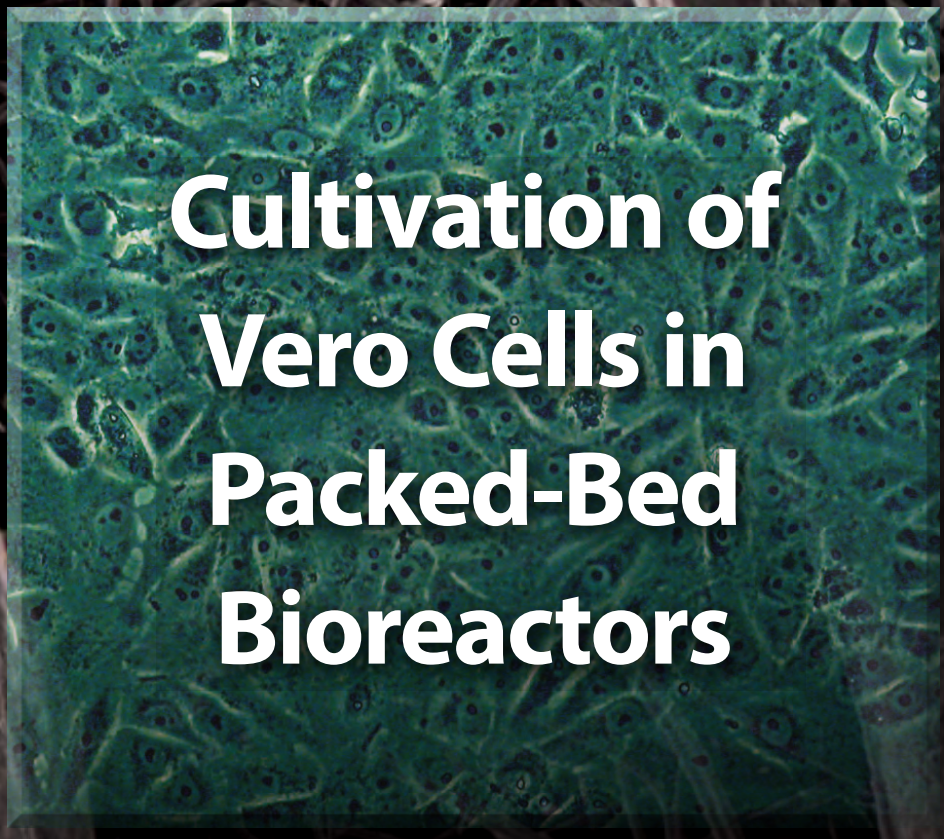
Viral Vaccine Production



Cultivation of Vero Cells in Packed-Bed Bioreactors

by Joseph Duffy, Shannon Guertin, Chris Bellerive, Dan Mardirosian, Ma Sha, and Kamal Rashid

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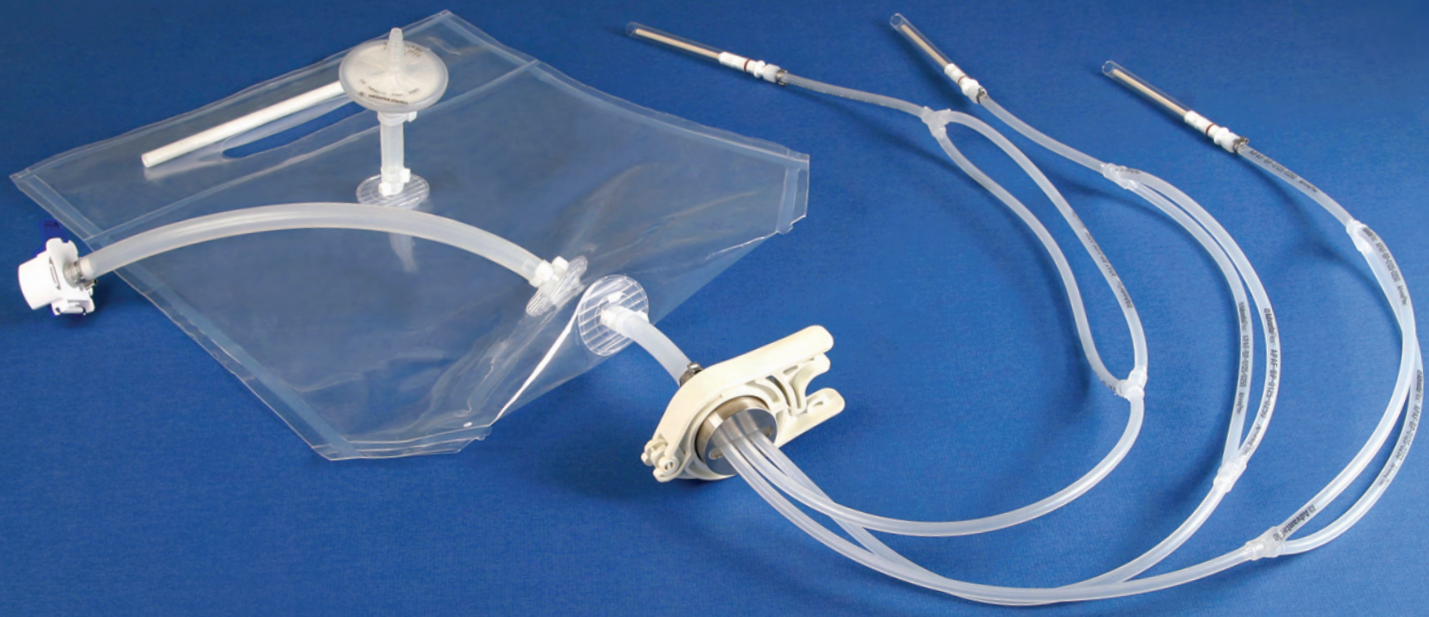

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Cover: Background shows magnified Fibra-Cel disk matrix (Eppendorf). Foreground shows Vero cells (Wikimedia)

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Abstract

*Vero cells are anchorage-dependent cells that are used widely as a platform for viral vaccine production (**1**). In stirred-tank bioreactors, they are grown ordinarily on microcarriers. Fibra-Cel disks are an alternative attachment matrix because they provide a three-dimensional environment that protects cells from damaging shear forces. However, such disks have not been tested for the cultivation of Vero cells. We tested whether benchtop single-use and glass bioreactors with a packed bed made of Fibra-Cel disks would be suitable for cultivation of Vero cells. To monitor the culture, we quantified glucose and lactate concentrations in the medium daily and inferred from those parameters about cell growth. We concluded that the cells multiplied efficiently in the packed-bed bioreactors. Cell yields in the glass and single-use bioreactors were equivalent. Our results suggest that benchtop, packed-bed bioreactors filled with Fibra-Cel disks are well suited for the cultivation of the Vero cell line — and hence vaccine production — in small batch sizes.*

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Current vaccine production relies on two principal methodologies: embryonated chicken eggs and mammalian cell culture. Although producing with chicken eggs has been the favored method of viral production, it has several shortcomings, including inadequate viral yields and difficulty of obtaining reliable supplies of high-quality, pathogen-free eggs (**2**, **3**). Moreover, when viruses are cultivated for extended periods in eggs, an evolutionary process in the amnion or allantoic cavities of the eggs causes selection of a virus subpopulation that is antigenically and biochemically distinct from the original inoculum.

By contrast, the advantages of using mammalian cell culture for viral production have long been recognized: a shorter lead time, a production process that can be more tightly controlled, and reduced risk of microbial contamination. Use of specially engineered and monitored cell lines provides distinct advantages over use of chicken eggs in vaccine production. Some experts argue that such lines will supplant production with chicken eggs completely in the near future (**4**).

The Vero cell line is derived from epithelial kidney cells of African green monkeys. It is effective for primary virus isolation and can replicate high infectivity titers. This cell line provides genetic stability of the hemagglutinin molecule while maintaining the antigenic properties of human-derived viruses, and it has worldwide regulatory acceptance. It is highly susceptible to a wide range of viruses and bacterial toxins and has been licensed for production of both live (e.g., rotavirus, smallpox) and inactivated (e.g., poliovirus) viral vaccines as well as a number of other viruses, including rabies virus, reovirus, and Japanese encephalitis virus (**5**). The Vero cell line remains one of the most attractive platforms for cell-based viral vaccine production.

Vero cells are anchorage-dependent and are grown conventionally in roller bottles or T-flasks. However, their cultivation in stirred-tank bioreactors provides major advantages in process development and industry-scale production. A stirred-tank design simplifies process scale-up, and corresponding bioprocess control systems facilitate tight process monitoring and control.

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Stirred-tank bioreactors typically are used to cultivate nonadherent cell lines. To cultivate anchorage-dependent cells in these bioreactors, a growth surface has to be provided. Microcarriers are one type of attachment matrix, on which cells can adhere to be kept in suspension by culture agitation. In packed-bed bioreactors, cells are grown on an attachment matrix that creates a bed through which growth medium circulates (Figure 1). Fibra-Cel disks (Eppendorf) are one example of an attachment matrix. They are made of a polyester mesh with polypropylene support. During their manufacture, the disks are electrostatically treated to facilitate cell adherence. The shear-free environment provided by a packed-bed impeller allows for robust cell growth and facilitates attachment. Different disposable fixed-bed bioreactor systems have been investigated for their ability to support the production of viral vaccines using Vero and MRC-5 cell lines (**6**). However, to our knowledge, the performance of Vero cells in packed-bed bioreactors filled with Fibra-Cel disks has not been reported.

We tested whether bioreactors with a packed-bed made of Fibra-Cel disks would be suitable for cultivation of Vero cells. To accommodate the increasing importance of using single-use bioreactors, we compared culture performance in a conventional glass bioreactor with that in a single-use vessel.

Materials and Methods

Medium: We cultivated Vero cells in in Dulbecco's modified eagle medium (containing GlutaMAX and 4g/L glucose) supplemented with 5% fetal bovine serum (FBS) (DMEM and 5% FBS). All media components were Gibco brand purchased from Thermo Fisher Scientific.

Bioreactor Setup: After preculture in T-flasks as described below, Vero cells were cultivated in packed-bed bioreactors with a 5-L total volume. We used both glass and single-use bioreactors.

Preparation of Glass Bioreactor: A 5-L glass bioreactor containing a basket impeller (Eppendorf) was assembled and loaded with 150-g Fibra-Cel disks. We autoclaved the pH and dissolved oxygen (DO) sensors inside the unit. The sensors were calibrated 24 hours before inoculation, and an overnight hold provided for equilibration. After sterilizing the unit, we pumped 3.2 L DMEM and 5% FBS into the bioreactor.

Preparation of Single-Use Bioreactor: We connected a BioBLU 5p single-use vessel (Eppendorf) preloaded with 150 g of Fibra-Cel disks to the controller according to the manufacturer's instructions. DO and pH sensors were autoclaved separately from the unit and installed within a biosafety cabinet. We pumped 2.9 L DMEM and 5% FBS into the vessel.

Process Parameters and Analytics: Process parameter setpoints were the same for cultures in both the glass and single-use bioreactors. Culture temperatures were adjusted to 37 °C, and DO was set at 40% saturation. Medium pH was adjusted to 7.1, and sodium carbonate was used as a base. We set agitation to 80 rpm and air flow at 0.004–1.0 standard liters per minute (SLPM). The recorded temperature, DO, and pH before inoculation provided a baseline. Using a Cedex Bio Analyzer

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Figure 1: Packed-bed, single-use bioreactor used in this study

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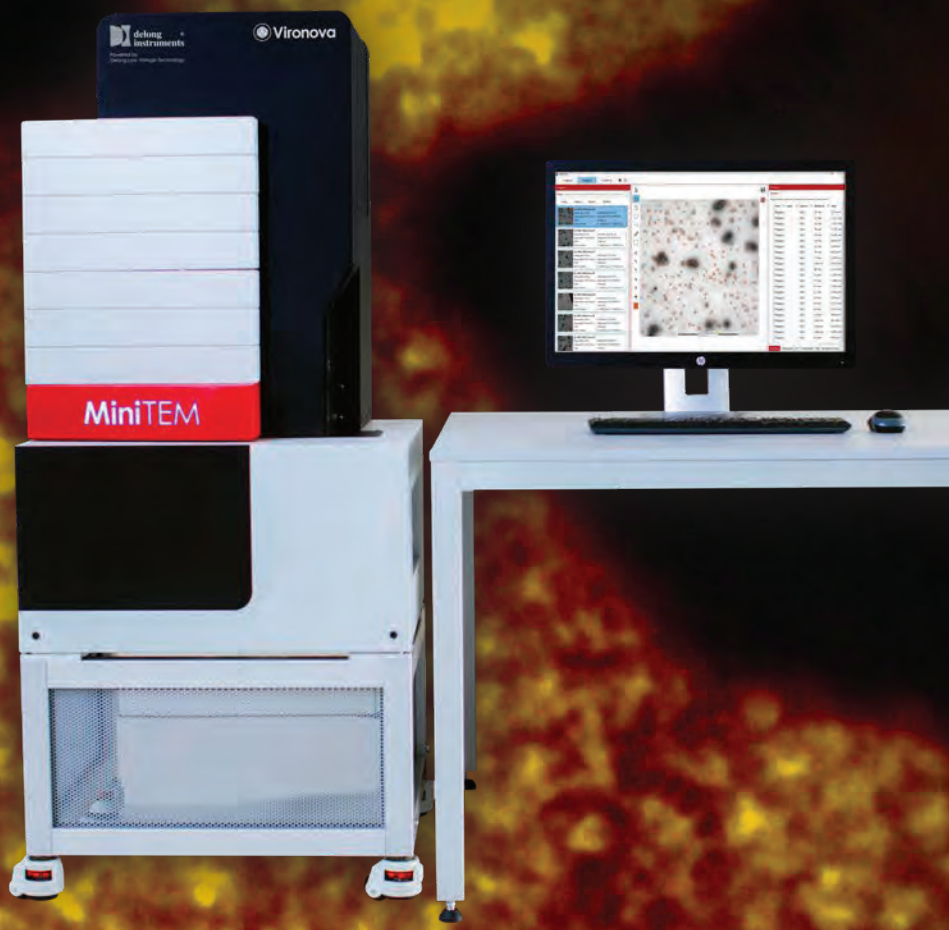

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(Roche Diagnostics), we measured glucose and lactate concentrations offline before inoculation, 30 minutes after inoculation, and daily during the cell culture process.

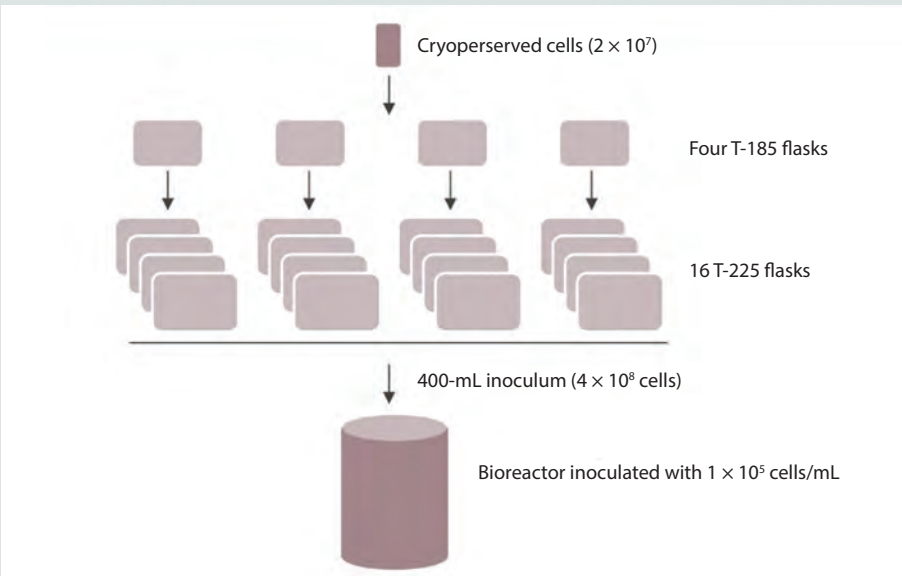
Preculture and Inoculation: We prepared the inoculum for one bioreactor as follows: 2×10^7 cells from one cryovial were split into four T-185 flasks. When the cells had grown to confluency, we split them into 16 T-225 flasks. Those flasks produced a 400-mL inoculum at a density of 1×10^6 cells/mL, which corresponds to a total number of 4×10^8 cells. Cell viability was 99.3%. Adding that inoculum to the medium into the bioreactor resulted in a working volume of 3.6 L with a cell density of 1.1×10^5 cells/mL for the glass bioreactor and a working volume of 3.3 L with a cell density of 1.2×10^5 cells/mL for the single-use bioreactor. Figure 2 shows this expansion protocol.

Cell Culture: We inoculated the cultures as described above. To track cell adherence to the Fibra-Cel disks, we took media samples at 15 and 30 minutes postinoculation and analyzed them for cells in suspension. The cultures ran for 21 days. On day 6, we changed the working volume of the single-use vessels from 3.3 L to 3.5 L to match that of the glass vessel.

Through media exchanges and glucose additions, we adjusted glucose concentration to 5 g/L. For media exchange, half the working volume of the old media was pumped out and replaced with fresh media. To add glucose, we pumped a feed solution with 200 g/L glucose into the bioreactor. To determine what volume had to be added, we measured glucose concentration in the medium and calculated the volume of feed solution required to adjust that concentration to 5 g/L.

For glass-bioreactor culture, we performed media exchanges on days 6, 11, and 16. Glucose was added by pumping a feed solution with 200 g/L glucose into the bioreactor on days 4, 8, 13, and 18. For the culture in the single-use bioreactor, we performed media exchanges on days 6, 11, and 16. We added glucose by pumping a feed solution with

Figure 2: Cell expansion protocol



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200 g/L glucose into the bioreactor on days 5, 10, 15, and 20. Antifoam was added in 1-mL volumes as needed.

Calculation of Glucose Consumption: We calculated the glucose consumption of the cultures as an indirect measurement of cell growth. To do so, we measured glucose concentration in the media daily as described above and calcuated the total amount of glucose present, taking into account the working volume. From those numbers, we determined the amount of glucose consumed in the interval between two measurements ($Gluc_i$). To calculate the total glucose consumption at time t ($Gluc_{total}$), we summed the amounts of glucose consumed in the intervals until this time. Hourly glucose consumption rate can be calculated as the ratio of the total glucose consumption rate to the elapsed time ($Gluc_{total}/t$). To display the development of $Gluc_i$ we plotted those values over time.

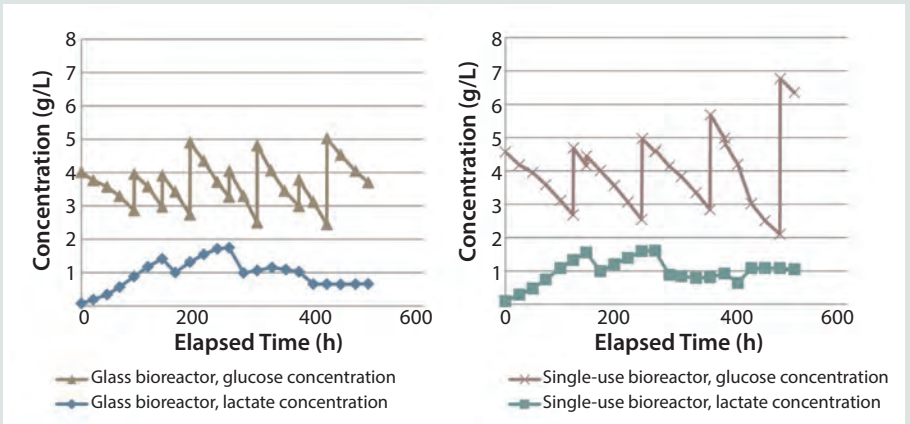
Results

To assess the suitability of a packed-bed bioreactor filled with Fibra-Cel disks for the cultivation of Vero cells, we analyzed cell attachment first. At 30 minutes postinoculation, no cells remained in suspension, indicating that they had attached to the matrix rapidly.

Analyzing cell expansion is critical to evaluating culture performance. Because Vero cells are anchorage dependent and grew on the Fibra-Cel disks, we could not obtain cell counts directly. For an indirect measure of cell growth and numbers, we took daily samples from the bioreactor and measured different metabolic activities (Figures 3–6). Daily measurements of glucose and lactate concentrations also helped us to determine whether we needed to perform a medium exchange and/or glucose addition.

Figure 3 shows glucose and lactate concentrations from both the single-use and glass bioreactors. Brown and purple lines indicate glucose concentration in the glass and single-use bioreactor, respectively, over a 21-day period. When glucose levels dropped below 3 g/L, we added glucose. Small spikes in glucose levels within the glass vessel are caused by glucose present in new media after media exchange. The blue and greeen lines chart lactate production over 21 days within the glass and

Figure 3: Glucose and lactate concentrations in the culture medium



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single-use vessels, respectively. Glucose concentrations in the culture decreased as lactate levels increased, indicating that the Vero cells grew. Glucose and lactate concentrations developed similarly in the glass and single-use vessels.

Figure 4 shows total glucose consumption over the course of 21 days. The blue line represents glucose consumption within the glass vessel, and the green line represents glucose consumption within the single-use vessel. In both vessel types, glucose consumption rose at a consistent rate over the course of 21 days. There was almost no variation between the total glucose consumption in the two types of vessels. Figure 5 plots glucose consumption in grams per hour within each vessel over the course of 21 days. The blue line represents glucose consumption within the glass vessel, and the green line refers to the single-use vessel. Consumption showed a lag phase at initiation of the culture, followed by a period of growth, reaching a plateau at around 300 hours. The increase of glucose consumption per hour suggests that the number of Vero cells in the culture increased over time. Those data show that during a period of growth, there was almost no variation between the glucose consumption in the two vessel types.

Figure 6 shows the glucose consumption per interval in the vessels as a function of time. These data establish that the grams of glucose consumed in the interval between two measurements indicate the cellular need for more glucose or media exchanges. Sharp peaks in glucose levels per liter consumption follow addition of glucose to the vessels.

Figure 4: Total glucose consumption ($Gluc_{total}$) in glass and single-use bioreactors over 21 days

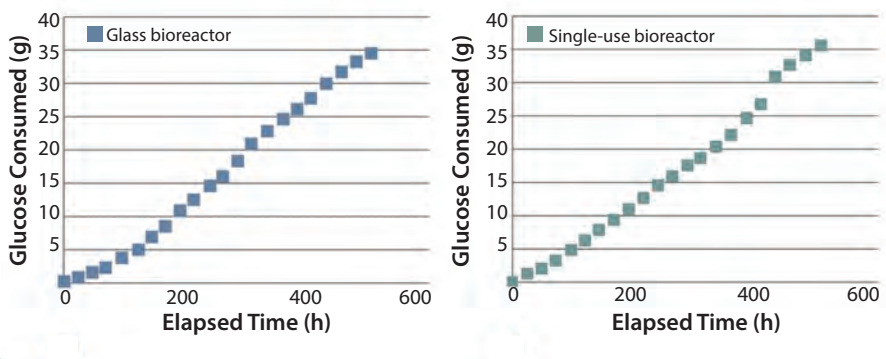

Figure 5: Grams of glucose consumed hourly ($Gluc_{total}$/h)

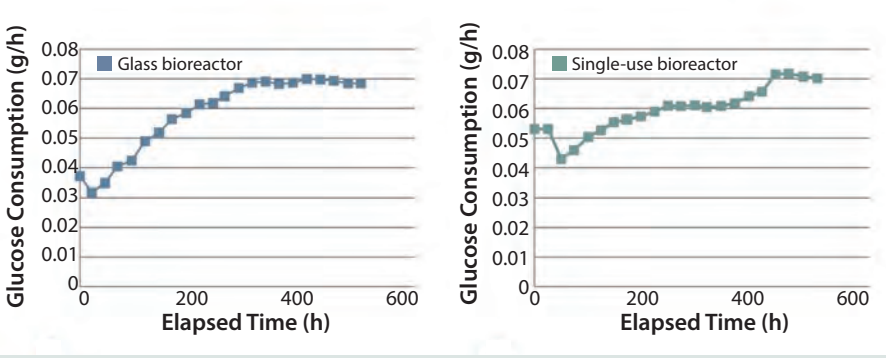

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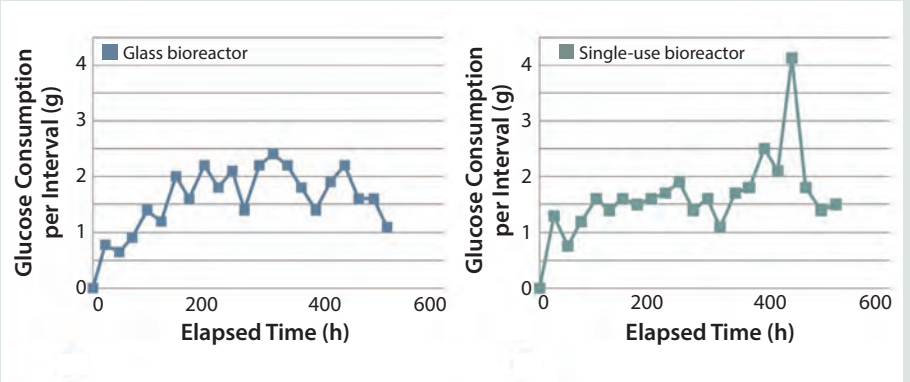
Figure 6: Glucose consumption per measurement interval ($Gluc_i$) plotted over time



Expansion in Packed-Bed Bioreactor

Our study focused on testing the utility of packed-bed bioreactors filled with Fibra-Cel disks for cultivation of Vero cells as a model for anchorage-dependent cell lines in general. This provided a head-to-head assessment of reusable and single-use bioreactor vessels.

Cells within a packed-bed bioreactor face less shear force than they would in conventional suspension culture. We have shown in previous studies that CHO cells grown in a packed-bed bioreactor filled with Fibra-Cel disks consumed more glucose and at a faster rate than CHO cells grown in suspension culture (**7**). That is consistent with increased cell numbers and metabolic activity. In addition, these cells produced more of the protein of interest, alkaline phosphatase, than had been produced by CHO cells grown in suspension culture (**7**). From the glucose consumption of our culture, we concluded that Vero cells continued to proliferate robustly over a 21-day period. So packed-bed bioreactors filled with Fibra-Cel disks also appear to be suitable for expansion of this anchorage-dependent cell line. Process handling is simplified because the packed-bed basket technology allows for easy exchange of culture media.

We used seeding densities of 1.2×10^5 cells/mL for the packed-bed bioreactors. Ordinarily, a density that low would be expected to prevent cell growth from reaching confluence because of a lack of effective cell-to-cell communication. In this protocol, however, cellular multiplication proceeded rapidly. Seeding at a density lower than in the typical protocol significantly decreases time, cost, and contamination risk during the seed train and scale-up process.

There is a slight difference in the sparge elements between the BioBLU and glass vessels that can cause minor bubble-size variations. Other than that difference, the two vessels are equivalent, and there was no difference in cell growth between them. In summary, our results establish that Vero cells can be expanded efficiently in packed-bed bioreactors containing Fibra-Cel disks as a growth support.

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