

Bioimpedance Microsensor for Cell-Density Monitoring in Small-Scale Bioprocessing

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Summary:

Small-scale bioprocessing systems are increasingly used in biopharmaceutical applications from bio-process optimization to patient-scale therapy manufacturing. In these confined formats, inline monitoring of process parameters such as viable cell density (VCD) is limited by the lack of miniature sensing probes. Here, we demonstrate in CHO cell cultures that bioimpedance VCD sensing can be miniaturized to a 1×10 mm footprint and 25 μ L working volume with performance close to conventional probes.

Keywords: cell density monitoring, process analytical technology (PAT), electrochemical impedance spectroscopy (EIS), bioimpedance, small-scale bioprocessing

Introduction

Small-scale bioprocessing systems are increasingly used in the biopharmaceutical industry to advance high-throughput bioprocess development, support intensified manufacturing, or enable flexible, patient-scale, production of personalized therapies [1-2].

Timely measurement of key process parameters is essential to ensure performance and product quality, as encouraged by the FDA's Process Analytical Technology (PAT) initiative. However, real-time PAT solutions compatible with small-scale bioprocessing systems remain limited. This is particularly true for viable cell density (VCD), a key parameter directly linked to process performance and product titer [2-3].

Inline VCD measurement relies on bio-capacitance probes that are too large for small-scale bioprocessing systems [1,4]. Alternatively, offline determination by trypan blue exclusion method is possible, but the need for sampling limits its practical use.

Here, we present a miniature VCD sensor with a reduced footprint of 1×10 mm and a working volume of 25 μ L. Using microfabrication techniques, tetrapolar microelectrodes for Electrical impedance spectroscopy (EIS) were patterned on a glass microchip. Bioimpedance analysis of Chinese hamster ovary (CHO) cell suspensions showed a linear correlation ($R^2=0.92$) with reference VCD in the range 10^6 - 10^8 cells mL^{-1} .

Materials and Methods

Miniaturized VCD sensors made of evaporated Pt thin films (250 nm) were fabricated on a 100 mm fused silica wafer using photolithography and lift-off processes, followed by dicing into 15×15 mm microchips. The chips were mounted in a holder providing fluidic routing and electrical connection to an impedance analyzer (Zurich Instruments) (Fig. 1a). Each chip consists of two VCD sensors positioned within two microfluidic channels with a sample volume of about 25 μ L each (Fig. 1b). Samples were manually dispensed into the channels using a pipette. EIS measurements were made from 10 kHz to 5 MHz with an amplitude of 200 mV in a tetrapolar configuration (Fig. 1c). Impedance spectra were calibrated using an open-short-load procedure with supernatant as reference.

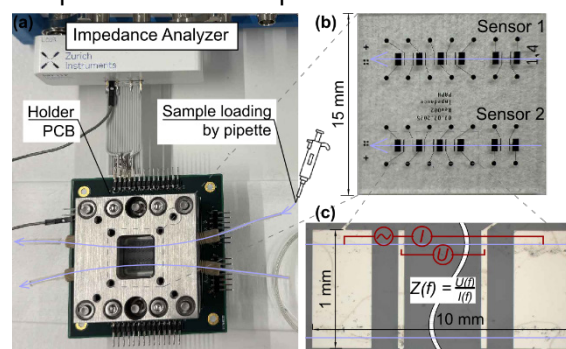


Fig. 1 Measurement setup. (a) Holder for fluidic and electrical interface to the chip. (b) Image of a chip with the two VCD sensors. (c) Close-up of a VCD sensor and schematic of the tetrapolar EIS measurement.

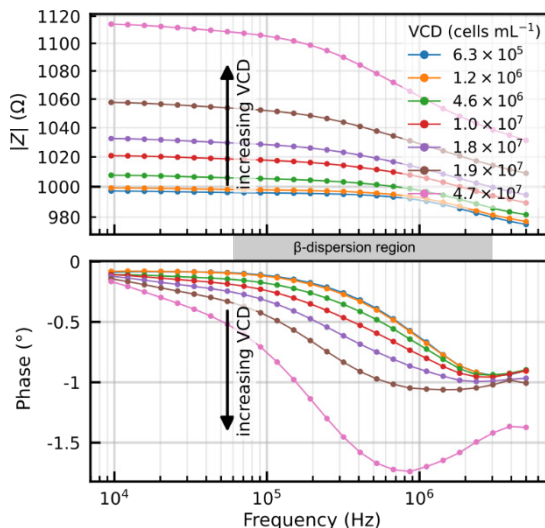


Fig 2. Bode plots showing calibrated impedance spectra of CHO cell suspensions at different VCDs. The lines are guides for the eye.

Finally, the biocapacitance $C(f)$ was derived at each frequency from a parallel RC model circuit.

ExpiCHO-S cells were cultured in Efficient-Pro Medium (Thermo Fisher Scientific) as described previously [5] and sampled during the exponential growth phase at VCD of 4.6×10^6 cells mL^{-1} and mean cell diameter of $13.6 \mu\text{m}$. Cells were then centrifuged at 300 RCF for 5 min and resuspended in supernatant at defined concentrations. Reference VCD measurements were made using a Cedex HiRes Analyzer (Roche).

Results and Discussion

VCD measurement by bioimpedance models cells as core-shell spheres suspended in an electrolyte. Under an alternating electric field, such cell suspensions exhibit β -dispersion (kHz to MHz) due to Maxwell-Wagner interfacial polarization, resulting in VCD-dependent permittivity changes [6]. This behavior is visible in the calibrated EIS Bode plots shown in Fig. 2, where CHO cell suspensions of increasing VCD exhibit stronger capacitive effects.

As shown in Fig. 3, the biocapacitance $C(f)$ extracted from the calibrated EIS spectra at 55 kHz

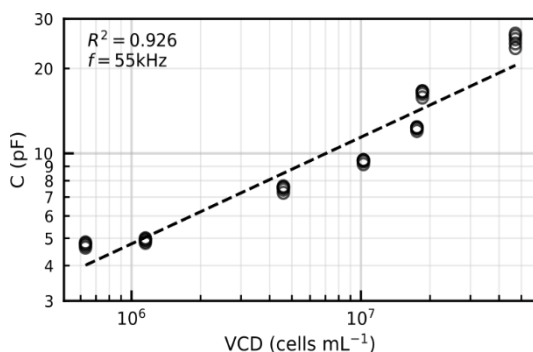


Fig 3. Extracted biocapacitance $C(f)$ at 55 kHz versus reference VCD, with linear fit (dashed line). Each sample was measured 5 times.

shows a coefficient of linearity $R^2=0.92$ with reference VCD measurements, matching the performance of conventional probes [4]. Likewise, deviations from linearity below 0.8×10^6 and above 2×10^7 cells mL^{-1} are similar to commercial probes, reflecting detection limits ($\sim 0.5 \times 10^6$ cells mL^{-1}) and crowding effects beyond Maxwell-Wagner assumptions [4].

Conclusion and Outlook

Addressing the lack of inline VCD monitoring in small-scale bioprocessing systems is essential for their broader adoption. Here, we demonstrate that chip-based miniaturization of VCD sensing can achieve performance comparable to conventional probes with a significantly reduced footprint, enabling monitoring at the smallest scale.

Future work will focus on continuous monitoring over up to 14 days, with focus on biofouling effects. Ultimately, our miniaturized chip-based approach will allow integration of additional sensors, paving the way for multi-parameter PAT microchips tailored to small-scale bioprocessing.

References

- [1] J. I. Betts and F. Baganz, Miniature bioreactors: current practices and future opportunities, *Microb Cell Fact*, 5 (2006); doi: 10.1186/1475-2859-5-21
- [2] R. K. Iyer, P. A. Bowles, H. Kim, and A. Dular-Tulloch, Industrializing Autologous Adoptive Immunotherapies: Manufacturing Advances and Challenges, *Frontiers in Medicine* 5 (2018); doi: 10.3389/fmed.2018.00150
- [3] M. M. Hewitt, N. Trainor, N. Ostrout, and E. Abraham, Cell therapy manufacturing: process analytic technologies needed to achieve flexible, feedback-driven automation, *Current Opinion in Biomedical Engineering*, 20 (2021); doi: 10.1016/j.cobme.2021.100358
- [4] A. Bergin, J. Carvell, and M. Butler, Applications of bio-capacitance to cell culture manufacturing, *Biotechnology Advances*, 61 (2022); doi: 10.1016/j.biotechadv.2022.108048
- [5] J. Ott, V. Ott, P. Neubauer, D. Eibl, and R. Eibl, Process intensification by inoculating antibody production bioreactors directly from cryovials, *Chemie Ingenieur Technik* (2024); doi: 10.1002/cite.202300189
- [6] H. P. Schwan, Determination of biological impedances, *Electrophysiological Methods* (1963); doi: 10.1016/B978-1-4831-6743-5.50013-7

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