BIOTHRUST

HAMILTON

Proof of Principle Study

In-line Dielectric Spectroscopy for T-cell and Jurkat Cultures in Membrane-Aerated Bioreactors

www.biothrust.com Version 1.0

Authors

Felipe Veliz Valenzuela[1], Yasemin van Heuvel[1], Katharina Dahlmann[2], Volker Donath[2], Patrick Bongartz[1]

1 BioThrust GmbH, Pauwelsstraße 17, 52074 Aachen, Germany
2 Hamilton Germany GmbH (Höchst), Forsteler Strasse 13, 64739 Höchst im Odenwald, Germany

Abstract

Continuous bioprocess monitoring is essential for cell therapy manufacturing, yet offline sampling provides limited temporal resolution. In-line dielectric spectroscopy offers real-time, non-invasive biomass tracking without samples or reagents. This application note evaluates dielectric spectroscopy as a monitoring tool for process development in two immune-cell culture modes: fed-batch expansion of primary T cells and perfusion cultivation of Jurkat cells. Permittivity closely correlated with offline viable cell density and revealed biomass changes between sampling intervals. In fed-batch culture, continuous monitoring identified candidate windows for earlier feeding interventions. In perfusion culture, the signal detected process deterioration during an offline sampling gap, improving temporal resolution of culture decline. Frequency-domain parameters showed cell- and process-specific dynamics, supporting dielectric spectroscopy as a complementary PAT tool for real-time process monitoring and bioprocess development.

Objective

To evaluate the use of the Hamilton in-line dielectric spectroscopy system for continuous process monitoring in two exploratory T-cell culture case studies: a fed-batch primary T-cell culture, in which permittivity was assessed for retrospective identification of candidate feeding windows, and a Jurkat-cell perfusion culture, in which the signal was used to characterise the onset and progression of an unexpected process disturbance. Frequency-domain parameters (f_c and α) were additionally explored as potential descriptors of culture-state transitions. The resulting feeding strategies and frequency-domain interpretations were considered hypothesis-generating and require prospective validation in replicated experiments.

Key Findings

1. In-line dielectric spectroscopy closely tracked viable cell density across fed-batch and perfusion immune-cell cultures (R^2 = 0.87 and 0.98).
2. Continuous permittivity monitoring revealed biomass declines between offline samples, identifying candidate windows for earlier fed-batch intervention.
3. In Jurkat perfusion culture, permittivity detected biomass decline during an offline sampling gap, improving temporal resolution of process deterioration..
4. Frequency-domain parameters showed cell- and process-specific dynamics, supporting dielectric spectroscopy as a complementary PAT tool for process monitoring and development.

Introduction

T-cell expansion is a central and time-intensive step in many cell therapy manufacturing processes, with performance affected by starting-material variability and sensitivity to culture conditions (Amini et al., 2020). Viable cell density, viability and fold expansion are typically assessed using discrete offline samples, which provide limited temporal resolution and may miss growth slowdowns or rapid process disturbances between measurements (Yee et al., 2018). This is particularly relevant in fed-batch and perfusion processes, where intervention timing can influence subsequent culture performance (Chen et al., 2021).

Dielectric spectroscopy provides continuous, non-destructive monitoring of the electrical response of cells in suspension. Viable cells with intact membranes polarise in an alternating electric field, producing a permittivity signal that generally increases with viable biomass (Opel et al., 2010). Because this relationship is influenced by cell size, membrane properties and population composition, process-specific comparison with offline measurements is required for quantitative interpretation (Schini et al., 2023).

Multi-frequency dielectric analysis can additionally provide parameters such as characteristic frequency (f_c) and the Cole–Cole exponent (α), which may reflect changes in cellular electrical and morphological properties (Bergin et al., 2022). These parameters are therefore of interest as complementary descriptors of culture-state transitions, although their interpretation remains cell- and process-dependent.

Within a process analytical technology framework, continuous dielectric monitoring can improve temporal understanding of culture behaviour and support investigation of feeding transitions or unexpected process disturbances (Gillespie et al., 2022). In this work, a BioThrust membrane-aerated stirred-tank bioreactor was combined with a Hamilton multi-frequency in-line dielectric spectroscopy system. The BioThrust platform provides bubble-free gas transfer through hollow-fibre membranes integrated into the rotating element and has previously supported immune-cell culture and high-cell-density mammalian-cell perfusion (von Werz et al., 2026; Jacobtorweihe et al., 2026).

Materials & Methods

Cell sources and culture preparation

Primary CD3+ T cells (Cellex GmbH) were activated using anti-CD3/CD28 stimulation and cultured for 3 days before transfer to the fed-batch bioreactor at 0.15 x 10^6 cells/mL with >95% viability (initial volume 150 mL).

Jurkat E6.1 cells (Cytion GmbH) were expanded in flask culture and seeded into the perfusion bioreactor at 0.17 x 10^6 cells/mL with >95% viability (initial volume 300 mL).

Bioreactor configuration and operating conditions

Fed-batch cultures were performed in a BioThrust *ComfyCell* SU300 single-use vessel (150–300 mL working volume). Perfusion cultures were performed in a 300 mL prototype vessel with integrated 3 µm bottom retention membrane.

Both were operated at 40 rpm using membrane aeration (no conventional sparging). Temperature was maintained at 37°C, pH at 7.2, and dissolved oxygen at 60% air saturation. Samples were collected daily via dip tube sampling.

Feeding and perfusion procedures

For fed-batch culture, fresh medium was added on day 2 (volume doubling) and partial media exchanges were performed on days 3–4 (50% volume exchange). The feeding schedule was predefined rather than signal triggered.

For perfusion culture, cell-specific perfusion rate (CSPR) was calculated from viable cell density and volumetric perfusion rate, and the perfusion rate was adjusted to maintain a target CSPR of approximately 100 pL/cell/day.

Dielectric spectroscopy measurement and data acquisition

Dielectric measurements were acquired using a Hamilton Incyte Arc 120 Expert probe installed in a PG13.5 port of the BioThrust bioreactor. The probe recorded permittivity and frequency-domain parameters (characteristic frequency fc and Cole–Cole exponent α) at 1 min intervals throughout each culture. The signal was zeroed after bioreactor inoculation according to the instrument operating procedure.

For analysis, offline viable cell-density measurements and dielectric data were aligned using recorded acquisition timestamps, and the nearest dielectric measurement was used for comparison with each offline sample. Detailed signal-processing and Cole–Cole fitting procedures are available from Hamilton upon request.

Offline analytics and data analysis

Offline viable cell density and viability were measured using a CytoDirect automated cell-counting system from daily bioreactor samples. Fold expansion was calculated relative to the total viable cell number at bioreactor inoculation.

The relationship between permittivity and viable cell density was assessed separately for each culture using ordinary least-squares linear regression and Pearson correlation. Regression equations, coefficients of determination (R^2), and Pearson correlation coefficients (r) were reported.

Candidate feeding windows were identified retrospectively by visual inspection of periods in which the permittivity signal plateaued or declined before predefined feeding interventions.

Results

Case study 1:

Retrospective identification of candidate feeding windows during fed-batch T-cell culture

Primary T cells were cultured in fed-batch mode for 115 h, reaching approximately 60-fold expansion while viability remained above 90% throughout (Figure 1). Cell numbers increased progressively over the culture period. Real-time permittivity measurements followed the overall trend of offline viable cell density (Figure 2, left). At matched sampling time points, permittivity showed a strong positive relationship with viable cell density (R^2 = 0.87, r = 0.93, n= 7; Figure 2, right). Transient decreases in permittivity were observed in association with each feeding intervention.

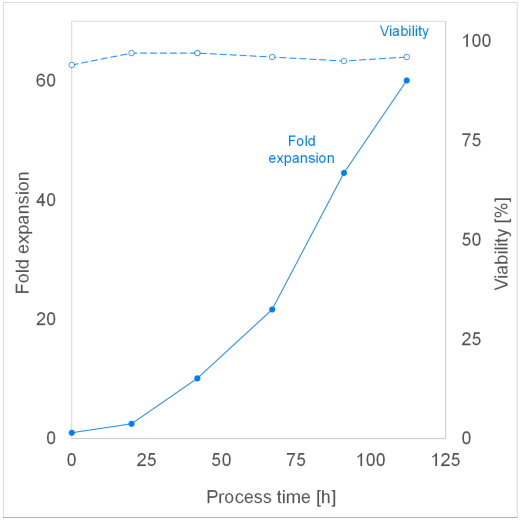

Figure 1: T-cell expansion in the single-use ComfyCell bioreactor. Fold expansion and viability of T-cells cultured in a 150 mL SUB300 BioThrust vessel. Cells were seeded at 3 days post-activation.

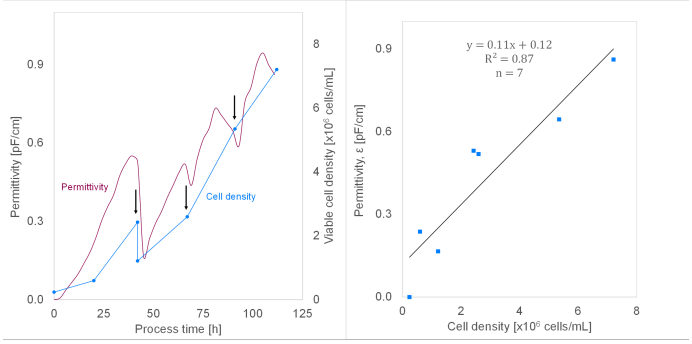

Figure 2. Left: Continuous permittivity signal (red line) and offline viable cell density measurements (blue points/line) during fed-batch T-cell culture. Black arrows indicate the predefined feeding interventions. **Right:** Relationship between matched offline viable cell density and permittivity measurements. The solid line shows the ordinary least-squares linear regression

Furthermore, the continuous permittivity profile revealed periods in which the biomass-related signal slowed, plateaued or declined between discrete offline sampling points, particularly before the later feeding interventions. These transitions were not fully resolved by the offline sampling schedule and were therefore examined retrospectively as potential indicators of earlier intervention windows.

Figure 3 presents a retrospective comparison between the predefined feeding schedule and the continuous permittivity trajectory. Candidate feeding windows were identified visually during periods in which the permittivity signal began to plateau or decline before the scheduled interventions. These windows represent hypotheses for earlier intervention timing and were not prospectively tested in the present study. Two additional hypothetical intervention points were included to illustrate how the continuous trajectory could be used to define prospective feeding experiments beyond the original culture schedule.

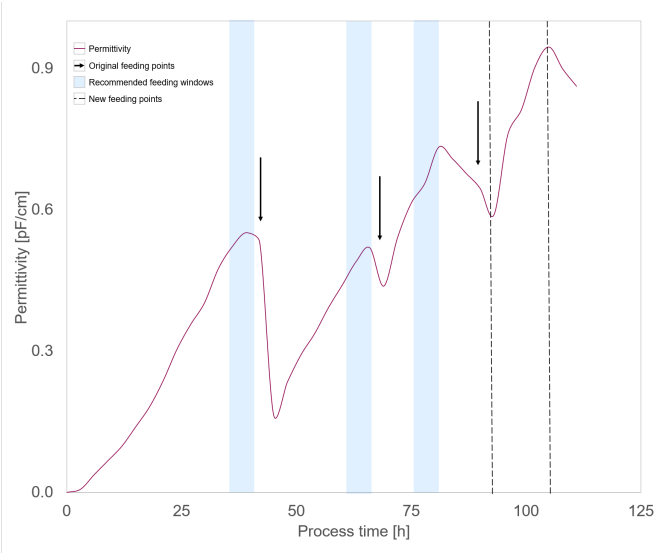

Figure 3. Permittivity-guided fed-batch process optimization. Black arrows show original feeding points. Blue shaded regions indicate suboptimal permittivity windows where feeding was delayed. Dotted lines show proposed feeding schedule to maintain higher permittivity and potentially extend culture duration.

Case study 2:

Real-time monitoring provides visibility into process failure during perfusion process development

Jurkat cells were cultured in a perfusion bioreactor for more than 250 hours (Figure 4). Fold expansion increased progressively during the first approximately 200 hours, reaching close to 100-fold, while viability remained above 95%. This was followed by a decline in culture performance, with fold expansion decreasing to approximately 40-fold by the end of the experiment.

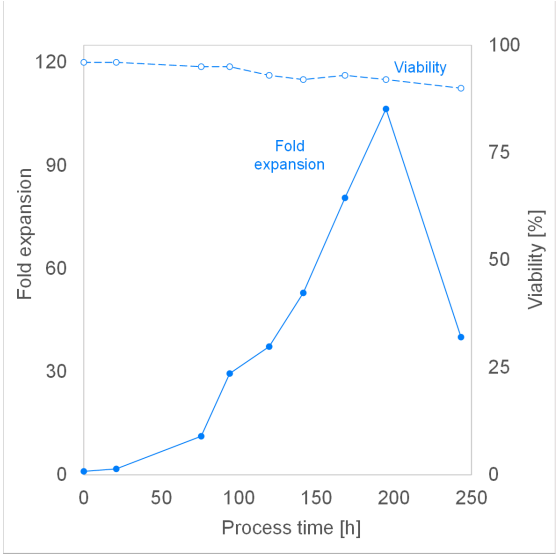

Figure 4. Jurkat cells perfusion culture with perfusion collapse. Fold expansion and viability of Jurkat cells cultured under perfusion for 250 h.

Continuous permittivity measurements followed the overall trajectory of offline viable cell density during both the growth and decline phase (Figure 5, left). At matched sampling time points, permittivity showed a strong positive relationship with viable cell density (R^2 = 0.98, r = 0.99, n= 9; Figure 5, right).

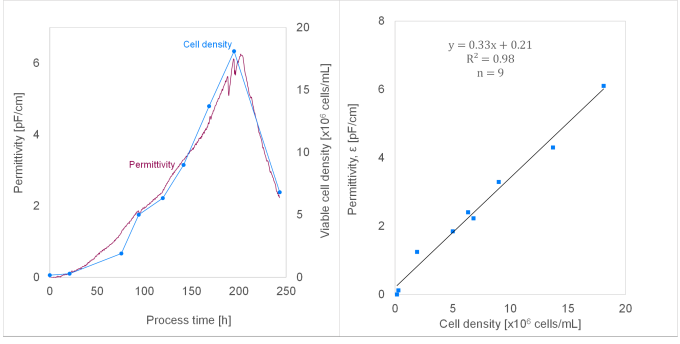

Figure 5. Left: Permittivity (red line) and viable cell density (blue points) measured during culture. Permittivity provides continuous real-time monitoring, while offline viable cell density measurements were collected at discrete sampling time points. **Right:** Relationship between matched offline viable cell density and permittivity measurements. The solid line shows the ordinary least-squares linear regression

A scheduled offline sample was not collected during the period in which culture performance began to deteriorate. During this sampling gap, the continuous permittivity signal showed that the biomass-related trajectory had already begun to decline and documented the progression of this change before the next offline cell-density measurement was available. The subsequent offline measurement confirmed a reduction in viable cell density but, by itself, could not resolve when the decline had started or how rapidly it had developed.

At approximately 240 h, complete loss of cell-retention performance was confirmed when the viable cell concentration measured in the permeate became comparable to that measured inside the bioreactor. Because pressure and transmembrane-pressure measurements were not available during this experiment, the mechanism and exact onset of the retention failure could not be established. The earlier decrease in permittivity therefore cannot be attributed specifically to membrane failure, but may reflect progressive loss of culture performance, developing retention problems, or a combination of process-related effects.

Exploratory analysis of frequency-domain parameters during fed-batch and perfusion culture.

Frequency-domain parameters were analysed retrospectively using the same datasets presented in Case Studies 1 and 2. In the fed-batch T-cell culture, the characteristic frequency, f_c, exhibited a pronounced early decrease from approximately 1.85 MHz at inoculation to approximately 1.50 MHz by 24 h, after which it fluctuated within a narrower range of 1.45 - 1.60 MHz (Figure 6A-1), with some local changes in f_c occurring around feeding interventions and periods of altered permittivity. The Cole-Cole α parameter (Figure 6A-2), increased progressively throughout the culture, from approximately 0.10 at inoculation to approximately 0.12 at 115 h. Transient decreases in α were observed around the feeding interventions and periods where the permittivity trajectory slowed or declined.

In the Jurkat perfusion culture, f_c decreased from approximately 1.75 MHz at inoculation to around 1.5 MHz during the early culture period, subsequently increased to a local maximum of approximately 1.65 MHz, and then declined progressively during later culture progression (Figure 6B-1). The α parameter increased more markedly than in the fed-batch culture, rising from approximately 0.10 at inoculation to approximately 0.22 during the high-density growth phase (Figure 6B-2). Both frequency domain parameters exhibited sharp decreases at the subsequent period of culture decline.

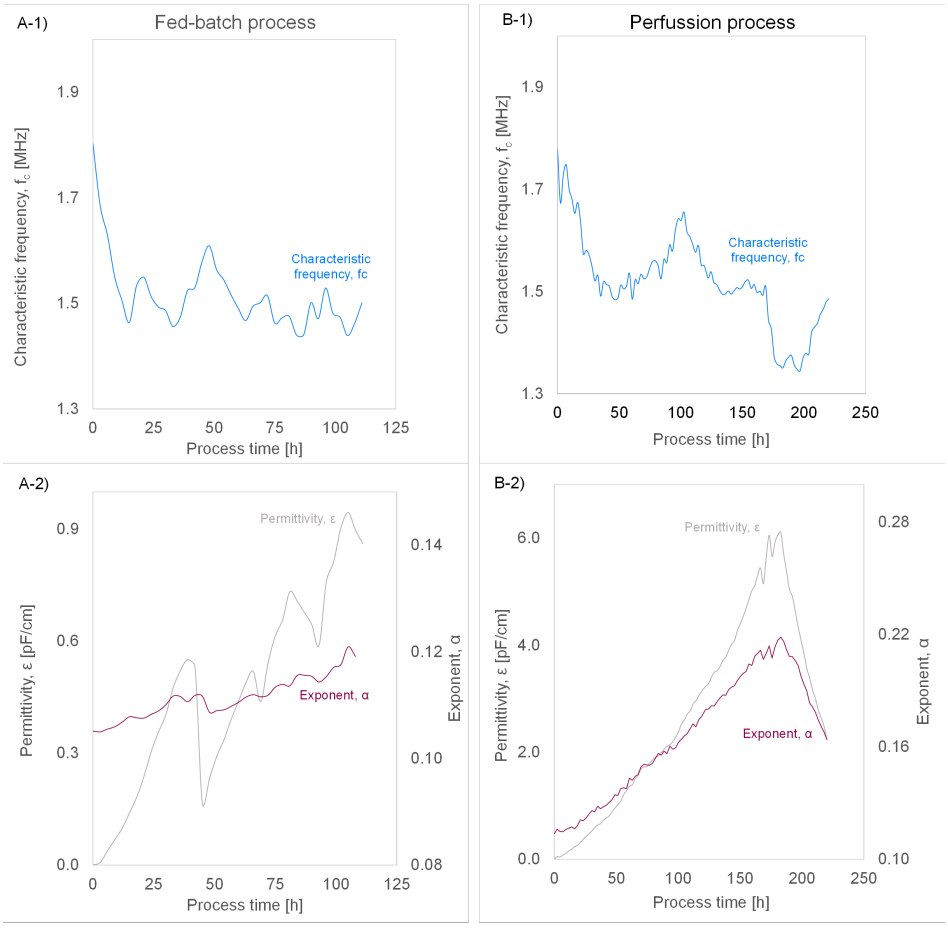

Figure 5. Frequency-domain parameters during T-cell fed-batch and Jurkat perfusion cultures. **(A-1)** Characteristic frequency (fc) measured during fed-batch culture. **(A-2)** Permittivity (gray line) and Cole-Cole exponent (α, red line) during fed-batch culture. **(B-1)** Characteristic frequency (fc) during perfusion culture. **(B-2)** Permittivity (gray line) and Cole-Cole exponent (α, red line) during perfusion culture. Frequency-domain parameters provide continuous real-time monitoring complementary to offline viable cell density measurements.

The temporal behaviour of f_c and α differed markedly between the fed-batch primary T-cell culture and the Jurkat perfusion culture and was not identical to the corresponding bulk permittivity trajectories. In the fed-batch culture, both parameters changed within relatively narrow ranges, whereas the perfusion culture showed a more pronounced increase in α and a distinct non-monotonic profile during the growth phase. The contrasting profiles may reflect differences in cell type, process operation, or the frequency and magnitude of process interventions, but these effects cannot be separated from the present datasets. Nevertheless, the distinct profiles illustrate why frequency-domain parameters should be interpreted using cell- and process-specific reference behaviour rather than applying a single universal trend across cultures.

The biological and operational meaning of these frequency-domain changes remains exploratory. Implementation requires establishing measurement reproducibility across runs, correlating frequency-domain trends with offline viability and metabolic data and developing process-mode-specific interpretation frameworks. Such validation would enable developers to integrate frequency-domain monitoring into routine process characterization and optimization workflows.

Discussion

Permittivity as a biomass proxy is process dependent

The positive relationships between permittivity and viable cell density observed in both cultures support the use of dielectric spectroscopy as a continuous indicator of changes in viable biomass. However, the strength and form of this relationship should not be considered universal. Permittivity is influenced not only by cell concentration, but also by cell size, membrane capacitance, intracellular conductivity and population composition (Opel et al., 2010; Schini et al., 2023). These factors are particularly relevant for activated primary T cells, which undergo substantial physiological changes during expansion.

The stronger linear relationship observed in the Jurkat perfusion culture compared with the primary T-cell fed-batch culture may therefore reflect differences in cell type, process operation, or both. Primary T-cell activation and differentiation have no direct equivalent in the Jurkat model, while fed-batch operation introduces discrete perturbations through media addition and exchange. By contrast, perfusion provides a more continuous culture environment. Because cell type and operating mode differed simultaneously between experiments, their individual contributions cannot be separated. The regressions should therefore be interpreted as process-specific descriptive relationships rather than universal calibrations.

Capacitance measurement provides process mode-specific insights

The principal value of continuous dielectric monitoring in these exploratory studies was its ability to resolve changes occurring between discrete offline measurements. This is consistent with the broader role of process analytical technology in improving temporal process understanding and supporting data-driven process development (Gillespie et al., 2022).

In fed-batch culture, the continuous trajectory provided additional information on when changes in biomass-related behaviour occurred relative to predefined feeding interventions. These observations do not demonstrate an optimised feeding strategy but provide a basis for prospectively testing signal-guided intervention timing. In perfusion culture, continuous monitoring provided information during a period without offline sampling. The signal narrowed the time window in which culture performance began to deteriorate, while subsequent offline measurements confirmed the reduction in viable biomass. These examples illustrate complementary uses of the same measurement principle: supporting hypothesis generation around planned fed-batch interventions and improving temporal resolution during unexpected perfusion disturbances.

The contrasting fc and Cole–Cole α profiles further demonstrate the context dependence of dielectric measurements. Differences may reflect cell-specific electrical and morphological properties, culture progression, operating mode, or process interventions (Bergin et al., 2022). Because these factors were not independently varied, mechanistic attribution is not possible; routine interpretation will therefore require cell- and process-specific reference behaviour.

Limitations of the current setup

The present work was exploratory and was not designed to establish validated monitoring or control strategies. The proposed feeding windows were identified retrospectively by visual inspection and were not tested prospectively. In addition, complementary measurements of cell size, membrane properties, phenotype, apoptosis and metabolic state were unavailable, limiting biological interpretation of changes in frequency-domain parameters.

The perfusion disturbance also highlighted a limitation of the initial prototype configuration. Loss of cell-retention performance was ultimately confirmed by the appearance of comparable viable cell concentrations in the permeate and reactor, but pressure and transmembrane-pressure measurements were not available. The pattern was consistent with a localised loss of membrane integrity rather than complete failure across the entire retention surface, but the exact mechanism could not be established. This observation motivated the inclusion of pressure monitoring in subsequent reactor designs to improve diagnosis of retention-related disturbances.

Integration into process development workflow

The combined BioThrust–Hamilton configuration illustrates a practical role for dielectric monitoring in early bioprocess development: using continuous in-line measurements to identify periods of interest for targeted offline analysis. In fed-batch development, future experiments should test whether interventions triggered by reproducible features of the permittivity trajectory improve expansion or other relevant cell attributes. In perfusion systems, combining dielectric measurements with pressure, flow and retention data could help distinguish biological changes from equipment or membrane disturbances. More generally, integration with complementary PAT measurements may improve the specificity of process-state interpretation, consistent with the use of multimodal monitoring strategies in advanced bioprocess development (Gillespie et al., 2022; Chen et al., 2021).

Future work should therefore focus on replicated cultures, prospective testing of signal-guided interventions, and correlation of dielectric parameters with complementary measurements such as viable cell density, cell size, phenotype, metabolites and retention-system performance. Establishing reproducible cell- and process-specific signal patterns would provide a basis for defining monitoring thresholds and, ultimately, incorporating dielectric measurements into automated process-control strategies.

References

Amini, A., Wiegmann, V., Patel, H., Veraitch, F. and Baganz, F. (2020) 'Bioprocess considerations for T-cell therapy: Investigating the impact of agitation, dissolved oxygen, and pH on T-cell expansion and differentiation', Biotechnology and Bioengineering, 117(10), pp. 3018–3028.

Bergin, A., Carvell, J. and Butler, M. (2022) 'Applications of bio-capacitance to cell culture manufacturing', Biotechnology Advances, 56, p. 107913.

Chen, G., Hu, J., Qin, Y. and Zhou, W. (2021) 'Viable cell density on-line auto-control in perfusion cell culture aided by in-situ Raman spectroscopy', Biotechnology Journal, 16(5), p. 2000290.

Dias, J., Garcia, J., Agliardi, G. and Roddie, C. (2024) 'CAR-T cell manufacturing landscape—Lessons from the past decade and considerations for early clinical development', Molecular Therapy Methods & Clinical Development, 32(2).

Gillespie, C., Wasalathanthri, D.P., Ritz, D.B., Zhou, G., Davis, K.A., Wucherpfennig, T. and Hazelwood, N. (2022) 'Systematic assessment of process analytical technologies for biologics', Biotechnology and Bioengineering, 119(2), pp. 423–434.

Hood, T., Springuel, P., Slingsby, F., Sandner, V., Geis, W., Schmidberger, T., Bevan, N., Vicard, Q., Hengst, J., Dianat, N. and Rafiq, Q.A. (2025) 'Establishing a scalable perfusion strategy for the manufacture of CAR-T cells in stirred-tank bioreactors using a quality-by-design approach', Bioengineering & Translational Medicine, 10(3), p. e10753.

Jacobtorweihe, L., Reichl, U., van Heuvel, Y., Scheiermann, K., Bongartz, P. and Genzel, Y. (2026) 'High cell density cultivation of HEK293 cells using a 2L membrane-stirred bioreactor', Biotechnology and Bioengineering, 123(2), pp. 436–448.

Opel, C.F., Li, J. and Amanullah, A. (2010) 'Quantitative modeling of viable cell density, cell size, intracellular conductivity, and membrane capacitance in batch and fed-batch CHO processes using dielectric spectroscopy', Biotechnology Progress, 26(5), pp. 1187–1199.

Schini, A., Loeffler, M., Müller, M., Bechmann, J., Herwig, C. and Neubauer, P. (2023) 'Influence of cell specific parameters in a dielectric spectroscopy conversion model used to monitor viable cell density in bioreactors', Biotechnology Journal, 18(5), p. e2300028.

von Werz, V., van Heuvel, Y., Hadrbolec, M., Wagner, M., Eibl, P., Huber, F., Meyer, M., Bongartz, P., Witz, C. and Spadiut, O. (2026) 'Homogeneous shear distribution improves NK-92 cell cytotoxicity in a clinically relevant 2 L membrane-stirred bioreactor', Frontiers in Bioengineering and Biotechnology, 14, p. 1804945.

Yee, J.C., Rehmann, M.S., Yao, G., Sowa, S.W., Aron, K.L., Tian, J., Borys, M.C. and Li, Z.J. (2018) 'Advances in process control strategies for mammalian fed-batch cultures', Current Opinion in Chemical Engineering, 22, pp. 34–4

BIOTHRUST

BioThrust GmbH
Pauwelsstraße 17
52074 Aachen
Germany

Dr.-Ing. Patrick Bongartz
patrick.bongartz@biothrust.de

Authors: Felipe Veliz Valenzuela, Yasemin van Heuvel, Katharina Dahlmann, Volker Donath, Patrick Bongartz

www.biothrust.com