact with certain tumor surface receptors such as nectin-4 [41]. The therapeutic concept takes advantage of both the specificity of the virus for tumor cells and of the lytic nature of a measles virus infection. Derivatives of measles virus vaccine strains are currently undergoing clinical testing for their efficacy in oncolytic virotherapy [42]. However, the broad application of measles virus in cancer therapy can only be successful if high titers of pure infectious virus particles can be manufactured. As discussed above for gene therapy vectors, oncolytic measles virus doses of 10^9 – 10^{12} particles are required per person and application [42].

The production of oncolytic measles virus was initially based on the process used to produce measles vaccines, but the requirements are quite different. First, animal-derived medium components are suitable for vaccine production but are not suitable for the manufacture of oncolytic vectors. Second, significantly higher titers of active, non-attenuated virus are required [43, 44]. This is by far the biggest issue in the field of oncolytic measles virus therapy because the therapeutic dose is 10^4 – 10^7 greater than the dose required for vaccination.

2. Bioprocess design

2.1. Product inactivation

Bioprocess design for the manufacturing of viral particles is strongly determined by the characteristics of the virus product. One major problem is the inactivation kinetics and the resulting negative effect on virus yield. In general, enveloped viruses are less stable against physicochemical stress than non-enveloped viruses [45]. Viruses suitable for gene therapy, e.g. herpesvirus particles, are sensitive towards heat [46, 47], low pH and low osmolality [48, 49]. The stability of some enveloped viruses against inactivation due to temperature or osmolality effects can be increased by adding stabilizer to the culture medium such as sucrose, glycerol, trehalose or a compatible solute [50–52].

In both of the case studies, thermal inactivation is a crucial factor that leads to the substantial loss of virus particles during cultivation. Typically, animal cell lines show the highest growth rate at a process temperature of 37°C. However, at 37°C, measles virus and retroviruses have half-lives of approximately 1 h and 6–7 h, respectively [46, 53]. There are two realistic options to avoid thermal inactivation: (a) reducing the process temperature or (b) harvesting the virus continuously, thereby achieving the rapid and efficient cooling of the product stream. However, a lower process temperature slows down host cell metabolism, which in turn reduces the virus titer. Therefore, the objective is to find an optimal temperature at which the virus production rate is higher than the inactivation rate. The former is dependent on the host cell and the latter on the virus. Retroviral vectors are known to be more stable if they are produced at 37°C. Vectors produced at 32°C are more rigid because of higher cholesterol content. This higher cholesterol content leads to a lower stability [54, 55]. In contrast, measles viruses showed a higher stability produced at 32°C [53]. The bioreactor system must eliminate as many factors as possible that reduce virus yields and should provide a suitable environment for the cells, avoiding additional stress caused by shear forces or nutrient limitation.

2.2. Bioreactor selection

The type of bioreactor system is primarily defined by the growth characteristics of the host cells. Animal cells grow either in suspension or as adherent cells, and several bioreactor systems have been established to meet their requirements. Subject to this distinction, bioreactor systems can be divided into three classes: static systems, semi-dynamic systems and dynamic systems (**Figure 1**).

Static cultivation systems are the simplest to handle, but the lack of power input (aeration or agitation) limits their use to screening studies rather than production. Semi-dynamic systems retain the cells while supplying them with fresh medium. This power input and convective mass transfer achieves a better nutrient supply at reasonable shear stress levels, but cell growth is heterogeneous and there is a lack of effective online/inline monitoring systems. Therefore, the most frequently used and well-characterized bioreactors are dynamic cultivation systems. These have a controlled power input, ensuring the homogeneous mixing of suspension cells and anchorage-dependent adherent cells growing on microcarriers. However, dynamic cultivation systems are a double-edged sword because the homogeneity

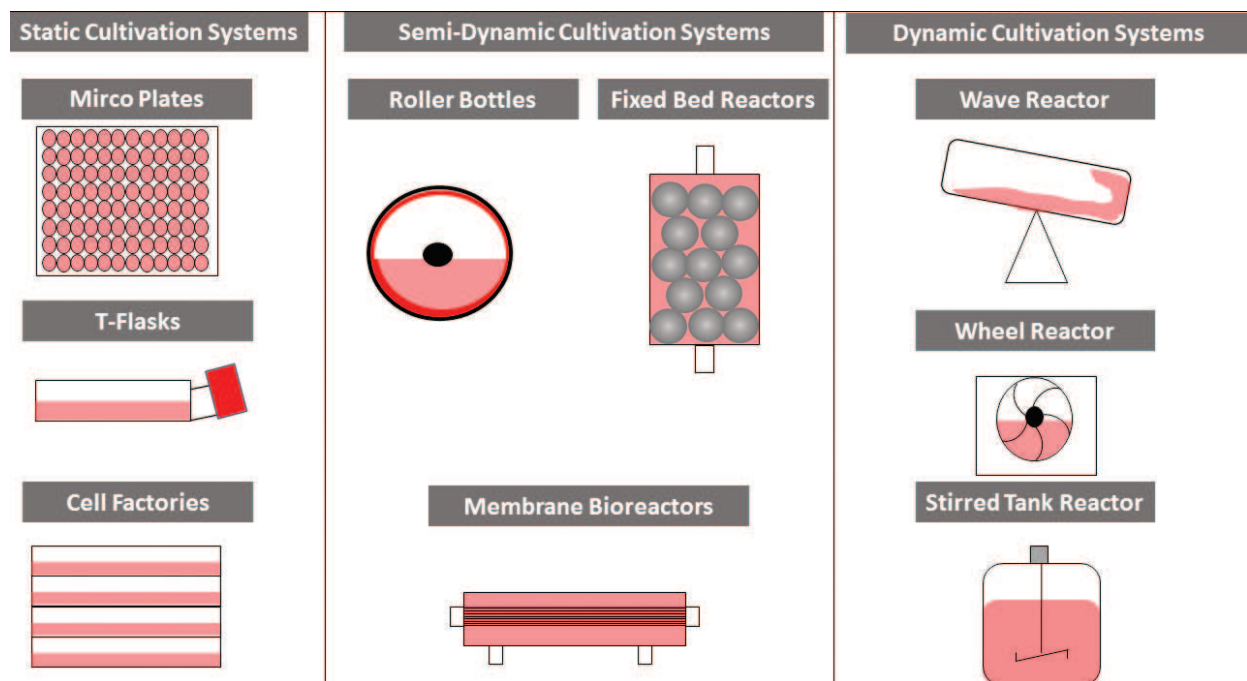


Figure 1. Overview of typical bioreactor systems for virus production.

of cell growth comes at the expense of high shear stress [56, 57]. Adherent cell lines can be shielded from shear forces by using porous carriers, and macroporous carriers are advantageous despite mass transfer limitations.

There is no optimal bioreactor for all virus production processes and the most suitable system must be chosen for each combination of virus and host cell, based on cell growth characteristics and virus infection kinetics. We will consider the most important parameters for our two case studies, seeking a balance between the advantages and disadvantages of the different systems.

2.2.1. Bioreactor selection for continuous retrovirus production

The MLV(HIV-1)-pseudotyped vector is released continuously by the adherent retroviral packaging cell line as discussed above. The harvesting strategy has a significant influence on the virus particle yield, so the bioreactor system must achieve the rapid and continuous collection of virus particles while ensuring total host cell retention. The best systems for this purpose are fixed-bed or hollow fiber bioreactors [17, 58]. Both systems immobilize cells to ensure complete cell retention. However, a fixed-bed bioreactor containing macroporous carriers can support a larger number of cells than a hollow fiber bioreactor because of the greater growth surface area, a key determinant of the product yield (**Figure 2**). Continuous virus production in fixed-bed bioreactors can maintain high performance over a production time of longer than 3 weeks. Virus purification and concentration were achieved using an integrated filtration system based on ceramic membranes [16–18]. Advantages of ceramic membranes are physical and chemical stability, durability (high expected lifetime), a narrow pore size distribution and a low potential for irreversible fouling. In addition, ceramic membranes are suitable for

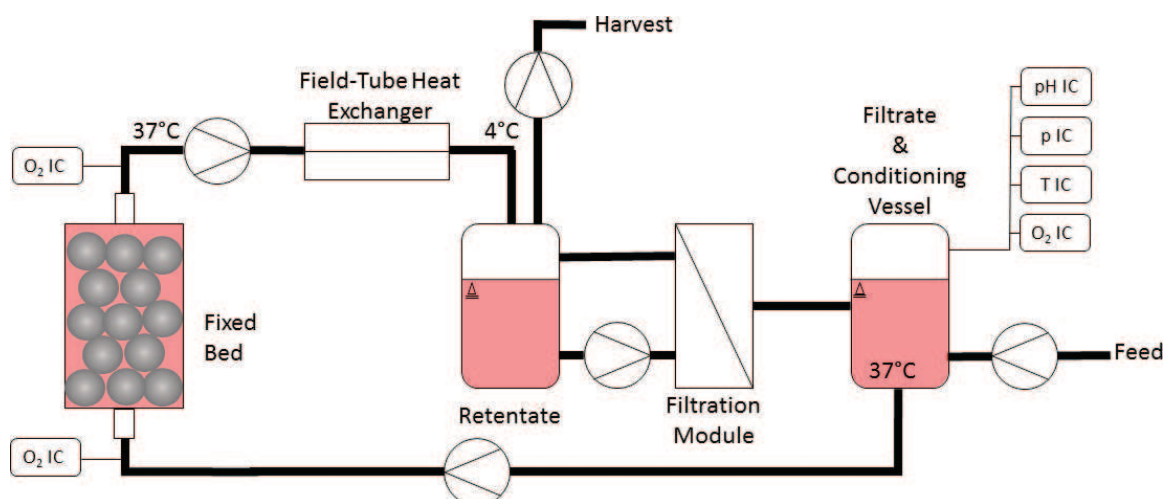


Figure 2. Continuous production process for MLV pseudotyped with the HIV-1 envelope protein in retroviral packaging cell line TELCeB6/pTr712-K52S (K52S).

sterilization in place (by, e.g., steam) [59]. However, the major advantage compared to organic membranes is the lack of organic extractables and leachables. For the use of a separation system in a continuous production process with a process time of several weeks, this fact is very important for the safety of the product [59, 60].

This process setup is superior to standard processes for the continuous production of degradable bioactive products because the product can be transferred from the culture medium to more suitable conditions (e.g. low temperature) and less handling is required compared to cultivation in standard culture flasks.

2.2.2. Bioreactor selection for measles virus production

In contrast to pseudotype vector production using a continuously infected packaging cell line, measles virus infection causes the total lysis of the host cells. The production cycle is therefore much shorter and the supernatant contains a high content of host cell proteins and other cell debris. Measles virus particles are typically produced using adherent Vero, HeLa or MRC-5 cells [43, 61, 62], so the cultivation system must provide a large growth surface area. The need to separate lysed cells from the supernatant overcomes the advantage of host cell retention in a fixed-bed bioreactor, so hollow fiber bioreactors or dynamic systems are preferable. However, this means that the other limitations of each bioreactor must be considered. Hollow fiber bioreactors require an external oxygenation system and a high circulation rate to achieve an adequate oxygen supply [63]. These demands on process design together with the large titers required for oncolytic therapy make the scale up of hollow fiber bioreactors unfeasible. To meet the necessary production scale and the power per dose, microcarrier-based dynamic cultivation systems are more effective than semi-dynamic systems. A stirred tank bioreactor is therefore preferable for the production of oncolytic measles virus particles (**Figure 3**).

One major drawback of stirred tank bioreactors is the shear force applied to the cells, which places limits on the maximum power input. Even so, the efficient suspension of the

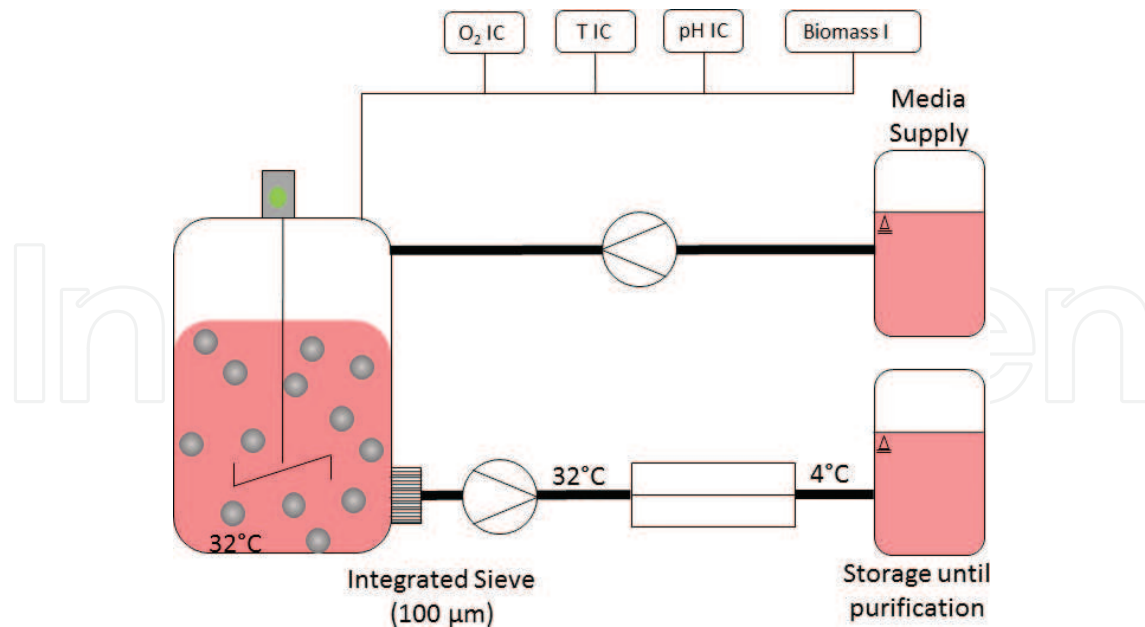


Figure 3. Bioreactor system for the production of oncolytic measles virus particles in perfusion mode.

microcarrier must be ensured to maintain homogeneous culture conditions. Proof of feasibility has been demonstrated by the cultivation of human mesenchymal stem cells at the 50 L scale without any deterioration in quality [64]. Because measles virus is characterized by a high rate of inactivation, the harvesting step must be carefully optimized. The continuous harvesting of lytic viruses produces large volumes of supernatant containing low concentrations of virus particles. Therefore, discontinuous harvesting strategies adapted to the lytic virus infection cycle can achieve better results. The best time for harvest has to be determined for each virus and host cell combination, and the optimal process mode depends on the kinetics of virus release.

2.3. Selection of the process mode

Process mode selection is one of the most challenging aspects of process design because all the advantages and drawbacks must be evaluated correctly. Virus production typically involves a two-step process: host cell expansion followed by the production phase (**Figure 4**). A deep understanding of the interaction between the host cell and virus is essential. Several critical parameters have been identified, including the host cell concentration at the time of infection (CCI) and the multiplicity of infection (MOI). These factors can be used to develop optimal strategies for process control.

2.3.1. Cell concentration at infection

The host cell is clearly a key factor in any virus production process, and for a wide range of processes, the mass of virus particles is linearly related to the number of host cells producing them. This is true for continuous production using packaging cell lines, but in other cases, increasing the cell density eventually causes the density-dependent inhibition of mitosis and

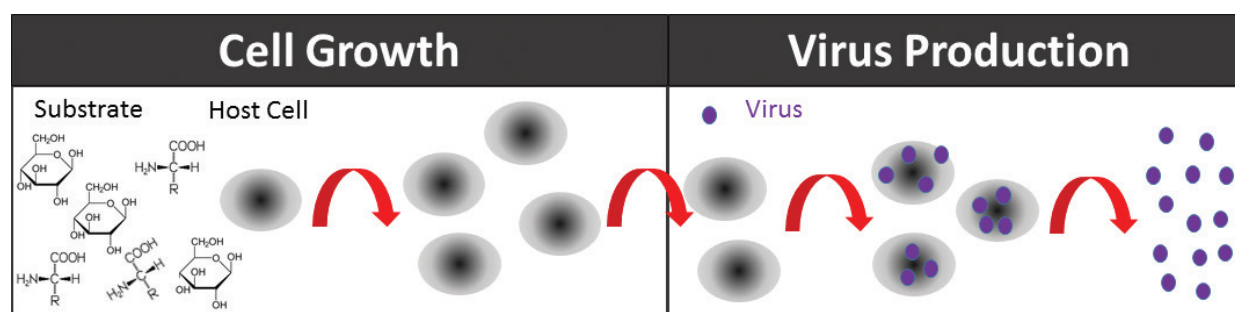


Figure 4. Substrate cascade during a virus production process. Initially, a sufficient supply of nutrients to the host cells must be ensured, but then the host cells become the substrate for virus production.

therefore reduces the virus yields [65]. The survival and growth of each cell line, as well as the production of viruses, are also dependent on a specific and individual minimum cell concentration [66]. This interdependency becomes more complex when additional factors such as virus infection kinetics, cytopathic effects are considered. Generally, a high virus yield requires an optimal CCI, although the CCI has only a minor effect on the yield of measles virus [44]. This is because a continuous supply of fresh uninfected host cells is required for the production of measles virus, to replenish the cells that are lost through lysis. Furthermore, several viruses (including measles virus) inhibit the cell cycle and thus affect cell growth [67, 68]. This can also influence the product yield, e.g., in the baculovirus-infected insect cell system the production of VLPs decreases when cells are infected during the late growth phase [69].

2.3.2. Multiplicity of infection

The MOI is the ratio of infective virus particles to host cell number and this can also influence the infection process. In the context of continuous production, the MOI is only of interest when generating a new packaging cell line [70]. In contrast, the MOI is a critical parameter when optimizing the production of lytic viruses used for the synthesis of recombinant proteins. Theoretically, in the best-case scenario, the termination of protein synthesis would coincide with medium depletion just prior to cell lysis [71]. This is also necessary during the production of lytic therapeutic viruses in order to recover active virus particles. Two infection strategies can be used for process optimization depending on the particular virus/host cell system. If the objective is a synchronous infection, then a large number of infective virus particles should be used ($\text{MOI} \gg 1$). A lower titer of infective viruses ($\text{MOI} < 1$) would result in a multiple-step virus amplification due to secondary infection of cells [72, 73]. Both strategies can amplify the virus yield by several hundred-fold when appropriately deployed. Aggarwal et al. [74] were able to increase the virus yield simply by using a lower MOI, but the characteristics of the virus strain should always be kept in mind because the outcome strongly depends on the biological system [75]. For the production of measles virus, a higher MOI can reduce the yield of active virus particles [44]. However, the behavior of each host cell/virus combination must always be studied in the context of the cultivation system. The MOI was shown to have a significant impact on virus yields in a static cultivation experiment in T-flasks, whereas in a stirred tank bioreactor the titer stayed within the same order of magnitude even if the MOI varied substantially (0.0005, 0.001 and 0.02) [44].

2.4. Process control

Reproducible, high-titer processes require appropriate measurement and control systems, including the established parameters of pH, temperature and oxygen concentration. However, real-time or online/inline monitoring and control systems for virus quantification are lacking, especially for the dynamic infection processes that characterize lytic virus production. Offline measurement methods are established and validated for several decades, but there are two major disadvantages. On the one hand, intervention in an operational system bears the risk of contaminations and on the other hand the lack of real-time data. Thus, it appears that online or inline measurement method should be preferred. The FDA has addressed this issue by launching the process analytical technology (PAT) initiative [76], whereas the European Medicines Agency (EMA) has implemented the similar concept of continuous process verification [77]. To fulfil these demanding requirements, bioreactor systems have been fitted with diverse monitoring systems including near infrared spectroscopy, and in situ microscopy [78–80]. Multivariate data analysis is then applied to determine a benchmark known as the “golden batch”. Subsequent batches are evaluated against this standard, and if the signals do not fall within certain tolerances then the process is aborted. This strategy usually lacks process control opportunities and large numbers of failed batches are generated. The in-line measurement of important media components, and parameters for the online evaluation of process reproducibility and stability, thus remain among the unsolved problems of industrial biotechnology [81, 82].

One example of the above is the measurement of cell numbers in static or semi-dynamic cultivation systems. Offline, cell number could be determined, e.g., by membrane leakage assays (e.g. Trypan blue staining) or mitochondrial activity assays (e.g. WST-1 assay). In small scale, microscopy-based cell counting devices for static cultivation systems are also available [83]. Typically online, these data are collected by indirect inline measurement, e.g. by estimating the cell number based on the oxygen demand or glucose consumption rate of adherent cells growing in semi-dynamic cultivation systems [58, 84, 85]. In a fixed-bed bioreactor, this is realized by the simultaneous measurement of oxygen concentrations at the inlet and outlet. Oxygen demand is tightly coupled to cellular metabolism and strongly affected by virus infection, so the estimates are often inaccurate. Dielectric spectroscopy is a promising alternative approach for the inline quantification of host cells because the measurement is decoupled from metabolism and is based on the passive dielectric properties of cells in a conductive medium. When an alternating electric field is applied to the cell suspension culture, the cell membranes act as small capacitors leading to a buildup of electrical charge (polarization). The overall capacitance is thereby dependent on the frequency of the alternating electric field (usually in the range 0.1–10 MHz), as well as the cell size, morphology and cell concentration [86]. For suspension cultures with a uniform cell size, this technique is appropriate for host cell quantification because there is a correlation between the recorded permittivity and cell number. Dielectric spectroscopy can also be used for the online quantitation of adherent cells, which usually requires cell detachment and offline cell counting. Dielectric spectroscopy can thus be used for the online monitoring of cell attachment and growth as shown during measles virus production (**Figure 5A and B**).

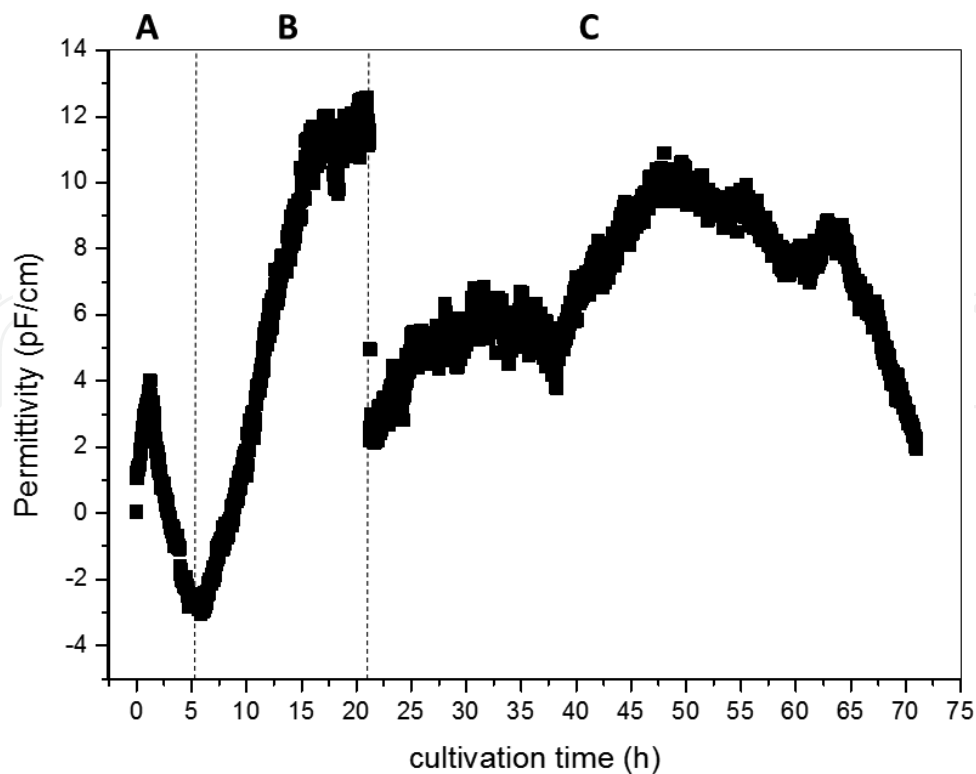


Figure 5. Impedance spectrometer readings during the production of measles virus using Vero cells growing on Cytodex 1 microcarriers in a 1-L stirred tank bioreactor. A: Cell adhesion to the microcarrier at a very high initial cell concentration of 80,000 cells per cm^2 . B: Cell growth in the bioreactor. C: Addition of 0.5-L fresh culture medium with simultaneous virus infection.

However, the permittivity signal is disrupted almost as soon as cells are infected. Dielectric spectroscopy readings are therefore strongly influenced by the cytopathic effect of the measles virus on the host cell (e.g. the formation of syncytia) and subsequent virus release. These changes in morphology also influence the permittivity signal (**Figure 5C**). Because the online quantification of virus particles is not possible, such morphological changes can be used for process control by calculating the Cole-Cole exponent parameter α . This is a dimensionless number with a value between 0 and 1 that nominally describes the distribution of relaxation times in the suspension, or the homogeneity of the cell population. In a homogenous cell suspension culture, the initial release of virus particles can be detected because the α parameter declines 24 h post-infection (**Figure 6**).

The value of α is also influenced by changes in cell morphology. This can be shown by comparing homogenous cell suspension cultures and adherent cells growing on microcarriers when both are infected with measles virus (**Figure 7**). The signal corresponding to virus release is superimposed on signals representing changes in cell morphology (e.g. the formation of syncytia), which makes data interpretation and process control more challenging. For the production of the lytic measles virus, dielectric spectroscopy can be used to ensure that cells are infected at the same point in the growth phase and to determine when virus particles are released, making it an ideal tool for process control in dynamic cultivation systems.

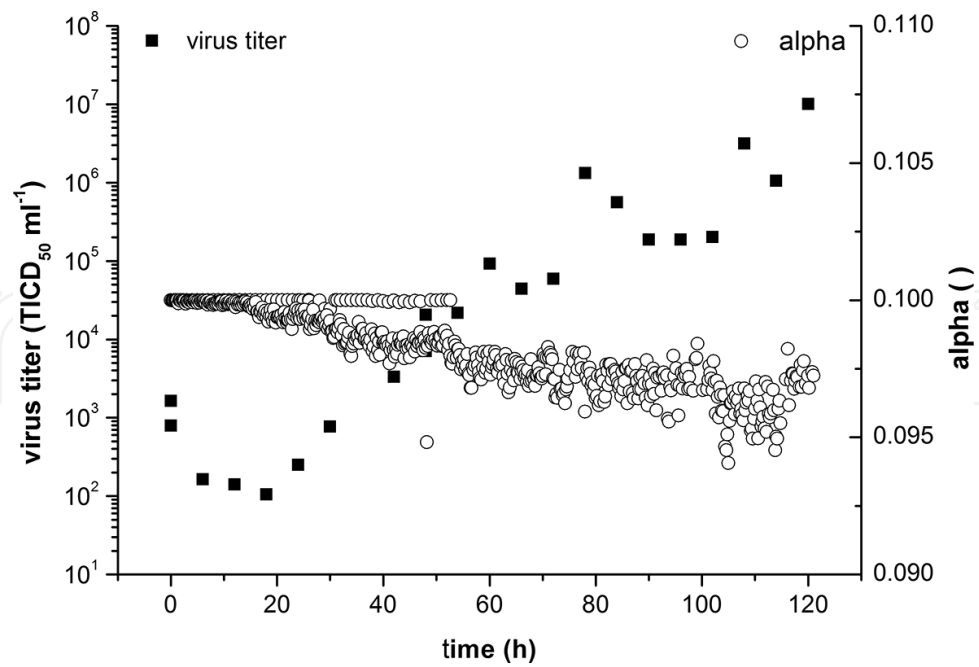


Figure 6. Virus release from a Burkitt's lymphoma cell line infected with measles virus.

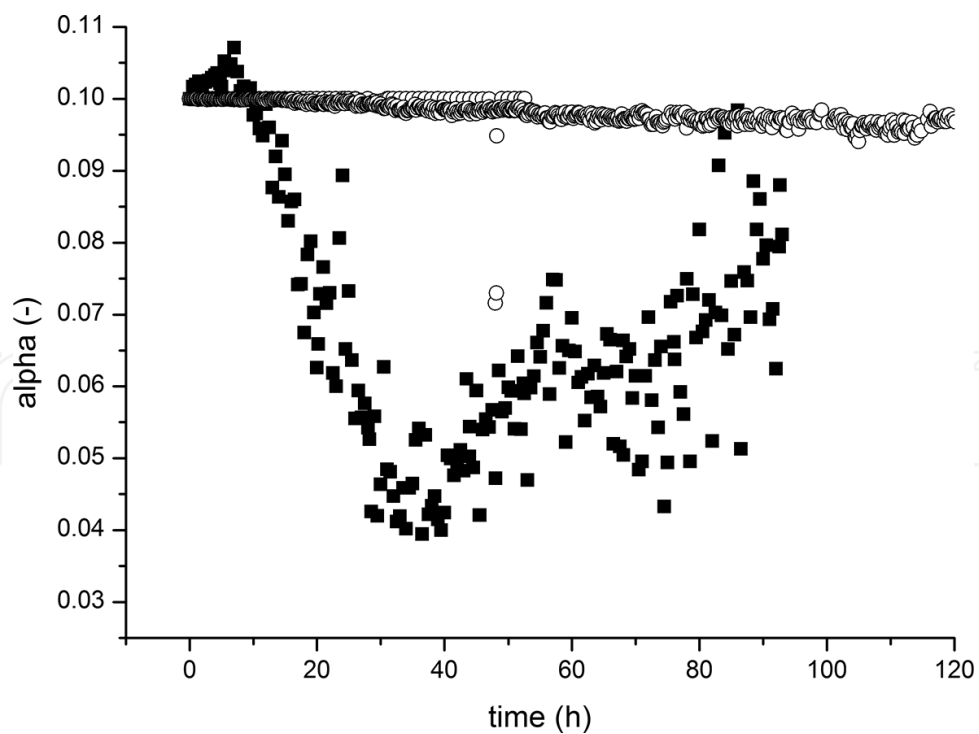


Figure 7. Distribution of relaxation times in the suspension (Cole-Cole exponent parameter α) of adherent cells (■) and cell suspension cultures (○).

3. Conclusion

Different production processes were established in each of our case studies by taking into account the requirements of the host cells and products, and the benefits and limitations of each type of bioreactor. The system of choice for the continuous production of pseudotype vector MLV(HIV-1) using the retroviral packaging cell line TELCeB6/pTr712-K52S (K52S) was the fixed-bed bioreactor (**Figure 2**). The cells were cultivated in a vessel packed with Fibracell macroporous carriers, with a working volume of 200 mL and an inner diameter of 56 mm. Perfusion of the immobilized cells was achieved using an additional vessel (working volume 2 L) for medium conditioning, and process parameters such as dissolved oxygen and pH were monitored and controlled in this vessel. Cell growth in the fixed-bed bioreactor could not be monitored using dielectric spectroscopy because of the small measuring volume, reflecting the fixed positions of the probe and the carrier. Cell concentration was therefore estimated based on the oxygen consumption rate, determined by the simultaneous measurement of oxygen concentrations at the inlet and outlet.

The perfused medium was preheated to 37°C, and the viral vectors were harvested continuously to improve vector stability and host cell productivity using an integrated filtration system [17]. To ensure optimal substrate consumption and yield, a portion of the virus-free filtrate was recycled to the conditioning vessel of the fixed-bed bioreactor [16, 18]. The tubular filtration unit comprised an Al₂O₃ support coated with asymmetric layers of ZrO₂ and TiO₂. The tubular membrane had an outer diameter of 25 mm and 19 inner tubes each with a diameter of 3 mm, with a molecular weight cut-off of 20 kDa (Innovations GmbH, Gladbeck, Germany). Filtration was performed at a flow rate of 2 L min⁻¹ and a transmembrane pressure of 0.4 bar. This combination of membrane type and operational mode allowed continuous filtration for more than 400 h. Virus production was tested in batch mode to confirm the efficiency of the reactor setup. The maximum virus titer of 2.76×10^9 cfu was achieved in perfusion mode, whereas the maximum yield in batch mode was only 1.4×10^6 cfu [16–18]. This probably reflects the higher volumetric throughput of perfusion mode and the low stability of the virus particles at the production temperature.

The design of a continuous production process for measles virus is more challenging because virus release depends on the lysis of the adherent, growing host cells. In contrast to packaging cell lines, the host cells are consumed during the production of lytic viruses, which limits the length of the production cycle and also the yields. A microcarrier based cultivation system was established to ensure the continuous supply of fresh host cells in a scalable system. Cells growing on Cytodex 1 microcarriers in a stirred tank bioreactor were detached by incubation for 10 min in PBS containing 0.025% TrypZean and 0.01% EDTA, thus enabling bead-to-bead transfer. This provided a seed train from T-flasks to a large-scale stirred tank bioreactor and the addition of up to 75% fresh microcarrier [87, 88]. As discussed above for the pseudotyped vector, the process temperature was the most critical process parameter for the production of measles virus. Process temperature optimization in different cultivation systems led to contradictory results [44]: in a static cultivation system (T-flasks) and in a stirred tank bioreactor, the highest virus titers were achieved at a cultivation temperature of 32°C, but in small-scale dynamic cultivation systems (spinner flasks) the highest titers were achieved at

37°C. However, both cultivation temperatures resulted in severe thermal virus inactivation and continuous harvesting was therefore necessary. Filtration during cultivation is challenging due to the rapid accumulation of host cell proteins (including proteases) and cell debris in the surrounding medium caused by cell lysis, resulting in the blocking of membrane pores. Furthermore, measles virus particles range in size from 300 nm to 1 µm [89]. The blocking of filter pores by debris and the particle size range of the virus make discontinuous filtration more appropriate. Regardless of the cultivation system, the best process mode was repeated batch mode with a daily virus harvest [44]. Using this setup (**Figure 3**), virus titers of up to 7.4×10^9 median tissue culture infective dose (TCID₅₀) can be managed in a working volume of 500 mL containing 3 g L⁻¹ Cytodex 1.

The analysis of these two representative processes provides insight into the challenges of process design when using sensitive viral production systems. As stated above, there is no perfect process, but the best process for each combination of host cell and product can be determined by considering as many parameters as possible in order to maximize the product yield. Many investigations are still required to satisfy the demands of large-scale virus production, for both the pseudotype vector and the oncolytic measles virus. The virus/host cell system must be understood in detail to facilitate the development of an appropriate production process which reproducibly produces sufficient yields of high-quality virus particles.

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References

- [1] Genzel Y, Reichl U. Vaccine production. In: Pörtner R, editor. *Animal Cell Biotechnology: Methods and Protocols*. Totowa, NJ: Humana Press; 2007. pp. 457–73.
- [2] Chiocca EA. Oncolytic viruses. *Nature Reviews Cancer*. 2002;2(12):938–50.
- [3] Kotterman MA, Chalberg TW, Schaffer DV. Viral vectors for gene therapy: translational and clinical outlook. *Annual Review of Biomedical Engineering*. 2015;17(1):63–89.

- [4] European Medicines Agency, Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products; EMA/CAT/80183/2014. 2015.
- [5] Mulligan R. The basic science of gene therapy. *Science*. 1993;260(5110):926–32.
- [6] Ginn SL, Alexander IE, Edelstein ML, Abedi MR, Wixon J. Gene therapy clinical trials worldwide to 2012 – an update. *The Journal of Gene Medicine*. 2013;15(2):65–77.
- [7] Thomas CE, Ehrhardt A, Kay MA. Progress and problems with the use of viral vectors for gene therapy. *Nature Reviews Genetics*. 2003;4(5):346–58.
- [8] Lai YH, Lin CC, Chen SH, Tai CK. Tumor-specific suicide gene therapy for hepatocellular carcinoma by transcriptionally targeted retroviral replicating vectors. *Gene Therapy*. 2015;22(2):155–62.
- [9] Eichler, F., et al., Interim Results from a Phase 2/3 Study of the Efficacy and Safety of Ex Vivo Gene Therapy With Lentiviral Vector (Lenti-D) for Childhood Cerebral Adrenoleukodystrophy. *Neurology*, 2016. 86; 16 Supplement PL02.002.
- [10] Spragg, C., H. De Silva Felixge, and K.R. Jerome, Cell and gene therapy strategies to eradicate HIV reservoirs. *Curr Opin HIV AIDS*, 2016. 11(4): p. 442–9.
- [11] Liao, H.-K., et al., Use of the CRISPR/Cas9 system as an intracellular defense against HIV-1 infection in human cells. *Nature Communications*, 2015. 6: p. 6413.
- [12] Cucchiari M, McNulty AL, Mauck RL, Setton LA, Guilak F, Madry H. Advances in combining gene therapy with cell and tissue engineering-based approaches to enhance healing of the meniscus. *Osteoarthritis and Cartilage*. 2016;24(8):1330–9.
- [13] Sanber KS, Knight SB, Stephen SL, Bailey R, Escors D, Minshull J, et al. Construction of stable packaging cell lines for clinical lentiviral vector production. *Scientific Reports*. 2015;5:9021.
- [14] Ghani K, Cottin S, Kamen A, Caruso M. Generation of a high-titer packaging cell line for the production of retroviral vectors in suspension and serum-free media. *Gene Therapy*. 2007;14(24):1705–11.
- [15] Cosset FL, Takeuchi Y, Battini JL, Weiss RA, Collins MK. High-titer packaging cells producing recombinant retroviruses resistant to human serum. *Journal of Virology*. 1995;69(12):7430–6.
- [16] Nehring D, Gonzalez R, Pörtner R, Czermak P. Experimental and modeling study of a membrane filtration process using ceramic membranes to increase retroviral pseudotype vector titer. *Journal of Membrane Science*. 2004;237(1–2):25–38.
- [17] Nehring D, Gonzalez R, Pörtner R, Czermak P. Experimental and modelling study of different process modes for retroviral production in a fixed bed reactor. *Journal of Biotechnology*. 2006;122(2):239–53.
- [18] Nehring D, Poertner R, Schweizer M, Cichutek K, Czermak P. Integrated inline filtration: a method to produce highly concentrated retroviral vector titer supernatant. *Desalination*. 2009;245(1–3):614–20.

- [19] Mühlebach MD, Schmitt I, Steidl S, Stitz J, Schweizer M, Blankenstein T, et al. Transduction efficiency of MLV but not of HIV-1 vectors is pseudotype dependent on human primary T lymphocytes. *Journal of Molecular Medicine*. 2003;81(12):801–10.
- [20] Thaler S, Burger AM, Schulz T, Schnierle BS. MLV/HIV-pseudotyped vectors: a new treatment option for cutaneous T cell lymphomas. *Molecular Therapy*. 2003;8(5):756–61.
- [21] Wirth T, Parker N, Ylä-Herttuala S. History of gene therapy. *Gene*. 2013;525(2):162–9.
- [22] El-Aneed A. An overview of current delivery systems in cancer gene therapy. *Journal of Controlled Release*. 2004;94(1):1–14.
- [23] Rammensee H-G. From basic immunology to new therapies for cancer patients. In: Britten MC, Kreiter S, Diken M, Rammensee H-G, editors. *Cancer Immunotherapy Meets Oncology: In Honor of Christoph Huber*. Cham: Springer International Publishing; 2014. pp. 3–11.
- [24] Guillerme JB, Boisgerault N, Roulois D, Menager J, Combredet C, Tangy F, et al. Measles virus vaccine-infected tumor cells induce tumor antigen cross-presentation by human plasmacytoid dendritic cells. *Clinical Cancer Research*. 2013;19(5):1147–58.
- [25] Coffin R. S, McNeish M.H.I, Sibtain A, Hamilton B, Love C, Nutting C, Harrington K, Phase I/II trial of OncoVEXGM-CSF combined with radical chemoradiation (CRT) in patients with newly diagnosed node-positive stage III/IV head and neck cancer (HNC). *Journal of Clinical Oncology (ASCO Annual Meeting Proceedings)*, 2007. 25(18): p. 14095.
- [26] Patel MR, Kratzke RA. Oncolytic virus therapy for cancer: the first wave of translational clinical trials. *Translational Research*. 2013;161(4):355–64.
- [27] McDonald CJ, Erlichman C, Ingle JN, Rosales GA, Allen C, Greiner SM, et al. A measles virus vaccine strain derivative as a novel oncolytic agent against breast cancer. *Breast Cancer Research and Treatment*. 2006;99(2):177–84.
- [28] Zhang, S.C., et al., Engineered measles virus Edmonston strain used as a novel oncolytic viral system against human hepatoblastoma. *Bmc Cancer*, 2012. 12.p. 427.
- [29] Wong HH, Lemoine NR, Wang Y. Oncolytic viruses for cancer therapy: overcoming the obstacles. *Viruses*. 2010;2(1):78–106.
- [30] Jurgens, E.M., et al., Measles Fusion Machinery Is Dysregulated in Neuropathogenic Variants. *mBio*, 2015. 6(1):e02528-14, doi:10.1128/mBio.02528-14.
- [31] Maisner A, Schneider-Schaulies J, Liszewski MK, Atkinson JP, Herrler G. Binding of measles virus to membrane cofactor protein (CD46): importance of disulfide bonds and N-glycans for the receptor function. *Journal of Virology*. 1994;68(10):6299–304.
- [32] Segerman A, Atkinson JP, Marttila M, Dennerquist V, Wadell G, Arnberg N. Adenovirus type 11 uses CD46 as a cellular receptor. *Journal of Virology*. 2003;77(17):9183–91.
- [33] Kasuya H, Kodera Y, Nakao A, Yamamura K, Gewen T, Zhiwen W, et al. Phase I dose-escalation clinical trial of HF10 oncolytic herpes virus in 17 Japanese patients with advanced cancer. *Hepatogastroenterology*. 2014;61(131):599–605.

- [34] Cohen EE, Rudin CM. ONYX-015. Onyx Pharmaceuticals. *Current Opinion in Investigational Drugs*. 2001;2(12):1770–5.
- [35] Parato KA, Breitbach CJ, Le Boeuf F, Wang J, Storbeck C, Ilkow C, et al. The oncolytic poxvirus JX-594 selectively replicates in and destroys cancer cells driven by genetic pathways commonly activated in cancers. *Molecular Therapy*. 2012;20(4):749–58.
- [36] Miyamoto S, Inoue H, Nakamura T, Yamada M, Sakamoto C, Urata Y, et al. Coxsackievirus B3 is an oncolytic virus with immunostimulatory properties that is active against lung adenocarcinoma. *Cancer Research*. 2012;72(10):2609–21.
- [37] Goetz C, Dobrikova E, Shveygert M, Dobrikov M, Gromeier M. Oncolytic poliovirus against malignant glioma. *Future Virology*. 2011;6(9):1045–58.
- [38] Mansour M, Palese P, Zamarin D. Oncolytic specificity of Newcastle disease virus is mediated by selectivity for apoptosis-resistant cells. *Journal of Virology*. 2011;85(12):6015–23.
- [39] Thirukkumaran C, Morris DG. Oncolytic viral therapy using reovirus. In: Walther W, Stein U, editors. *Gene Therapy of Solid Cancers: Methods and Protocols*. New York, NY: Springer New York; 2015. pp. 187–223.
- [40] Greenwood KP, Hafiz R, Ware RS, Lambert SB. A systematic review of human-to-human transmission of measles vaccine virus. *Vaccine*. 2016;34(23):2531–6.
- [41] Mühlebach MD, Mateo M, Sinn PL, Prüfer S, Uhlig KM, Leonard VH, et al. Adherens junction protein nectin-4 is the epithelial receptor for measles virus. *Nature*. 2011;480(7378):530–3.
- [42] Russell SJ, Federspiel MJ, Peng K-W, Tong C, Dingli D, Morice WG, et al. Remission of disseminated cancer after systemic oncolytic virotherapy. *Mayo Clinic Proceedings*. 2014;89(7):926–33.
- [43] Trabelsi K, Majoul S, Rourou S, Kallel H. Development of a measles vaccine production process in MRC-5 cells grown on Cytodex1 microcarriers and in a stirred bioreactor. *Applied Microbiology and Biotechnology*. 2014;93(3):1031–40.
- [44] Weiss K, Gerstenberger J, Salzig D, Mühlebach MD, Cichutek K, Pörtner R, et al. Oncolytic measles viruses produced at different scales under serum-free conditions. *Engineering in Life Sciences*. 2015;15(4):425–36.
- [45] Firquet S, Beaujard S, Lobert P-E, Sané F, Caloone D, Izard D, et al. Survival of enveloped and non-enveloped viruses on inanimate surfaces. *Microbes and Environments*. 2015;30(2):140–4.
- [46] Andreadis ST, Roth CM, Le Doux JM, Morgan JR, Yarmush ML. Large-scale processing of recombinant retroviruses for gene therapy. *Biotechnology Progress*. 1999;15(1):1–11.
- [47] Wechuck JB, Ozuer A, Goins WF, Wolfe D, Oligino T, Glorioso JC, et al. Effect of temperature, medium composition, and cell passage on production of herpes-based viral vectors. *Biotechnology and Bioengineering*. 2002;79(1):112–9.

- [48] Grein TA, Michalsky R, Vega Lopez M, Czermak P. Purification of a recombinant baculovirus of *Autographa californica* M nucleopolyhedrovirus by ion exchange membrane chromatography. *Journal of Virological Methods*. 2012;183(2):117–24, doi: 10.1016/j.jviromet.2012.03.031.
- [49] Melnick JL. Virus inactivation: lessons from the past. *Developments in Biological Standardization*. 1991;75:29–36.
- [50] Croyle MA, Cheng X, Wilson JM. Development of formulations that enhance physical stability of viral vectors for gene therapy. *Gene Therapy*. 2001;8(17):1281–90.
- [51] Cruz PE, Silva AC, Roldão A, Carmo M, Carrondo MJT, Alves PM. Screening of novel excipients for improving the stability of retroviral and adenoviral vectors. *Biotechnology Progress*. 2006;22(2):568–76.
- [52] Kissmann J, Ausar SF, Rudolph A, Braun C, Cape SP, Sievers RE, et al. Stabilization of measles virus for vaccine formulation. *Human Vaccines*. 2008;4(5):350–9.
- [53] Weiss K, Salzig D, Röder Y, Gerstenberger J, Mühlebach MD, Cichutek K, et al. Influence of process conditions on measles virus stability. *American Journal of Biochemistry and Biotechnology*. 2013;3(9):243–54.
- [54] Carmo M, Faria TQ, Falk H, Coroadinha AS, Teixeira M, Merten OW, et al. Relationship between retroviral vector membrane and vector stability. *Journal of General Virology*. 2006;87(Pt 5):1349–56.
- [55] Beer C, Meyer A, Müller K, Wirth M. The temperature stability of mouse retroviruses depends on the cholesterol levels of viral lipid shell and cellular plasma membrane. *Virology*. 2003;308(1):137–46.
- [56] Grein, T.A., et al., Multiphase mixing characteristics in a microcarrier-based stirred tank bioreactor suitable for human mesenchymal stem cell expansion. *Process Biochemistry*, 2016. 51(9): p. 1109–1119.
- [57] Rewatkar VB, Rao KSMSR, Joshi JB. Critical impeller speed for solid suspension in mechanically agitated three-phase reactors. 1. Experimental part. *Industrial & Engineering Chemistry Research*. 1991;30(8):1770–84.
- [58] Tapia F, Vogel T, Genzel Y, Behrendt I, Hirschel M, Gangemi JD, et al. Production of high-titer human influenza A virus with adherent and suspension MDCK cells cultured in a single-use hollow fiber bioreactor. *Vaccine*. 2014;32(8):1003–11.
- [59] Grein TA, Kovacs Z, Ebrahimi M, Michalsky R, Czermak P. Membrane supported virus separation from biological solutions. *Chemie Ingenieur Technik*. 2013;85(8):1183–92.
- [60] Grein T, Michalsky R, Czermak P. Virus separation using membranes. *Animal Cell Biotechnology*. 2014;1104:459–91.
- [61] Betáková T, Svetlílová D, Gocník M. Overview of measles and mumps vaccine: origin, present, and future of vaccine production. *Acta Virologica*. 2013;57(2):91–6.

- [62] Langfield K., et al., Manufacture of Measles Viruses, in *Viral Vectors for Gene Therapy*, O.-W. Merten and M. Al-Rubeai, Editors. 2011, Humana Press. p. 345–366. Heidelberg.
- [63] Ala-Uotila S, Marjamäki A, Matikainen M-T, Jalkanen M. Use of a hollow fiber bioreactor for large-scale production of $\alpha 2$ -adrenoceptors in mammalian cells. *Journal of Biotechnology*. 1994;37(2):179–84.
- [64] Schirmaier C, Jossen V, Kaiser SC, Jüngerkes F, Brill S, Safavi-Nab A, et al. Scale-up of adipose tissue-derived mesenchymal stem cell production in stirred single-use bioreactors under low-serum conditions. *Engineering in Life Sciences*. 2014;14(3):292–303.
- [65] Stoker MGP, Rubin H. Density dependent inhibition of cell growth in culture. *Nature*. 1967;215(5097):171–2.
- [66] George, E.F., G.J.D. Michael A Hall Klerk, and P.D. Sherrington, *Plant Propagation by Tissue Culture: The background*. 2008: Springer. Heidelberg.
- [67] Schang LM. The cell cycle, cyclin-dependent kinases, and viral infections: new horizons and unexpected connections. *Progress in Cell Cycle Research*. 2003;5:103–24.
- [68] Wang M, Libbey JE, Tsunoda I, Fujinami RS. Modulation of immune system function by measles virus infection. II. Infection of B cells leads to the production of a soluble factor that arrests uninfected B cells in G0/G1. *Viral Immunology*. 2003;16(1):45–55.
- [69] Maranga L, Rueda P, Antonis A, Vela C, Langeveld J, Casal J, et al. Large scale production and downstream processing of a recombinant porcine parvovirus vaccine. *Applied Microbiology and Biotechnology*. 2002;59(1):45–50.
- [70] Kafri T, van Praag H, Ouyang L, Gage FH, Verma IM. A packaging cell line for lentivirus vectors. *Journal of Virology*. 1999;73(1):576–84.
- [71] Aubrit F, Perugi F, Léon A, Guéhenneux F, Champion-Arnaud P, Lahmar M, et al. Cell substrates for the production of viral vaccines. *Vaccine*. 2015;33(44):5905–12.
- [72] Enden G, Zhang YH, Merchuk JC. A model of the dynamics of insect cell infection at low multiplicity of infection. *Journal of Theoretical Biology*. 2005;237(3):257–64.
- [73] Power JF, Nielsen LK. Modelling baculovirus infection of insect cells in culture. *Cytotechnology*. 1996;20(1):209–19.
- [74] Aggarwal K, Jing F, Maranga L, Liu J. Bioprocess optimization for cell culture based influenza vaccine production. *Vaccine*. 2011;29(17):3320–8.
- [75] Isken B, Genzel Y, Reichl U. Productivity, apoptosis, and infection dynamics of influenza A/PR/8 strains and A/PR/8-based reassortants. *Vaccine*. 2012;30(35):5253–61.
- [76] FDA, U.F.a.D.A., *Guidance for Industry PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance*, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug

Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), Office of Regulatory Affairs (ORA), 2004, Rockville.

- [77] European Medicines Agency, EMEA, Guideline on process validation for finished products - information and data to be provided in regulatory submissions EMA/CHMP/CVMP/QWP /BWP/ 70278/2012- Rev 1,Corr.1, Nov. 2016.
- [78] Lee-Montiel FT, Reynolds KA, Riley MR. Detection and quantification of poliovirus infection using FTIR spectroscopy and cell culture. *Journal of Biological Engineering*. 2011;5:16.
- [79] Druzinec D, Salzig D, Brix A, Kraume M, Vilcinskas A, Kollewe C, et al. Optimization of insect cell based protein production processes - online monitoring, expression systems, scale up. *Advances in Biochemical Engineering/Biotechnology*. 2013;163:65–100.
- [80] Copin R, Vitry M-A, Mambres DH, Machelart A, De Trez C, Vanderwinden J-M, et al. In situ microscopy analysis reveals local innate immune response developed around Brucella infected cells in resistant and susceptible mice. *PLoS Pathogens*. 2012;8(3):e1002575.
- [81] Sandor M, Rudinger F, Solle D, Bienert R, Grimm C. NIR spectroscopy for process monitoring and control in mammalian cell cultivation. *Bioprocess International*. 2013;11:40–50.
- [82] Luttmann, R., Borchert, S.-O., Müller, C., Lögering, K., Aupert, F., Weyand, S., Kober, C., Faber, B., Cornelissen, G., 2015. Sequential/parallel production of potential Malaria vaccines – A direct way from single batch to quasi-continuous integrated production. *J. Biotechnol.*, doi:10.1016/j.jbiotec.2015.02.022
- [83] Johnston ST, Shah ET, Chopin LK, McElwain DS, Simpson MJ. Estimating cell diffusivity and cell proliferation rate by interpreting IncuCyte ZOOM™ assay data using the Fisher-Kolmogorov model. *BMC Systems Biology*. 2015;9(1):1.
- [84] Weber C, Freimark D, Portner R, Pino-Grace P, Pohl S, Wallrapp C, et al. Expansion of human mesenchymal stem cells in a fixed-bed bioreactor system based on non-porous glass carrier--part A: inoculation, cultivation, and cell harvest procedures. *The International Journal of Artificial Organs*. 2010;33(8):512–25.
- [85] Justice C, Brix A, Freimark D, Kraume M, Pfromm P, Eichenmueller B, et al. Process control in cell culture technology using dielectric spectroscopy. *Biotechnology Advances*. 2011;29(4):391–401.
- [86] Yardley JE, Kell DB, Barrett J, Davey CL. On-line, real-time measurements of cellular biomass using dielectric spectroscopy. *Biotechnology and Genetic Engineering Reviews*. 2000;17(1):3–36.
- [87] Weiss K, Freimark D, Mühlebach MD, Pörtner R, Czermak P. Measles virus production process for the use in cancer therapy. *The International Journal of Artificial Organs*. 2011;34(8):698.

- [88] Weiss K, Salzig D, Mühlebach MD, Pörtner R, Czermak P. Investigations on measles virus production for cancer therapy in a serum-free medium. *Human Gene Therapy*. 2011;22(10):A124–5.
- [89] Daikoku E, Morita C, Kohno T, Sano K. Analysis of morphology and infectivity of measles virus particles. *Bulletin of the Osaka Medical College*. 2007;53:107–14.

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