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# Continuous manufacturing of viral particles

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Cell-based production of viral particles has gain interest in the last years due to promising therapeutic applications. In the field of vaccination, novel vaccines are required to face the new pathogen outbreaks, and rapid and more efficient processes are required to respond as fast as possible to the vaccination demand worldwide. On the other hand, viral vector-driven gene therapies have demonstrated to be efficient and safe in numerous clinical trials, and three of them are already approved for commercialization. However, viral vector production is still a bottleneck in the road to the clinic. Although batch and fed-batch culture modes are preferred in industry, continuous culture strategies have demonstrated to improve viral titers and to reduce the bioprocessing costs. Therefore, there is increasing interest in exploring and optimizing continuous strategies in order to intensify viral vector production bioprocesses. This review is a summary of how continuous cultures have been applied to viral particle production (viral vaccines and viral vectors), the improvements achieved so far and the future perspectives in this field.

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## Introduction

Unlike batch and fed-batch cultures, a continuous cell culture is fed with fresh medium, while spent medium is removed to maintain a constant volume in the bioreactor. Continuous cultures are often performed to achieve high cell densities, which means that medium perfusion rates are higher than the cell growth rate and cells need to be retained inside the bioreactor. The variety of cell retention devices has been previously reviewed [1]. However, the most commonly used are Tangential Flow Filtration (TFF), Alternating Tangential Flow (ATF), and acoustic filters.

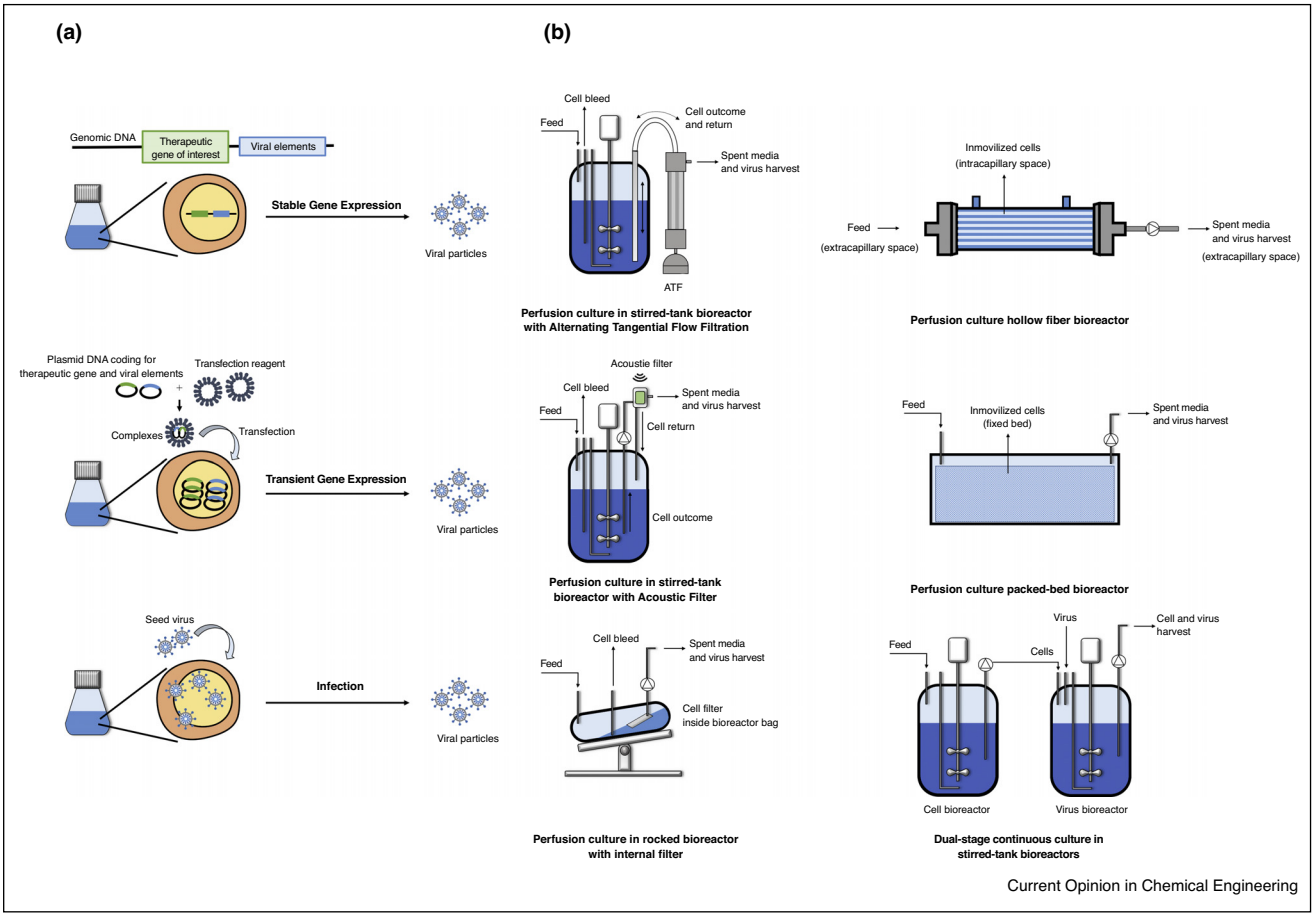
Continuous cultures were considered in biomanufacturing in the late 80s and early 90s. However, the developments in cell biology led to a substantial increase of cell-specific productivities, and batch and fed-batch cultures provided enough material to meet the demand, dominating biopharmaceutical production. Continuous bioprocesses were reduced to the manufacturing of unstable proteins [2]. As the demand of biopharmaceuticals and market competition grow, industry is seeking for more efficient and more flexible processes and facilities. As a solution for these new challenges, industry has renewed the interest for continuous cultures [3,4,5••]. Although they have a more complex operation, continuous cultures have clear advantages over batch and fed-batch culture modes. Continuous feeding of media allows achieving higher cell densities. At the same time, metabolic by-products that may inhibit cell growth or protein production are continuously removed. This is often translated into high volumetric productivities, which lead to reduced bioreactor sizes, reducing plant footprint and investment costs. The size of the production lots is determined by the time of operation, rather than by the size of the bioreactor vessels. The product can be continuously harvested, which is especially beneficial for labile molecules that lose their quality attributes in the culture conditions if the residence time is too long. Overall, continuous culture allows to intensify processes while enhancing operational flexibility with reduced costs compared to other culture modes [3,4,5••,6] (Figure 1).

Continuous cultures have been explored in protein and monoclonal antibody production in the past years [4,7–9]. This review focuses on the production of viral particles (vaccines and viral vectors) using continuous cultures, and how this culture mode is implemented and optimized to improve the yield and quality of cell culture-based viral products. Table 1 summarizes some examples of continuous production of viral particles.

## Continuous manufacturing of viral vaccines

Cell culture-based viral vaccines include the more classical attenuated and inactivated viruses and the new-generation non-infective virus-like particles (VLPs) [10]. This variety is reflected as well in their production methodologies: some vaccines are produced by infection of the host cells, and others are produced by stable or transient heterologous protein expression. In either case, manufacturing challenges need to be overcome. These challenges are, mainly, increasing the titers to have more

**Figure 1**




Outline of the different approaches to obtain viral particles. **(a)** Illustration of the most common production strategies (transient transfection, virus infection and stable cell lines). **(b)** Different bioreactor configurations used to perform continuous culture for viral particle production.

cost-effective processes, while guaranteeing product quality, which will have a direct impact on the vaccine immunogenicity and safety profiles [11•]. Continuous culture offers solutions to improve cell-based viral vaccine manufacturing processes. Different continuous cultures culture approaches have been reported, including perfusion cultures, two-stage continuous bioreactors or hollow fiber bioreactors. A summary of some of these examples follow, focusing on the improvements provided by continuous technologies.

## Perfusion cultures for vaccine production

Opposite to recombinant proteins and monoclonal antibodies, the complexity of the viral structures hinders the capacity of the host cells to produce very high amounts of viruses, so the cell-specific virus yields are usually low. For this reason, providing continuously fresh nutrients to the cells and removing at the same time, metabolic by-products helps to increase the number of viruses that the cells can produce. Nikolay *et al.* [12] produced Zika virus in BHK-21 suspension cells with low success in batch shake flasks ($9 \times 10^3$ PFU/mL), compared to the standard production in adherent Vero cells ($\sim 10^7$ PFU/mL). To increase the titers, the authors performed a perfusion culture using the ATF system. Before infection, cells grew up to $12 \times 10^6$ cells/mL with an increasing perfusion rate of 0.15–0.42 reactor volumes (RV)/day. Perfusion was maintained after infection for six days, reaching a production of $3.9 \times 10^7$ PFU/mL. Even though the cell-specific virus yield was 10-fold lower compared to adherent cells, they achieved a sufficiently productive scalable manufacturing process for Zika virus. Cervera *et al.* [13] developed a novel methodology to intensify Human immunodeficiency virus (HIV) VLP production by repeated transient transfection and medium replacement in HEK293 cultures, named Extended Gene Expression (EGE). Medium replacements every 48 hour after transfection increased protein production by 8-fold compared to a 72-hour batch culture, while the combination with two retransfections resulted in a 12-fold improvement. This methodology was successfully performed in a 1.3 L bioreactor using perfusion to continuously replace the