



Application Note

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Control and scale-up of a microcarrier-based viral vaccine process using BioPAT® ViaMass for inline viable cell density measurement

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Abstract

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Viable cell density (VCD), representing cell growth, is a key process attribute essential to define time and multiplicity of viral infection during viral vaccine production. VCD is still commonly measured by manual offline methods, which are time-consuming, error-prone and bare the risk of contamination. Manual sampling can be avoided by using the inline capacitance sensor BioPAT® ViaMass to determine VCD. Here we show that BioPAT® ViaMass can be used to in-situ monitor the growth of vero cells attached to microcarriers in a viral vaccine process. This is demonstrated in a scale-up process, performed in a 2L Univessel® SU and BIOSTAT STR® 50L bioreactors in which BioPAT® ViaMass models the VCD reliably across scales and independent of whether a conventional multi-use or integrated single-use sensor is used. We conclude that the implementation of inline capacitance in viral processes allows for a fully automated, continuous measurement of viable cell density providing timely information on the status of the cell culture. The use of inline capacitance sensors does not only increase process robustness and prevent faulty batches, but can further be used in advanced control strategies to determine the optimal time points for viral infection or product harvesting, with the potential of significantly increasing the yield while reducing the costs of goods.

Introduction

Over the last decades, a significant number of human and veterinary viral vaccines have been brought to the market and still represent a major part of the current pipeline (1). One critical issue to be considered in the development of vaccines is their Costs of Goods (CoG), especially in veterinary use. Therefore, disposable technologies have gained significance over the last years, as they reduce CAPEX investments and CoGs, while increasing flexibility and production capacity (2). Overall, single-use technologies bring a significant number of benefits in vaccine production: reduce time to market, decrease validation efforts, and increase process security by reducing the risk of cross contamination in multi-purpose facilities. Nowadays, mammalian cell cultures have become the main platforms for viral vaccine productions (3). Therefore, Single-Use Bioreactors (SUB) systems are becoming an industry standard for the production of viruses, both in suspension and adherent cells (4).

Process Analytical Technologies (PAT) and Quality by Design (QbD) principles gain popularity in viral processes. PAT and QbD enable better process understanding and control, improving process robustness, product quality and hence patient safety. This potential has been underlined by regulatory agencies that have put in place a framework for the use of PAT and QbD in bioprocessing (5). The availability of reliable inline sensors for process monitoring is essential for the implementation of PAT, which enable QbD approaches and advanced control strategies. In recent years, sensor technologies have matured and more sensors are available as single-use technologies, readily integrated in SUBs (6). The use of such inline technologies provide advanced process understanding, early detection of process deviations, and high process robustness, resulting in high quality products with shorter time-to-market and lower CoGs (7).

The present work is focused on the application of the inline capacitance measurements, to monitor cell growth – one of the most important process attribute in viral processes as it used to determine the amount of virus to add to the culture according to the multiplicity of viral infection (MOI). Despite the importance of cell growth in manufacturing processes, it is still commonly measured by traditional offline methods, which are time-consuming, error-prone and bare the risk of contamination. Here we show, that the inline capacitance sensor BioPAT® ViaMass can monitor in real-time the growth of vero cells attached on microcarriers. A scale-up process from 2L Univessel® SU bioreactor to 50L BIOSTAT STR® bioreactor acknowledges the consistently reliable performance of BioPAT ViaMass in monitoring cell growth, irrespective of scales and whether a single-use or multi-use sensor is used.

Principle of biocapacitance measurement by BioPAT® ViaMass

The underlying principle of capacitance measured by BioPAT® ViaMass is that live cells get polarized by an electric field, as charged molecules migrate towards the electrodes, but cannot pass the cell membrane (see figure 1). Thus, cells act as small capacitors, giving a capacitance signal that is proportional to the membrane-bound cell volume, representing the viable cell volume. In contrast, dead cells do not have an intact membrane, become leaky and cannot longer be polarized. Therefore, capacitance selectively assesses viable cells by measuring capacitance (pico-Farad (pF)) through radio-frequency impedance measurement (8). The capacitance is typically normalized to the distance of the electrodes and represented as permittivity (pF/cm).

The viable cell density (VCD) can be calculated from the obtained capacitance signal using a simple scaling factor determined through correlation with offline VCD measurements. Moreover, this factor changes, when cells change in diameter, which is often the case in the stationary phase, when the proliferation decreases and cells increase in diameter (9).

Capacitance is widely regarded as the most reliable method for real-time monitoring cell growth due to its simplicity, robustness and fast signal acquisition (10, 11). It has proven specifically useful for high-density fed-batch processes requiring complex nutrient feed (12 – 18). Meanwhile, control strategies based on capacitance sensors have found their way into GMP manufacturing for feed-control (13, 17, 18) and perfusion rate control (8).

Sartorius Stedim Biotech offers inline monitoring of capacitance through BioPAT® ViaMass. It comes as a SU sensor, readily integrated in its rocking motion BIOSTAT® RM for all sizes from 2 – 200L and in its single-use stirred-tank reactors BIOSTAT STR® from 50L to 2000L (see figures 2 and 4). The serial integration in the Sartorius Digital Control Unit (DCU) with an intuitive user interface for sensor set-up and calibration makes BioPAT® ViaMass a true plug-and-play solution. To facilitate up- and down-scaling, multi-use probes for conventional stainless steel and benchtop reactors are also available (see Figure 3).

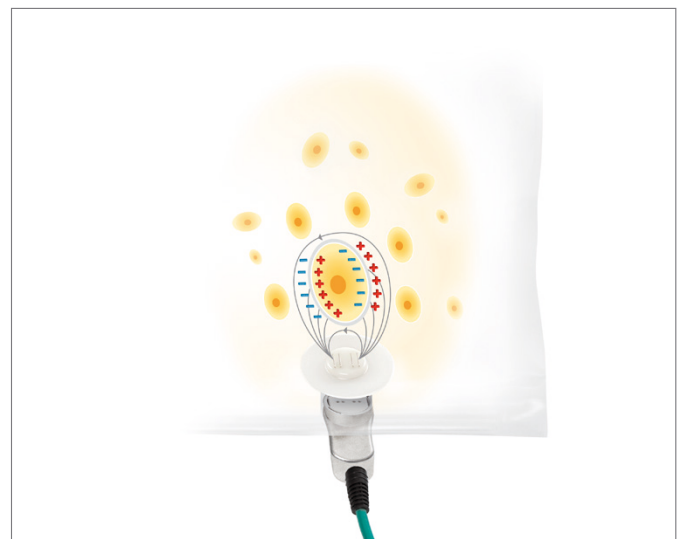


Figure 1: Principle of measurement of biocapacitance measured by the SU sensor BioPAT® ViaMass

Materials and Methods

Cell line and virus strain

Vero cells were cultured at 37°C in DMEM medium supplemented with serum. Cells were grown on microcarriers Cytodex-3 following the optimized parameters (pH, temperature, stirring speed and aeration rate).

Cells were infected with an Avian virus.

Culture system

Cultures were conducted in:

- BIOSTAT® B Twin (Sartorius Stedim): Univessel® SU 2L bioreactor with a reusable BioPAT® ViaMass probe.
- BIOSTAT STR® 50L (Sartorius Stedim): Flexsafe STR® single-use bag with the BioPAT® ViaMass sensor disc incorporated.



Figure 2: BIOSTAT® B Twin and BIOSTAT STR® 50L

Three batch-mode processes per bioreactor were performed, using the same temperature, DO and pH setpoints. Optical sensors were used to monitor and control DO and pH. The stirrer and gas flow were scaled accordingly based on the bioreactor size.

Determination of cell growth

Culture samples were regularly collected to determine offline cell concentration while the capacitance signal was used for inline measurement, as summarized below. The capacitance signal was set to zero before inoculation of the cultures.

- OFFLINE method: Nucleocounter-cell counter system (ChemoMetec) was used to determine offline data.
- INLINE method: A reusable BioPAT® ViaMass capacitance sensor was used to measure cell growth in the 2L Univessel® bioreactor (Figure 3). The BioPAT® ViaMass single-use capacitance probe was used to measure viable cells into cell culture processes in 50L STR SU bioreactor (Figure 4).

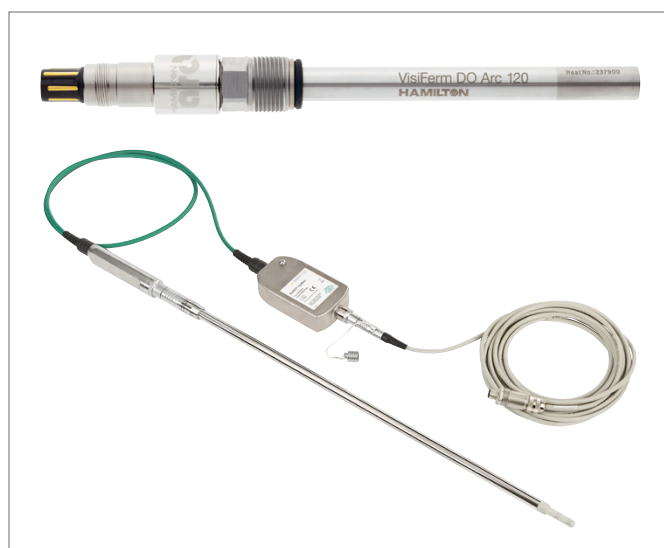


Figure 3: BioPAT® ViaMass reusable capacitance sensor

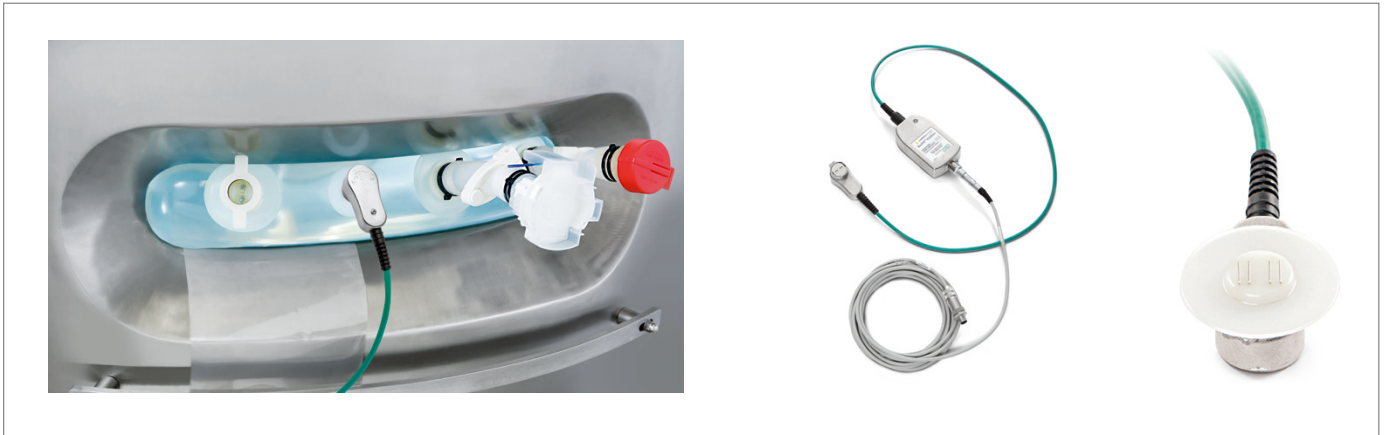


Figure 4: BioPAT® ViaMass single-use capacitance probe. Sensor disc and sensor connector.

Virus infection and titration

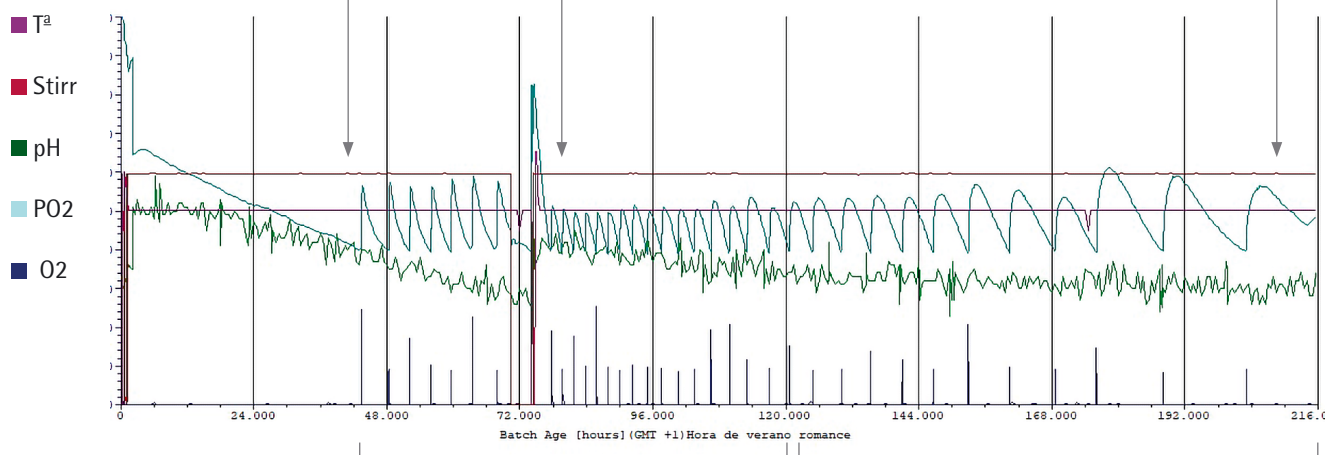
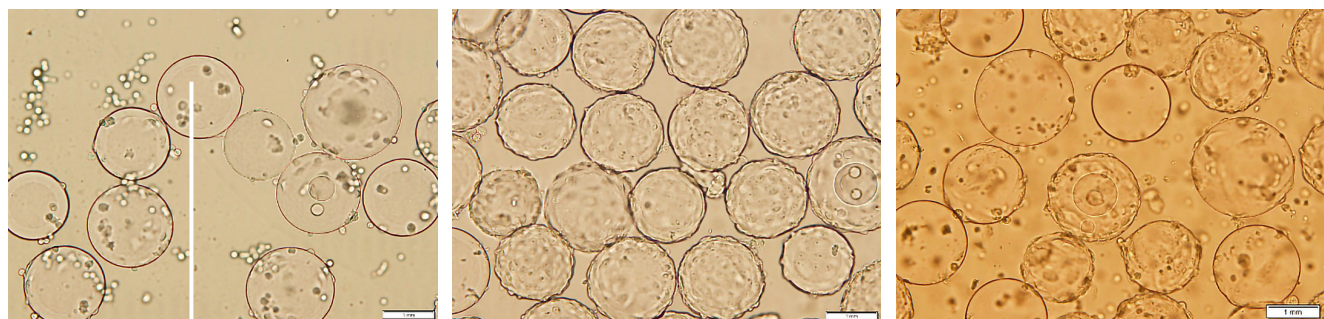
Cells were observed under microscope to verify health and confluency on microcarriers. Confluent cells were infected at a constant MOI, after 3 days of infection, during growth phase. Harvesting was done after 6 days post-infection.

Virus production was calculated according to the Spearman-Kärber method, expressing the result in tissue culture infectious doses (50%) (TCID₅₀).

Results and Discussion

Process development. Scalability

Vero cells were grown on microcarriers until day 3 (more than 90% of microcarriers are confluent). On day 3, the microcarriers were left to settle and the serum-containing cell growth medium was replaced by serum-free virus maintenance medium. At this specific point, the biomass sensors could not detect the cells due to microcarriers sedimentation, resulting in a drop in signal. After the viral infection, cells started lysing or detached from the microcarriers (Figure 5).



Microcarrier settling down ↑ O2 ↓ O2
 ↑ Cell growth ↓ Cell growth

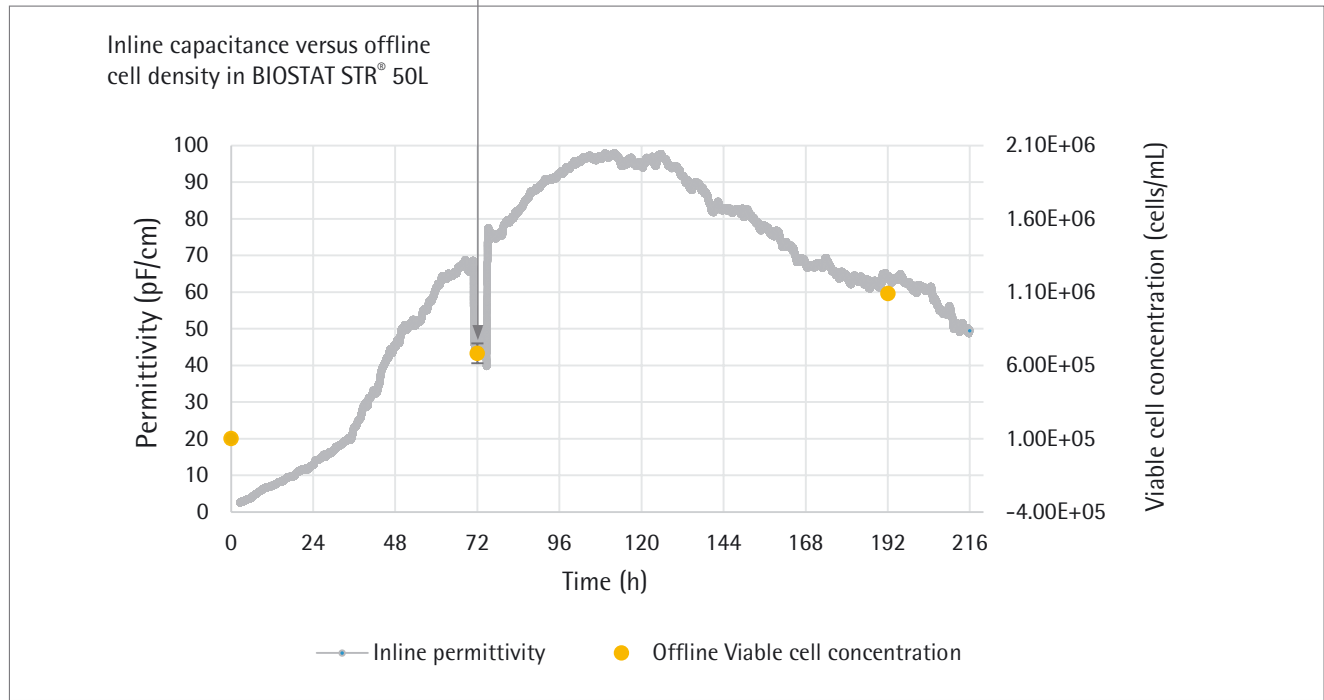


Figure 5: Process development. Pictures of cell attachment on microcarriers Measurement and control of physicochemical parameters during the process. Cell density during process.

Figure 6 shows the average cell concentration at time of infection and the average virus titer at time of harvest of 3 batches performed in 2L Univessel® SU and 3 batches performed BIOSTAT STR® 50L. Comparable cell growth is obtained at differ-

ent scales, with high viral productivity in both cases from $10^{7.4}$ to $10^{7.8}$ TCID50/ml, demonstrating the scalability and the consistency of the process from 2L to 50L.

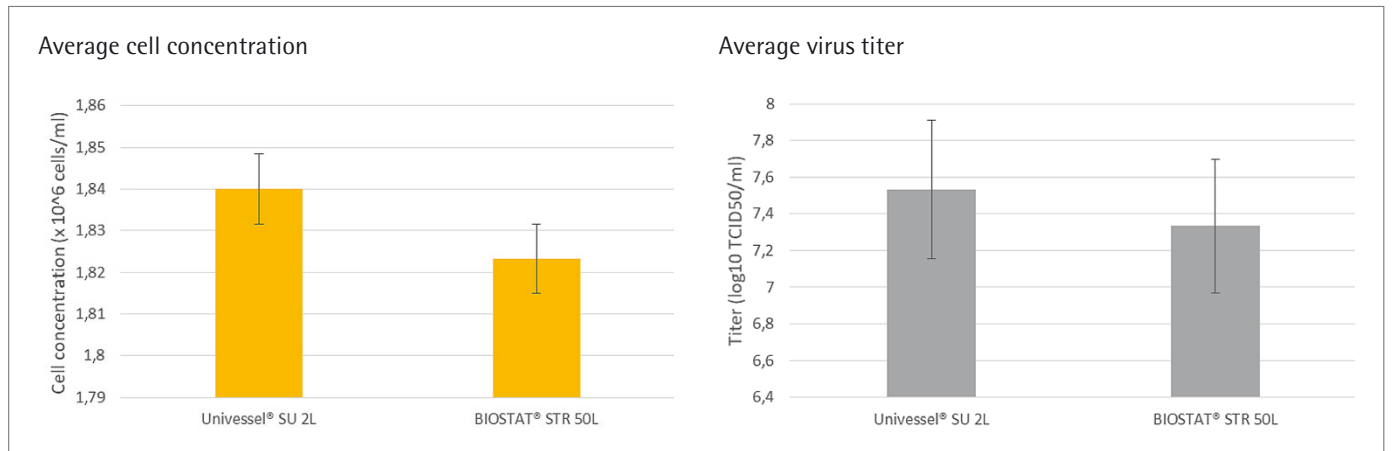


Figure 6: Batch cultivation of vero cells in 2L and 50L bioreactors. Left: Average Viable cell concentration at time of infection. Right: Average virus titer at time of harvest. Error bars represent the standard deviation of the three batches.

Cell growth inline measurement

The performance of the biocapacitance sensor BioPAT® ViaMass for monitoring cell growth is shown in figure 7 for the reusable probe in a 2L benchtop reactor, and in figure 8 for the single-use probe in the 50L SUB. Sections (A) show the raw permittivity values from BioPAT® ViaMass over the process time including the offline VCD measurements. From this data, one can determine the conversion factor to translate permittivity to VCD. As presented in sections (B), the permittivity values are plotted against the measured offline VCD values and fit with a linear fit through the origin. The labels on the data points show the chronological order from (A). The resulting linear equation yields the conversion factor. The linear regressions in figure 7(B) and 8(B) show high coefficients of determination $R^2 > 0.94$, underlining the linear relationship between permittivity measured by BioPAT® ViaMass and offline VCD. Note that the red data points in figure 7(B) were not included in the linear fit, as they were identified as outliers. The data point 3 was obtained while the microcarriers were settling and the sensor could not measure the cell concentration.

Using the conversion factor determined in sections (B), the capacitance signal can be converted to VCD, as plotted in sections (C). The agreement between the predicted VCD from the capacitance signal and the measured offline VCD values confirm that BioPAT® ViaMass can reliably predict the viable cell density of cells growing on microcarriers throughout the entire viral process.

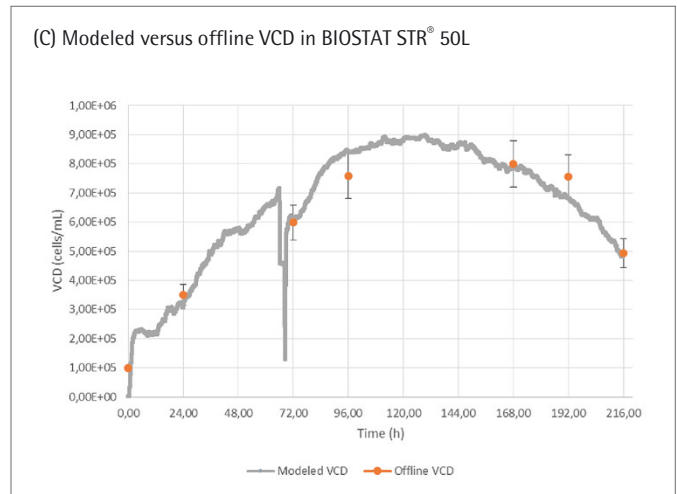
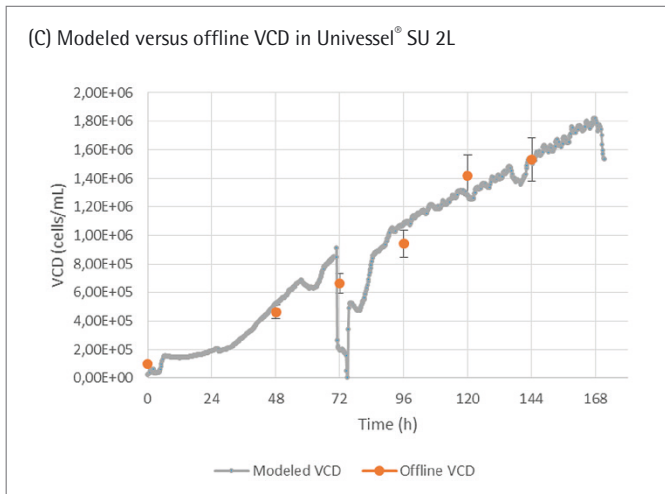
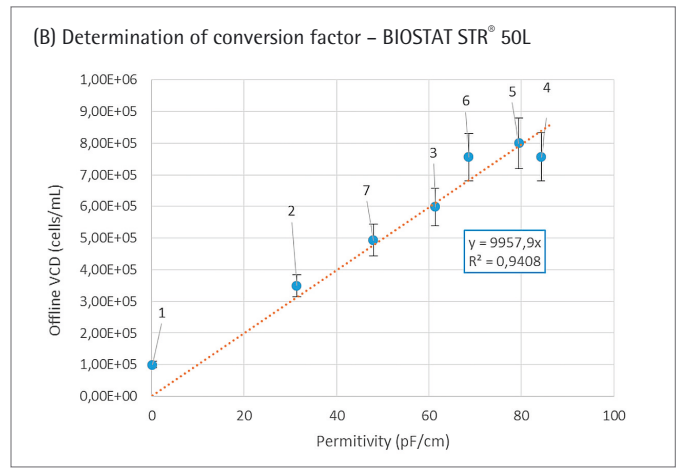
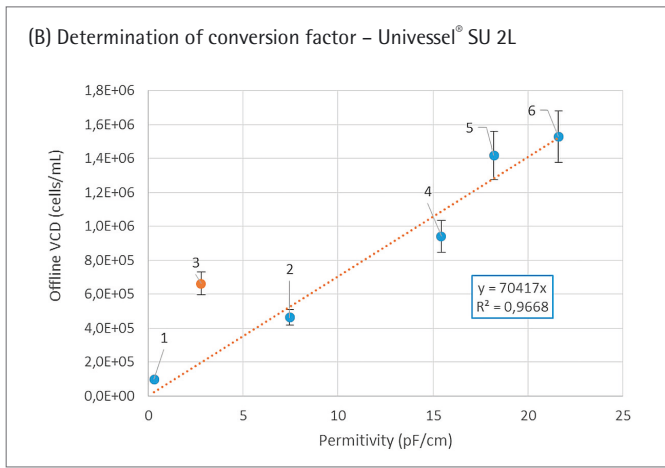
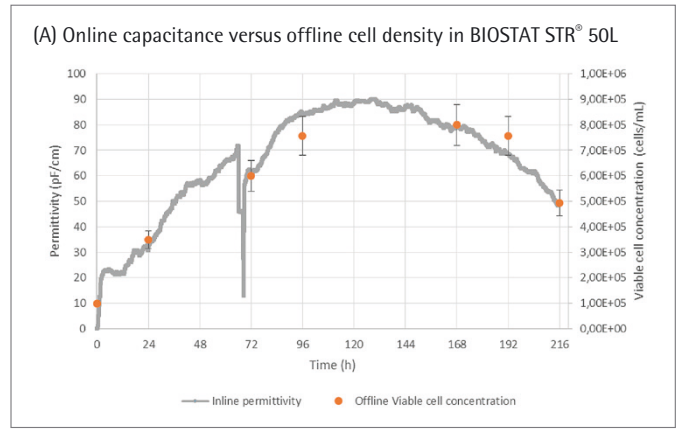
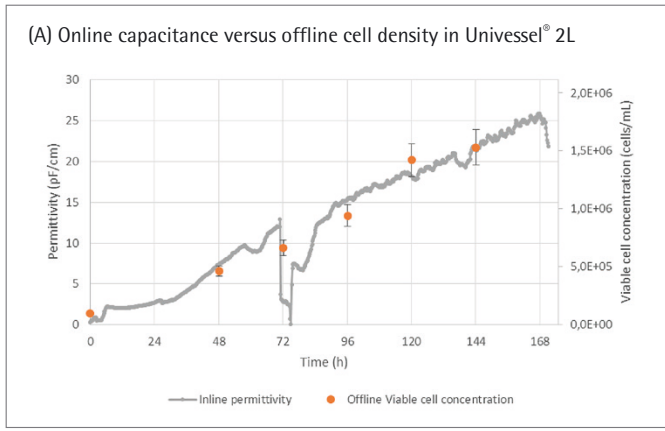


Figure 7: Performance of the reusable capacitance sensor BioPAT® ViaMass in a 2L benchtop Univessel®

Figure 8: STR Performance of the single-use capacitance sensor BioPAT® ViaMass in a 50L BIOSTAT STR®.

(A) Raw inline permittivity values over the process time.

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(B) Obtained offline VCD values plotted over the corresponding inline permittivity values to determine the conversion factor of permittivity to VCD. The data points are labelled in chronological order. The red data point 3 was taken during the settling of the micrcarriers and not included in the linear fit.

(B) Obtained offline VCD values plotted over the corresponding inline permittivity values to determine the conversion factor of permittivity to VCD. The data points are labelled in chronological order.

(C) Calculation of VCD through linear scaling is in agreement with offline measurements. Error bars of the offline measurement represent the accuracy of the method assumed to be 10%.

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Conclusions

In this study we demonstrated:

- The correlation between inline monitoring of the permittivity and viable cell density throughout the process for cells attached to microcarriers
- The feasibility and scalability of the process from 2L to 50L for cell growth and virus production
- The consistency of results between the reusable and the single-use BioPAT® ViaMass probes

The advantage of inline monitoring

This study underlines the value of inline monitoring. Compared to the conventional method, the implementation of cellular monitoring during process allows a continuous and accurate measurement of cell density providing timely information on the status of the cell culture. Process deviations can be identified and eliminated at the point of occurrence, reducing the number of failed batches. It reduces the need of manual offline sampling, thereby freeing up operators, while reducing contamination risks. The continuous monitoring leads to increased process knowledge, which can be leveraged to increase process robustness and to better determine the time points of viral infection and harvest, which optimizes the product quality and consistency. When used in advanced control strategies, the inline biocapacitance sensor can automate feeds, inoculation, infection and harvest. Furthermore, the data from inline sensors can be used by a Multi Variate Data Analysis (MVDA) software, such as SIMCA, to analyze process variations, identify critical parameters and predict final product quality (19, 20). In summary, inline sensors help producing safer drugs faster and at lower costs.

The advantage of single-use

The demonstrated comparability of reusable and single-use sensors support the industry's trend toward single-use technologies. Vaccine manufacturers are increasingly investing in single-use production facilities, due to the lower investment costs and higher flexibilities. However, the fact that many analytical probes used in conventional bioreactors are not available as a single-use versions had been a drawback. Here we show that the single-use biocapacitance sensor shows identical performance to the multi-use probe and successfully measures the cell density throughout the entire process. The single-use integration comes with reduced change-over time and reduced risk of contamination when compared to its multi-use version.

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