

REVIEW

Advances in sensor developments for cell culture monitoring

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Abstract

Cell culture encompasses procedures for extracting cells from their natural tissue and cultivating them under controlled artificial conditions. During this process, various factors, including cell physiological/morphological properties, culture environments, metabolites, and contaminants, have to be precisely controlled and monitored for the survival of cells and the pursuit of the desired properties of the cells. This review summarizes recent advances in sensor technologies and manufacturing strategies for various cell culture platforms using traditional plastics, microfluidic chips, and scalable bioreactors. We share the details of newly developed biological sensors, chemical sensors, optical sensors, electronic chip technologies, and material integration methods. The precise control of parameters based on the feedback by these sensors and electronics enhances cell culture quality and throughput.

KEYWORDS

biosensor, cell culture monitoring, cell physiology monitoring, electrochemical sensing, metabolite, optical sensing

1 | INTRODUCTION

Implementing sensing systems has revolutionized monitoring and control in the biotechnology industry, benefitting various academic and industrial fields such as biological science, biomedical and clinical manufacturing, fermentation, biotransformation, and related downstream processes.^[1] Thanks to new

analytical tools and biosensing techniques, monitoring cell culture no longer requires expensive, heavy machinery. The selection and assembly of the necessary analytical instruments can now be made easily.^[2,3] Cell culture technique is mainly considered as cultivation in a regulated artificial environment. Since the early era of cell culture experiments, enormous efforts have been made to create optimal conditions for a controlled

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microenvironment supporting the culture of the primary target cell or cell lines. This effort involves media composition,^[4–6] temperature,^[7,8] gas level,^[9,10] pH,^[11,12] mass transfer,^[1] and agitation.^[13,14] Therefore, an optimally regulated environment allows cells to get designed functionality and move on to further applications, including cell culture expansion, metabolite study using omics technologies, genetic transfection, drug screening, and biomanufacturing of products.^[1,14,15] While specific profiles may vary depending on the type and origin of the cells, it is essential to control key metabolic profiles and avoid exceeding certain thresholds in the cell culture environment. In this context, the depletion of nutrients and accumulation of metabolic byproducts was monitored with traditional analytical tools such as the optical microscope, spectrophotometer, chromatograph, electrical sensor, and a mass spectrometer to prevent degradation or failure of cellular viability and functionality.^[16] Monitoring such a cell culture environment allows an understanding of the optimal cycle for media renewal and the balanced parametric profiles. Due to the time-consuming process of cell preparation, assessment of the culture environment and the related parameters is critical to achieve ideal cell viability, expansion quantity, functionality, phenotype variability, and differentiation. Frequent or continuous monitoring of the cellular physicochemical profiling of the culture environment and media is vital to manage this. Several traditional methods have been employed to monitor cell culture, such as the optical microscope, spectrophotometer, chromatograph, electrical sensor, and a mass spectrometer. Recent advancements in analytical methods have supplemented traditional monitoring techniques, relying on an understanding and application of microfabrication,^[17–19] optical^[12,20] and conductive^[21,22] materials, sensitive signaling probes,^[5] and microfluidic chip packaging.^[23,24]

This review summarizes the parameters used for monitoring cell culture into four main categories: cellular physiology, cellular environment, cellular metabolite, and contaminant monitoring (Figure 1). The first category, cellular physiology, involves assessing various aspects of cellular viability, behavior, and related characteristics. This includes parameters such as cell morphology, cell counts, cell density, cell viability, adhesion, and migration events. Traditionally, microscopy has been the most commonly employed method for observing these features, serving as a fundamental tool for identifying potential risks of deformation due to culture failure. However, along with technological advancements, image processing algorithms, and optical and electrical sensing technologies are now utilized to enable quantified and continuous monitoring of these cellular physiology parameters. The second category

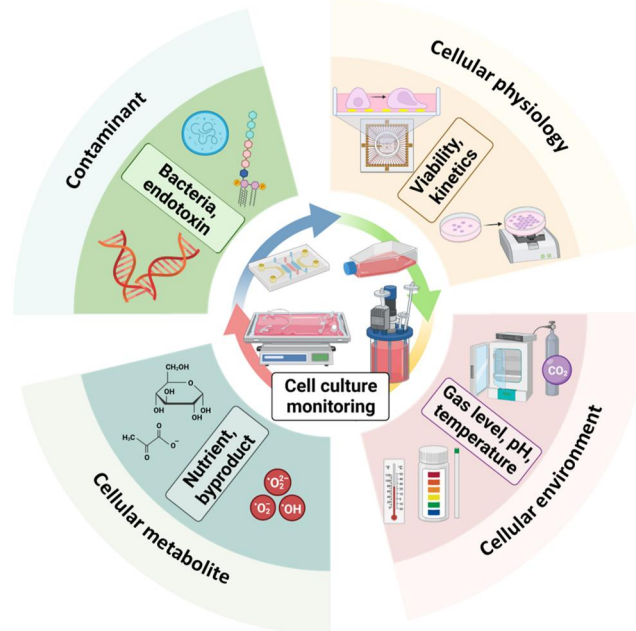


FIGURE 1 The overall schematic illustrates the four target parameters of cell culture monitoring. Firstly, cellular physiology monitoring parameters involve cell viability, morphological property, and cell kinetic property. The cellular environment monitoring parameters comprise dissolved oxygen, carbon dioxide, pH, and temperature. Cellular metabolite parameters are constituted by nutrient and byproduct levels in the cell culture medium. Finally, the contaminant is the external adventitious agents such as bacterial infection and its residual molecules. Created with [BioRender.com](https://www.biorender.com).

pertains to monitoring the cellular environment parameters crucial in cell culture. Critical factors in this category include environmental properties such as temperature, pH, carbon dioxide, and dissolved oxygen (DO) levels. Monitoring these environmental conditions is essential for ensuring optimal cell growth and performance. The third category focuses on assessing cellular metabolites as a significant parameter for evaluating cell quality and predicting yield. To achieve this, it is vital to quantitatively monitor chemicals and biological metabolites, such as nutrients, byproducts, and cell-secreted products. Additionally, tracking the presence of byproducts is important for identifying and maintaining optimal culture conditions. Moreover, the fourth category also encompasses monitoring contaminants, such as bacteria and endotoxins, which can potentially threaten the successful cell culture and product. The four categories for monitoring cell culture are cellular physiology, cellular environments, cellular metabolites, and contaminants monitoring.

Each category encompasses specific parameters and methodologies crucial for optimizing cell culture

processes and ensuring the desired cell behavior and product yield. Analyzing metabolites in media samples can be costly and time-consuming with traditional chromatography and mass spectrometer methods. Alternative methods like electrochemical and optical sensing-based catalytic reactions offer a reliable and fast way to profile target molecules. Emerging fields of electrical sensing manufacturing, including novel materials, nanoscale fabrication, packaging, and electronics integration, have become alternative options for evaluating physiological properties. These developments overcome challenges in monitoring localized regions, lowering the sampling volume, and de-risking contamination in traditional bioreactors. Electrical sensors can detect a wide range of molecules and be embedded in microelectronic devices by applying techniques such as voltammetry, potentiometry, amperometry, and electrical impedance spectrometers. Similarly, the availability of optical sensing components driven by the sensitive optical probes (e.g., absorption, plasmonic, luminescent, Raman) and modular spectrometer gives more applicability in various formats.

Our review has investigated biosensing and analytical methods, explicitly focusing on optical and electrochemical analysis systems to monitor a wide range of cell culture monitoring parameters. Our study offers an overview of the monitoring parameters involved in cell culture processes, including cellular physiology, environment, metabolite, and contaminant, highlighting the corresponding analysis strategies for each specific target. Additionally, we explore cutting-edge technologies in cell culture monitoring, emphasizing the importance of collaboration with various technological advancements for continuous development in this field.

2 | CELLULAR PHYSIOLOGY

In this section, we review the recent advances in cellular physiology monitoring applications, including parameters related to cell viability, proliferation, and kinetic behavior. Monitoring cell morphological and physiological properties during the culture environment is the most basic and vital mission to be accomplished. One of the many ways to gather cell physiological information is through visualization, essentially imaging. This is the initial step toward comprehending cellular viability, morphology, motile properties, and other cell culture events. This section outlines representative target elements and kinetic events for cellular physiology monitoring in several sensing strategies regarding optical and electrical sensors. The corresponding sensing strategies are elaborated in detail in Table 1.

2.1 | Cell viability and proliferation

2.1.1 | Visualization and optical sensing techniques

Optical density: Owing to assess cell culture status in terms of proliferation, researchers can conduct one of the simplest and earliest methods to measure the optical density of the cell culture environment. For instance, with floating cell culture, it is necessary to frequently monitor the optical density of cell suspensions during the exponential growth phase.^[27,28] While this review mainly focuses on animal cells, optical density has been used to measure most types of plants, bacteria, and viruses. Recent developments in optical density analysis include sampling methods and direct analysis through in situ microscopic, UV-vis, and near-infrared (NIR) spectroscopic analysis.^[29,30]

Cellular imaging: Cell culture experiments require continuous human intervention, which can be laborious and subjective. Frequent human intervention often leads to significant variations and inconsistent results, particularly when visually assessing cell confluency to determine the optimal time for subculturing. Therefore, traditional microscopic imaging is frequently utilized during the whole cycle of the cell culture process. Nevertheless, alternative instrumentation exists, such as a miniaturized automatic microscopic device that decreases human involvement during visual monitoring of cell culture. These products were capable of real-time cell imaging and time-lapse imaging. One recently commercialized product, Provi CM20 (Olympus), is for static culture at the CO₂ incubator (Figure 2a). These incubation monitoring devices act as digital microscopic scanning devices in a 2d culture, providing time-lapsed images at a defined duration to track morphological properties.^[25] However, 3d cell scaffolds or spheroid imaging mainly relies on the optical microscope. Unlike traditional visualization techniques involving the optical microscopic method, direct imaging of the culture niche can be a powerful tool for the intrinsic understanding of the cell microenvironment.^[18,28,38,39] As one of the examples, in situ, visualization on the organ-on-chip designed for airway tissue monitoring was suggested by Mir et al.^[26] The developed monitoring chip platform comprises the gradient-index (GRIN) lens and imaging-enabled PTFE custom valve type bioreactor (Figure 2b). Stem cell differentiation on tissue was visualized by monitoring with bright field and fluorescence imaging inside the tissue that possesses the capability of an imaging-assisted bioreactor platform. Using image processing strategies, recent examples of determining the cell confluency through visualization on the microscope are also notable. Assuming the automated high throughput

TABLE 1 Sensor preparation methods for cellular physiology monitoring.

No.	Targets	Source	Culture format	Sensing surface shape & material	Chip/sensing surface fabrication method	Ref.
1	Microscopic imaging	Microscopy	Various type	Independent device	- Commercial products (Provi CM20, Olympus)	[25]
2	Optical density	In situ microscopy	Organ-on-chip	Gradient-index lens on optical fiber	- Optical fiber for PTFE custom valving bioreactor	[26]
		NIR spectroscopy	Batch culture	Microscope	- Commercial products	[27, 28]
3	Counts	Optical coherence tomography	Batch culture	Optical fiber	- NIR LED (880 nm) with photodiode detector	[29, 30]
			Flask culture	Optical fiber	- Optical fiber probe with 890 nm two diode, 2048-pixel spectrometer	[31]
4	Viability, confluency	LSPR	Cell-on-chip	Gold nano dot array	- Lithography for nano dot array (dia. 184 nm) on glass substrate	[32]
		Electrical	Petri dish culture	Al electrode array	- Solder two square plate electrodes	[33]
5	Adhesion	Impedance	Petri dish culture	Ga ₂ O ₃ nanorod field-effect transistor	- Chemical bath deposition of Ga ₂ O ₃ nanorods on ITO	[21]
			96 well plate	Micro-electrode array	- ITO substrate on PMMA, Au/Ti deposition and patterning	[34]
5	Adhesion	Quartz crystal microbalance	Microfluidic channel	Electrode array	- Lithography with e-beam deposition	[23]
			MEA	Micro-electrode array	- ITO substrate on glass, Au/Ti deposition and patterning	[35]
			Micro culture chamber	Vertically aligned electrodes	- Four pair of PCB with gold plating electrode	[36]
5	Adhesion	Quartz crystal microbalance	Custom chamber	QCM-D	- Galactose-modified polycaprolactone-chitosan blended thin film	[37]

Abbreviations: ITO, indium tin oxide; LSPR, localized surface plasmon resonance; MEA, microelectrode array; PCB, printed circuit board; PMMA, polymethyl methacrylate; PTFE, polytetrafluoroethylene; QCM, quartz crystal microbalance with dissipation.

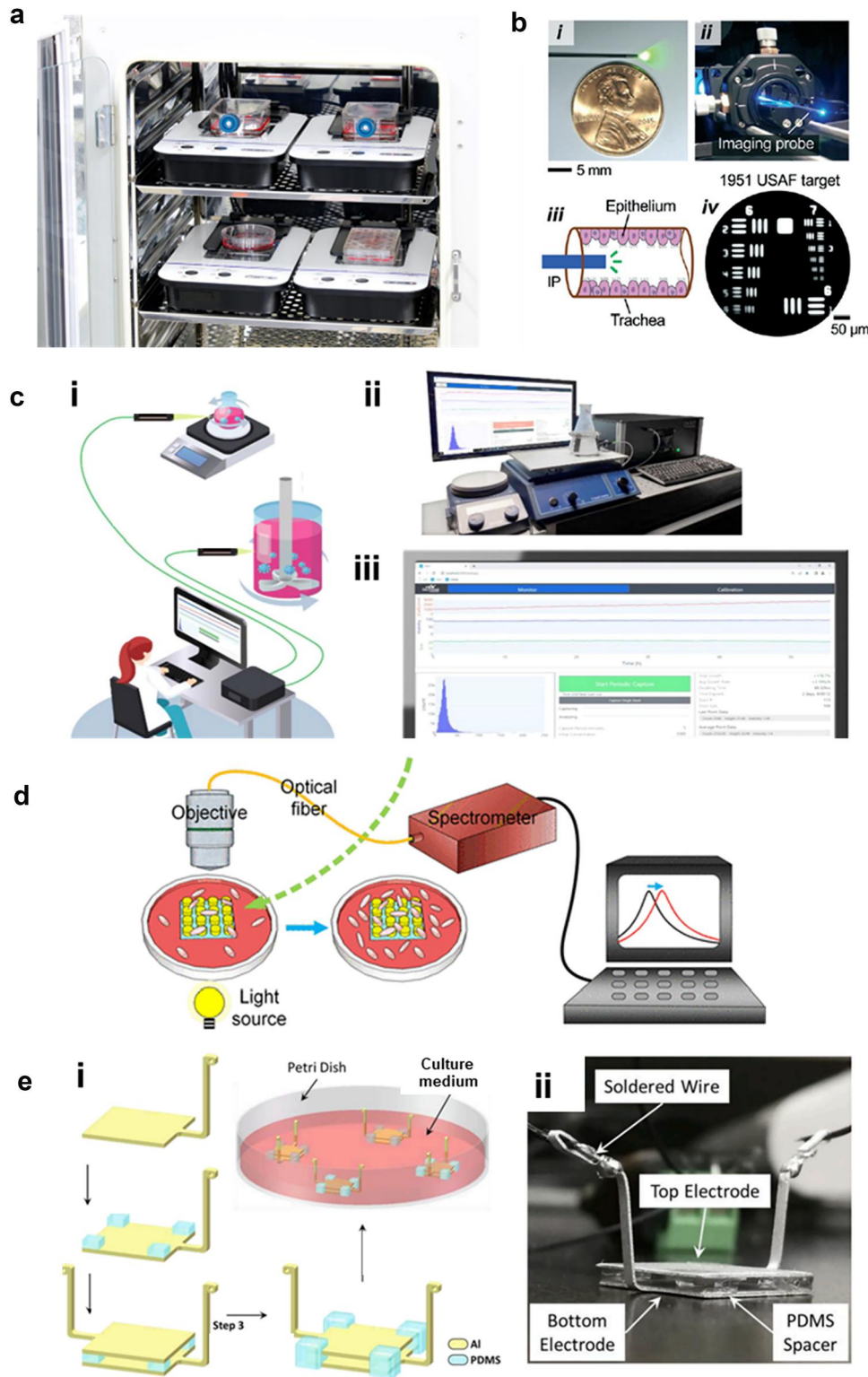


FIGURE 2 Legend on next page.

cell culture system is required, computer vision will be the crucial part of the visual assessment of the cell culture. Although the visual inspection by a human operator is intuitive, there is a limitation for computer vision due to the low contrast between the cell cytoplasm area

(foreground) and the denuded area (background), which poses significant challenges. To overcome this limitation, Wang et al. demonstrated the 2d culture microscopic analysis-based automated quantification of cell confluency through a custom algorithm on a bright field

microscope.^[40] Image reconstruction, segmentation, and filtering processes enable robust and accurate confluence measurements under a low-cost brightfield microscope. For the 3d culture microscopic analysis, Farrel et al. conducted micro-flow imaging (MFI)-based cell confluency quantification on the microcarrier with image processing algorithms.^[41] MFI has its advantages in directly observing the bioreactor with precision cell flow with or without fluorescent labeling. Cell confluency from the spherical microcarrier was classified by size measurement on the microscopic images. The image process starts with identifying microcarriers and edge detection-based background exclusion. Additionally, the aggregated particles can be identified and measured for their confluency. Resultingly, the quality of confluency assessment was compared with static light scattering and exhibited more accurate results due to the classification ability from processed data from visualization to quantify the defected and uneven microcarrier. Without labeling, automation-based cell visualization can be a more effective and trustworthy method for the case of aiming for high throughput.

Optical coherent tomography: Optical coherent tomography (OCT) can provide deep visualization of environmental information inside the bioreactor on the setup of the inline cell growth monitoring method. One example of monitoring cell growth was suggested by Brehove et al. The authors conducted OCT imaging of various types of bioreactors, including shake flasks, impeller bioreactors, and rocking-bag bioreactors, using commercially available OCTiCell (ChromoLogic) (Figure 2c).^[31] OCT runs an interferometric imaging process to provide cross-sectional images from the microenvironmental of cell culture.^[42–44] Resulting images can also produce the 3d profile and are frequently utilized on organoid, 3d scaffold culture, and kinetic and viability properties. This optical imaging platform enables non-invasive culture monitoring, preventing potential contamination during the culture process. OCT does not require consumable materials or interaction with the user, and continuous operation can be drawn out to obtain highly accurate imaging results.

Surface plasmon resonance: Surface plasmon resonance (SPR) is a technique that profoundly reflects the surface affinity binding of the molecules or cells, and these binding-induced refractive index changes make the wavelength shift. Specifically, the difference in the evanescent field wavelength from the metal layer where the reaction occurs on the surface capture molecules can be acquired. Thus, SPR can measure the quantitative signal difference at the beginning of the reaction and the end and guides the user to understand the affinity or dissociation of target molecules from the sensing surface.^[45] Among variations of SPR techniques, localized surface plasmon resonance (LSPR) can serve the same applications, such as sensing, imaging, and optoelectronics.^[46] A significant difference is that plasmonic resonance will occur only on the metallic nanoparticles or nanostructures on the sensing surface and can optimize the frequency signal properties from the design of the nanoparticle size, shape, and patterns on the surrounding substrate. Therefore, in the case of the cell-on-chip application, one recent study from Chang et al. demonstrates the real-time quantification of cell confluency on the plasmonic nanodot array using LSPR detection (Figure 2d).^[32] The fabrication of a 5×5 mm areal gold nanodot array with a dot diameter of 184 nm was constructed on a glass substrate via the nanoimprint process. Gold nanodot array chips were tested for assuring cytotoxicity and viability with ARPE-19 cells and were applied to monitor the cell culture cycles from the G1 phase to the M phase. Regarding the sensing principle, LSPR redshift caused by the cell attachment on the nanodot surface that affected plasmonic wavelength shifted toward the longer wavelength. The cell confluency assessment was compared with the intra-cell DNA content quantification to ensure correspondence with the cell cycle. The visualization of cellular physiology, the most fundamental monitoring method, continues to hold significant relevance. Various tools ranging from conventional microscopes to OCT and SPR have their specialty depending on the scale and format of the cell culture. However, direct microscopic methods combined with optical probes have the potential for application in diverse

FIGURE 2 Application for monitoring cellular physiological parameters, including morphological properties, cell viability, and cell population. (a) Miniaturized and automated cell culture visualization microscope for incubator. (b) Cellular visualization using in situ optical fiber-based direct microscopy for fluorescent and bright field imaging in 3D tissue culture. (c) Cell culture visualization using optical tomography. i) schematic illustration of the versatility of optical coherent tomography, ii) application in the culture flask for in situ imaging, iii) acquired data format on a PC screen. (d) Cell adhesion and expansion monitoring using localized surface plasmon resonance-based redshift detection on gold nanodot array. (e) Cell population monitoring using impedimetric sensor during suspended cell culture system. i) Schematic illustration of sensor fabrication and utilization, ii) picture of the impedimetric metallic disposable sensor. Rights and permission. (a) Reprinted from the courtesy of Evident Scientific. (b) Reprinted with permission. Copyright 2022 Royal Society of Chemistry.^[26] (c) Reprinted with permission. Copyright 2023 from Elsevier.^[31] (d) Reprinted with permission. Copyright 2022 American Chemical Society.^[32] (e) Reprinted with permission. Copyright 2023 Elsevier.^[33]

scenarios and scales. While wide-angle capture is necessary for machine vision or computer vision in a cell culture environment for industrial uses, highly integrated electronics-based imaging applications are highly anticipated for research purposes.

2.1.2 | Electrical sensing

Unlike the visualization-based direct observation of the cell confluency test, the impedance sensor array can serve as a cell growth monitoring quantitatively.^[47] Liu et al. reported a disposable impedance sensor array that monitors cell growth during CAR-T manufacturing.^[33] The application is simple, inserting the sensor array into the cell culture medium. The impedance sensor composed of two aluminum plates with a polydimethylsiloxane (PDMS) spacer that presents cost-effective disposable sensing for a small working volume culture system. The impedimetric signal data are acquired by an LCR meter, and the processed data with a physics-inspired model can deduce a trained model that deduces the cell density of the suspension cell culture (Figure 2e). Additionally, optical microscopy is commonly utilized to assess cell coverage, confluence, and viability. However, it falls short of capturing the intricate tightness of cell-cell junctions. As an alternative, a less invasive approach is suggested to use electrical measurements to evaluate the barrier function of epithelial cells on a porous cell culture-supported substrate. The electrical cell-substrate impedance sensing technique is one example of the cell-to-cell electrical signaling method^[21,48,49] that indirectly interprets the cell status in microscale chip format.

2.2 | Kinetic cell property

In the starting process of the adherent cell culture, frozen or isolated cells are put into the cell medium-contained vessel and make adhesion to the culture ware surface. The determination of the cell adhesion property on the culture ware is essential to identify the cell surface property of sufficient proliferation or cell physiological property is functional for further process. Cell adhesion starts with fibrinogen formation toward the surface, making intracellular structures hold the position. Subsequently, cells begin to proceed to the expansion phase for division. To identify this property, researchers can have two strategies. (1) Visual confirmation of cell attachment on the surface with z -axis information. (2) Electrical property changes via cell contact with a surface with impedance measurement. Similar motile activity of cells, such as

migration and invasive, induced by chemical or biological stimuli can also be identified with visual confirmation and impedimetric analysis.

2.2.1 | Visualization and optical sensing techniques

Understanding optical imaging results from the cell culture microenvironment is a fundamental approach to cell physical and kinetic properties. A decade ago, from traditional optical spectroscopy, there was a trend to establish an independent imaging device for long-term cell dynamic behavior tracking and monitoring.^[50,51] Specifically, to achieve simplicity, lens-free holographic live-cell imaging is being diversely studied by the Ozcan group.^[52–55] From this footstep, 3d holographic lens-free imaging techniques reach the level for conducting system reliability tests toward the dynamic tracking of various types of cells (MIN6 cell line, P. minimum, and neuroblastoma [SH-SY5Y]).^[56] Also, Kesavan et al. reported the high-throughput monitoring of cell physiological properties through lens-free video microscopy.^[57] From the cell adhesion and spreading on the culture ware, the following study monitored division and death of the cell on petri dish cultured human mesenchymal stem cells and human osteosarcoma. As depicted in Figure 3a, an independent device can visualize through the complementary metal-oxide semiconductor RGB imaging sensor to obtain raw lens-free holograms along with the aspect-ratio measurement of cell groups to identify the z -axis information through the device through the reconstruction of data set with computing kurtosis, which is the determination method of peak or flat point from gray value distributed image. In the foreseeable future, automated direct microscopic and holographic microscopic visualization will become the standard. Currently, for industries aiming for high-throughput applications or even diagnostics, the expectation leans toward commercial devices offering miniaturized and superior image processing for 3d imaging.

2.2.2 | Electrical sensor

Electrical cell-substrate impedance sensing Among the electrical sensors that invasively contact the cell culture environment, the micro-scale working volume on the electrode array chip for cell monitoring has been typical from the birth of the microelectrode array (MEA) chip.^[17–19,34,58–61] However, the most common approaches toward the cell-to-culture ware interface impedimetric

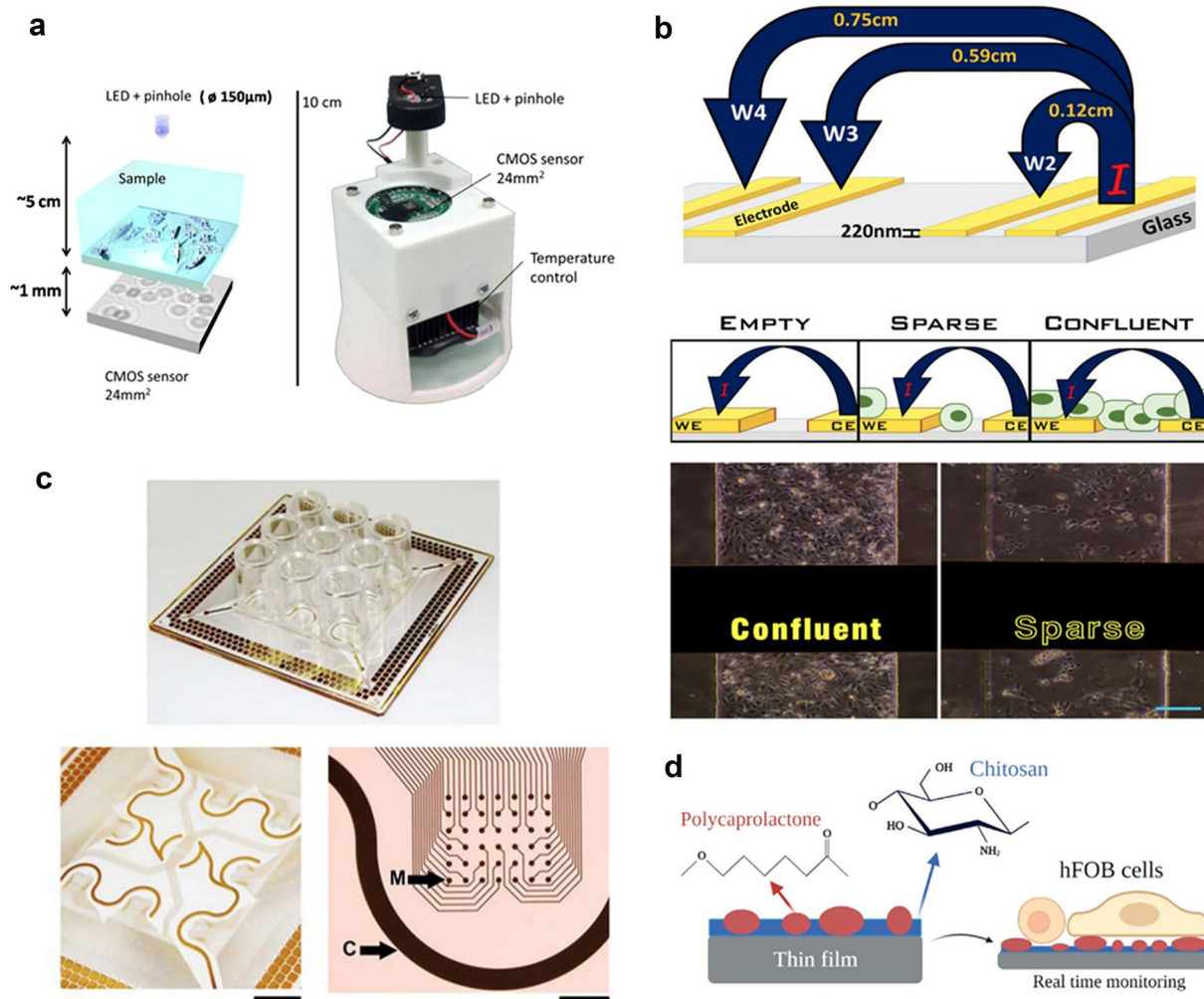


FIGURE 3 Application for monitoring cellular physiological parameters, including confluency and kinetic properties. (a) Cell confluency detectable lens-free pinhole microscope (left) schematic illustration of acquiring holographic images on cell culture, (right) picture of the device and its components. (b) Cell location and confluency detection using an impedimetric sensor. Measurement can utilize different sets of electrodes placed at different points to quantitatively determine the cell-expanded confluency. (c) Highly dense microelectrode array chip for cell migration monitoring. Upper) complete array view, lower left) top view of the array with circuit path and contact pads (lower left bar = 10 mm), lower right) 6 × 7 microelectrode array (lower right bar = 1 mm). (d) Polycaprolactone-chitosan film and quartz crystal microbalance-based real-time cell adhesion monitoring sensor. Rights and permission a Reprinted with permission. Copyright 2014 from Springer Nature.^[57] Creative Commons CC-BY-NC-ND license. (b) Reprinted with permission. Copyright 2022 from Springer Nature. Creative Commons CC BY license.^[23] (c) Reprinted with permission. Copyright 2019 from Springer Nature.^[35] Creative Commons CC BY license (d) Reprinted with permission. Copyright 2023 from American Chemical Society.^[37]

sensing target involve cell contact or motile ability.^[47,62,63] One example is Rapier et al. reported a simple and intrinsic microfluidic sensory system based on impedimetric sensing for determining cell location and confluency.^[23] The developed method was integrated with four bands of gold electrodes spanning the width of 500- μm -wide-microfluidic channels. These electrode probes were placed at different distances along the channel length to probe for cells (Figure 3b). Thus, it is possible to utilize different sets of electrodes placed at other points along the device length. Analyzing the impedance spectra of the microfluidic channels makes it possible to distinguish between various

levels of occupancy, including empty, sparsely populated, and densely populated areas.^[36] Jahnke et al. reported an impedance sensor for monitoring cell migration in a highly dense MEA chip^[35] (Figure 3c). Cell migration monitoring can indicate chemo- bio- tactic kinetics of immune cells and stem cells.

Moreover, migration is a crucial indicator of tumor cell metastatic behavior. To this end, the impedimetric sensor array was fabricated on a glass substrate with multiple cleanroom microfabrication processes, including sputter coating of gold on an indium tin oxide layer. Tumor spheroid T30.6.9, T12.8.10ZII, and MDA-MB-231 were

chosen for a model study of cell spreading and migration on the MEA. To assess cell migration, the authors monitor the increased covered electrodes resulting from cells spreading over the MEA from the tumor spheroid.

Quartz crystal microbalance: Certain types of animal cells require a specific surface environment, such as extracellular matrix coating. In other cases, cell adhesion analysis identifies new surface modifications in cell culture environments. To sense the cell attachment, visual confirmation of the floating cells in the medium is the simplest way; however, to prevent physical impact on the cell medium, a culture surface embedded sensor can provide the quantified information about the cell attachment through the detect impedance changes on electrodes and quartz crystal microbalance (QCM).^[64–67] In one approach, Sert et al. proposed a polycaprolactone-chitosan film and QCM-based real-time cell adhesion monitoring sensor that captures characteristic frequency and dissipation signal pattern differences. In contrast, cell adhesion is progressed.^[37] In detail, the human fetal osteoblastic (hFOB) cell was employed as a model cell adhesion, and the polycaprolactone-chitosan thin film was utilized as an interactive interface to the hFOB cell. QCM with dissipation (QCM-D) can serve as a signal indicator for the dissipation pattern between cell and polymer. QCM-D techniques were applied to determine the frequency changes, which is a label-free, highly sensitive detection of the surface interaction between the cell and the sensing surface. Figure 3d shows the schematic sensor composition for the chitosan-blended polycaprolactam film on the QCM sensor. Thin polymeric film induces cell fibrinogen production toward the sensor surface thus can be utilized for cell adhesion yield monitoring. When the hFOB cell initiated the sedimentation, a thin polymer film on the QCM device could detect the gradual drop of frequency and dissipation pattern for 1-h real-time monitoring. Unlike visualization, electrical sensing at the microscale area level still faces challenges due to inherent resistances posed by biomaterials. However, using specific patterns to maximize the signal from nanostructured electrodes with refined manufacturing processes can enhance the signal-to-noise ratio, thereby addressing the issues of resolution and sensitivity.

3 | CELLULAR ENVIRONMENT

This section outlines the sensing strategies for parameters in monitoring cell culture that contribute to cellular viability. It includes environmental detection such as DO, carbon dioxide, temperature and pH. As environmental elements, physical and chemical properties such as DO, carbon dioxide partial pressure, pH, and temperature exist.

These elements have been monitored since the beginning of cell culture technology. Thus, highly optimized and reliable sensors are currently utilized. However, the emergence of microelectronics and microfabrication-based cell culture platforms necessitates miniaturized and reliable sensing techniques for integration into the culture platform. Hence, the current trend is toward translational approaches that incorporate principles from traditional and complex sensing probe technologies for large-scale applications into new forms that are miniaturized, disposable, and non-invasive. While traditional instruments like chromatography, mass spectrometry, and spectrophotometry have been used for reliability, current technologies now see new adaptations in optical and electrochemical sensing principles. These strategies will help navigate changing cell culture systems and environmental factors, enabling further understanding of metabolic profiles and their impact. Details will be discussed in the following sections, and applications are listed in Table 2.

3.1 | Dissolved oxygen monitoring

DO in cell culture media is also a crucial factor. Undoubtedly, oxygen is the source of life's activities, and there are several ways to clarify the oxygen level in the media environment. Most animal cell cultures require a slightly low level of the atmospheric proportion of oxygen, and the same as the other environmental parameters, the required oxygen level will differ for every cell from the original niche.^[9,70] The bioreactor filter and control a gas mixture intake system. This system analyses gas modules that control nitrogen, oxygen, carbon dioxide, and intake gas mixtures with proper agitation methods to dissolve. Thus, static culture systems may require a specific gas monitoring system in the culture environment and incubator except for the bioreactor system.

3.1.1 | Optical sensing techniques

DO analysis with IR spectroscopy is now considered a gold-standard platform. One of the commercial analysis tools for DO sensing is PM-PST7 from the PreSens, which is a miniaturized probe-type IR spectrometer sensor (Figure 4a), because of the minimum invasive detection for microenvironment study regarded as a precaution measure for potential contamination issue.^[68] Thus, the automated cell culture platform has adapted optical fiber sensors with small measurement tips and analysis instruments on the top side of the culture ware to control the contact of fiber sensing probes.^[9] By utilizing minimally

TABLE 2 Sensors preparation for cellular environment monitoring.

No.	Targets	Sensing source	Culture format	Sensor performance	Sensing surface shape & material	Chip/sensing surface fabrication method	Ref.
1	Dissolved oxygen	IR spectrometer	Transparent culture vessel	Range: 0.0001–10 μ M, response time: N.A.	Independent device with the optical probe	- Commercial products (PM-PS7, PreSens, Germany)	[9, 68]
		Luminescent sensor	Digital microfluidic channel	Range: 0.5–8 μ M, response time: N.A.	Photoluminescent film (PtTFPP-Hyflon AD 60) on fluidic channel	- PtTFPP dye and Hyflon AD 60-film mixture - Fabrication of film was integrated into ITO glass	[20]
		Amperometry	Microculture chamber	Range: 0–200 μ M, response time: <1 s	Thin film platinum oxide electrode	- Lithography, Pt deposition on Ti adhesion promoting layer with the permeable 1,3-diaminobenzene membrane through physical vapor deposition (PVD)	[22]
2	Carbon dioxide level	IR sensor	Versatile	Limit of detection (LOD): 500 ppm, response time: <40 s	Near/Mid IR receiver with interferometric filter	- Commercial products	[10]
3	Temperature	Resistance sensor	Microelectrode array chip	Range: 24–38°C, response time: N.A.	Zigzag-shaped electrode (150 \times 150 μ m)	- Photolithography on an e-beam evaporated copper layer 275 nm thick, 500 nm Si_3N_4 layers with PECVD	[69]
			Micro cavity chamber	Range: 34–40°C, response time: real-time	Zigzag-shaped electrode (120 \times 150 μ m)	- Si_3N_4 insulated Pt thin film with self-assembled multilayer silane film for cell adhesion promotion	[24]
			Micro culture chamber	Range: 25–45°C, response time: real-time	Serpentine	- Poly (N-isopropylacrylamide) substrate with gold-graphene serpentine nanoribbon	[7]
4	pH	Luminescent sensor	Versatile	Range: 2–13, response time: N.A.	pH-responsive polymeric luminescent dye	- Luminescent optical receiver - Commercial products (SBI, PreSens)	[12]
		Amperometry	Culture flask	Range: 6.3–7.4, response time: <5 s	Iridium oxide	- Anodic iridium oxide film synthesis by cyclic voltammetry	[11]

Abbreviation: PECVD, plasma-enhanced chemical vapor deposition.

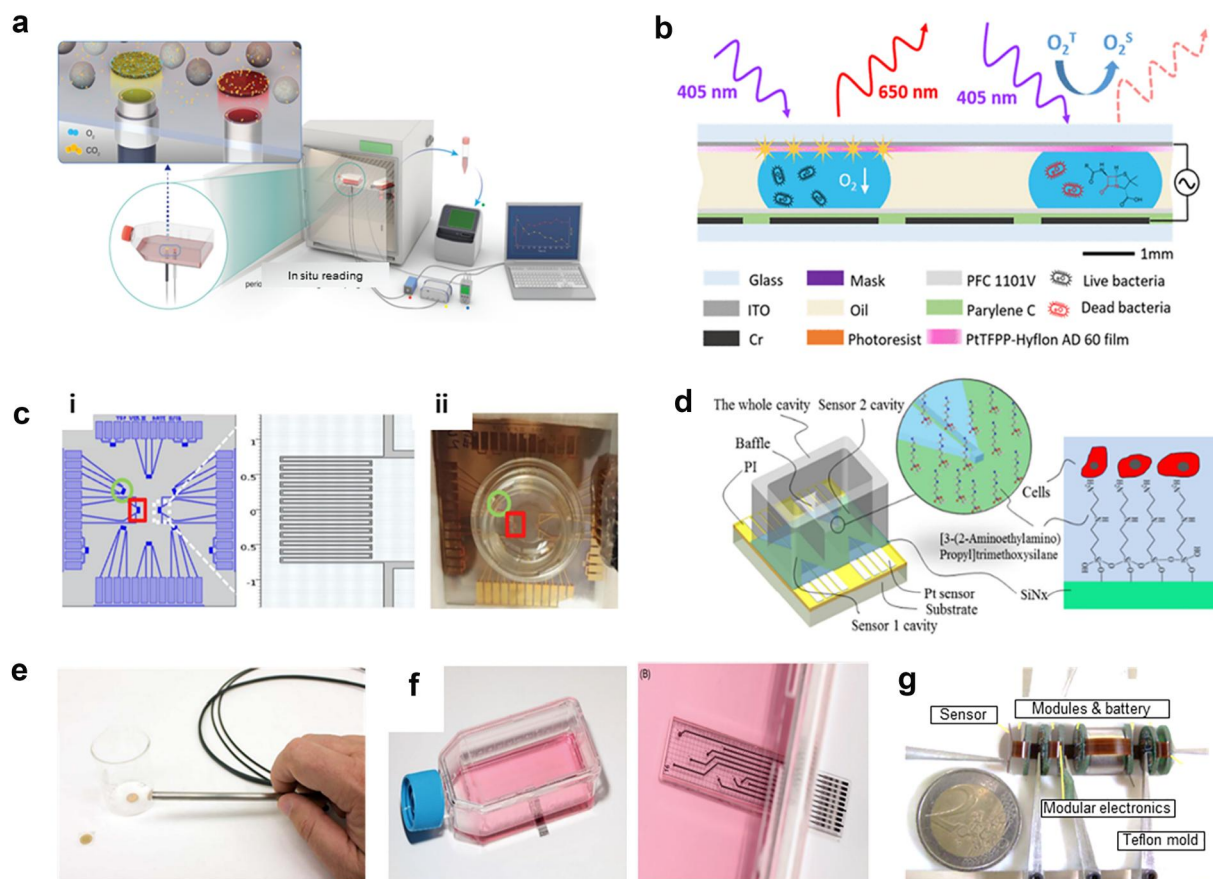


FIGURE 4 Application for monitoring cellular environment parameters, including dissolved oxygen, temperature, and pH. (a) Simplified schematic illustration exhibiting a macroscopic view of the commercialized luminescent DO sensor spots attached to the culture vessel. (b) Schematic side view of luminescent DO sensor in a digital microfluidic chip for *Escherichia coli* culture. (c) Temperature sensor design in cell culture microsystems. i) Designed sensor layout, ii) temperature sensor plate and cell culture chamber. (d) Temperature sensor comprised with Pt electrode layer for microcell culture chip. (e) Commercialized luminescent pH detection sensors comprise pH-sensitive dye and optical fiber spectroscopy. (f) pH sensing electrode embedded for sensing cell culture flask system. (g) Multisensor-embedded capsule type modular sensor for the PATsule project. Rights and permission (a) Reprinted with permission. Copyright 2022 from Springer Nature.^[68] Creative Commons CC-BY license. (b) Reprinted with permission. Copyright 2021 from American Chemical Society.^[20] (c) Reprinted with permission. Copyright 2018 from Elsevier.^[69] Creative Commons CC-BY-NC-ND license. (d) Reprinted with permission. Copyright 2023 from American Chemical Society.^[24] (e) and (g) Reprinted with permission. Copyright 2018 from Elsevier.^[3] (f) Reprinted with permission. Copyright 2018 from MDPI.^[11] Creative Commons CC-BY license. DO, dissolved oxygen.

invasive micro-profiling techniques, it is possible to measure the concentration of oxygen in a specific area along the z -direction by programming a specific step size. The z -directional operation allows for accurately determining spatial oxygen gradients, providing oxygen distribution within a given microenvironment. Optical, luminescence-based oxygen sensing relies on the effect of oxygen quenching the luminescence intensity. Quenching is measured using luminescent probes, which can determine oxygen concentrations or pressures through two distinct methods. The luminescence intensity and excited-state lifetime are both affected by oxygen, leading to accurate readings. Several indicators are sensitive to oxygen and have various applications.^[71] These indicator compounds can be categorized into two main

groups: (1) molecules based on ruthenium and (2) molecules based on metalloporphyrin. One of the examples, Qiu et al. utilized a film-type DO sensor on the digital microfluidic platform for the microbial assay system.^[20] The author's Digital microfluidic platform has the requirement to monitor DO depletion levels to identify the cell viability under the treatment of the antimicrobial susceptibility test. The utilized dye for the DO sensing was platinum (II)-5,10,15,20-tetrakis-(2,3,4,5,6-pentafluorophenyl)-porphyrin (PtTFPP), and it was mixed with Hyflon AD 60 polymer to form a transparent film. The luminescent profile was 405 nm UV excitation and 650 nm red luminescence (Figure 4b), and the sensor provided a luminescent signal under UV radiation on a digital microfluidics platform.

3.1.2 | Electrical sensor

Oxygen sensors in the electrochemical sensor can be conducted through the amperometric reduction of DO at the platinum electrode with a three-electrode system. Weltin et al. designed cell culture monitoring microsystems that include thin platinum oxide electrodes to detect DO.^[22] Ag/AgCl pseudo-reference electrodes were prepared by anodic oxidation in KCl solution. The sensor chip was fabricated on a glass wafer with silicon nitride, epoxy resin, and polyamide film with metal deposition processes of titanium/platinum to construct the electrode patterns. DO sensing was calibrated in air-tight boxes to materialize the air at 0% and 100%. The sensing performance of the DO range was found to be 0–200 μM . Despite the authors addressing a slight (ca. 10%) decrease in sensitivity during the first injection of culture medium, sensor performance exhibited no significant change in sensitivity. In the chemical or dissolved gas sensing field, there is still a trend toward miniaturizing traditional instruments or catalytic reactions derived from optical probes. Permanent utilization necessitates conventional methods, while disposable bioreactors or microfabricated chips can employ luminescent disposable probes. Nonetheless, it can be predicted that the original format will continue to be maintained for culture methods that require specific DO conditions.

3.2 | Carbon dioxide level monitoring

Carbon dioxide gas contributes to the pH buffering systems of the cell culture medium. Due to the dissolved carbon dioxide in the medium culture forms carbonic acid (H_2CO_3), dissociated bicarbonate ions (HCO_3^-), and hydrogen ions (H^+) from carbonic acid will establish pH balance with sodium bicarbonates in the medium during the cell cultivation process. Thus, in order to get optimized performance in cell culture, atmospheric partial pressure in the incubator chamber should be maintained at around 5%. Therefore, the gas sensor in the chamber is also crucial for environmental analysis in the cell culture process. CO_2 sensor for cell culture monitoring has two types. One is the thermal conductivity (TC) sensor that measures the difference between a reference resistor and a gas flow-encountering resistor. The TC sensor detects the atmospheric conditions changes from gas flow in the chamber and is capable of sensing CO_2 , temperature, and humidity. Comparing resistance from flow-encountered and sealed reference resistors capable of exhibiting CO_2 concentration changes. However, as mentioned before, the TC sensor has been responsive to temperature and humidity changes, and CO_2 detection performance is

easily affected. Therefore, despite the cost advantages of TC sensors, interference-driven reliability issues are critical for sophisticated control. One other type of CO_2 sensor is an infrared sensor. Utilizing the NIR absorbance property of CO_2 , an optical sensor that comprises the NIR source and its receiver pairing can deduce the atmospheric CO_2 concentration. Specifically, the metal probe type is typical and inside the probe, there is a gas-permeable chamber with a NIR emission source and reflected light receiver called the Fabry-Perot interferometer. The absorbance wavelength for CO_2 can be selected by negating the water IR absorbance range, which is around 2.5–10 μm range.^[10] From this nature, the incubator and bioreactor system have NIR detection probes for independent spatial insert with the gas controller.

3.3 | Temperature monitoring

During the culture process, the media temperature is easily considered a constant parameter since the temperature has been automatically controlled and maintained by an incubator or reactor-embedded thermostat controller. Currently, there are two types of digital temperature sensors. One is thermocouples based on the Seebeck effect from bimetallic joints, causing voltage production upon temperature difference. A thermocouple sensor has reliability in high-temperature conditions but is not frequently utilized in a modern incubator. Furthermore, the other type is a resistive temperature detector (RTD) system with a coiled wire resistor wrapped around the ceramic core. RTD has a highly accurate performance with repeatability and a long history of industrial application and is further toward the microelectromechanical systems application for micro-scale.

Among the cell assays on cell-on-chip, the work of 2018 Mäki et al. reported the fabrication of an indirect temperature-controlling microscale cell culture system.^[69] In the system, the temperature sensor plate was constructed on a silicon dioxide substrate, including a resistor, tracks, and contact pad, and the patterning was implemented using photolithography on an e-beam evaporated Cu layer (Figure 4c). After that, the track area for the sensing pads was electrically insulated with silicon dioxide and a Si_3N_4 layer with PECVD. Further, as a heating element, an indium tin oxide plate was integrated into a chip to control the temperature. These approaches aim to obtain the dynamic temperature variation in the target cell culture system. Similar to these fabrication steps, the cell assay and monitoring the microscale system utilizes an RTD-like temperature sensor on the thin film in a flexible manner. Zhang et al. chose platinum thin film temperature sensors with a silicon nitride insulation layer grafted

with self-assembled multilayer silane film for cell adhesion promotion (Figure 4d).^[24] Notably, the RTD sensing system for the microscale volume-controlled sensing chip results in immediate and accurate performance that only works with proper calibration. Kim et al. reported a multifunctional cell culture platform including a temperature sensor fabricated with graphene nanoribbons.^[7] Due to the delicate fabrication control for the ultrathin film thermal responsive polymer, poly(N-isopropyl acrylamide) was chosen to fabricate gold-graphene serpentine nanoribbon to obtain better electrical and mechanical properties.

3.4 | pH monitoring

Nevertheless, the cell culture medium has its buffering system assisted with bicarbonate ions. PH level is gradually changed due to the catalytic or metabolic activity of cells. In order to artificially reproduce the original environments for the target cell, pH conditions should be maintained with a thorough analysis. Specifically, depending on where the cells were originally isolated from the organ or tissue, cells require specific pH levels to achieve successful proliferation and expansion.^[8] Thus, pH monitoring is also a critical environmental monitoring element in culture. The most used pH sensor is the combination electrode, which contains both Ag/AgCl and glass membrane electrodes within a single unit.^[72,73] This electrode for measuring pH levels is dependable and user-friendly, with a quick response time, and relatively low cost. However, it does have some drawbacks, such as being quite large, fragile, and prone to interference from electrical signals.

Additionally, it cannot be sterilized and is susceptible to biofouling and protein contamination, necessitating regular maintenance.^[74] Furthermore, there are optical sensing systems based on the pH-responsive luminescent dye molecules that the spectrometer can detect. There is a notable contrast between optical and electrochemical sensors regarding measuring techniques. Specifically, electrochemical sensors monitor the activity of H_3O^+ ions directly. In contrast, optical sensors that detect the concentration of the pH indicator^[69] as commercial products with optical spectroscopic modules and adhesive fluorescent indicator patches are now becoming usual cell biology research applications, especially in disposable bioreactors.

3.4.1 | Optical sensing techniques

NIR luminescent pH optical sensor for cell monitoring exhibited several advantages such as minimum-invasive

detection, continuous reading, maintenance-free, feasible for a variety of types of culture ware, and easily disposable. Commercially available luminescent and fluorescent sensors comprised the dye indicator matrix and fiber optics connected to the spectrometer. By immobilizing a pH-sensitive luminophore in a polymer matrix, spectrum, intensity, or luminescence lifetime changes can occur through reversible protonation or deprotonation.^[74] There are commercial products following this format, like DOTS (SBI GmbH) and SP-HP5-SA (PreSens) (Figure 4e).

3.4.2 | Electrical sensor

The pH indicator, phenol red, is already a basal additive in a commercial medium. Phenol red serves as pH, indicating gradual color development when purple, red, and yellow are represented as alkaline, neutral, and acidic, respectively.^[75,76] However, requirements in monitoring in a sophisticated manner with a quantitative results, pH measurement is implemented with potentiometric sensors and optical sensors.^[77] Traditionally, there is a glass membrane-associated Ag/AgCl electrode potentiometric sensor. Due to the glass membrane acting as an interface between sample liquid and membrane-sealed internal buffer, the potential difference can represent quantitative pH information. Examples of other specific materials that are functionalized on electrodes are conductive polymers and metal/metal oxides, for example, iridium, palladium, ruthenium, and tungsten with their oxides. The transition reaction of these metal oxides, accompanied by the production of hydrogen ions and several electrons, can indicate a potentiometric or amperometric relationship with pH level.^[78] Thus, probe or 2d film-type pH sensors are actively utilized for bioreactor and microscale cell culture systems (Figure 4e). Following this, Kienninger et al.,^[79] developed cell culture flask embedded multiparametric sensor capable of detecting DO and pH, which electrodes were obtained by electrodeposition of iridium oxide on chip-level on silicon nitride coated glass substrate. The conceptual goal of this platform is to provide electrical monitoring data from single-use cell culture flasks with an embedded sensor that has an external electrical interconnect. The model acidification during cell culture was selected as a T-47D cell to identify the gradual pH decrement (Figure 4f). While the multiplexing sensor module for 2D appearance expands the variety of shapes, sizes, and thickness in cell culture monitoring applications, a capsule-type wireless multiplex sensing device was proposed by O'mara et al.^[3] The device, PATsule, was initially designed to apply as a process analytical technology in bioreactors assisted by the impeller agitation.

Temperature, pH, and DO are target parameters for devices and aimed to provide real-time monitoring data to external system control and data analysis computer. The structural features are represented in Figure 4g, where the tight integration of the battery, modular circuitry, antenna, and sensor is packed in the capsule shell. Despite no operating results of sensor performances in action, new approaches for the co-existing sensor module in the cell culture environment are hoped to establish a new platform for cell monitoring technology.

4 | CELLULAR METABOLITE

Metabolites from cell culture are crucial evidence for understanding cellular biology. During cell culture, metabolites are consumed and produced, including nutrients, waste, byproducts, and other cell signaling molecules. Many metabolites interpret specific mechanisms for cellular activity, but in this section, only universal target molecules such as nutrients and waste will be discussed. Nutrient quantification for cell consumption is a critical parameter for understanding the cell growth rate, phenotype, and metabolomics.^[1,13,80–83] Thus, the remaining nutrient level quantification in cell culture media in and large scaled cultures is implemented. In the case of the bioreactor that has a larger volume scale than the lab experimental culture condition, bulky analytical instruments for chemical analysis tools such as LC-MS, HPLC, and NIR spectroscopy were frequently applied through the flow injection-based external sampling devices.^[14,84–87] Over the decades, online and inline sampling-based nutrient assessment for cell culture ecosystems has changed. For example, the micro-scale cell culture system has limited options to adapt the small volume analysis through the traditional analytical instruments, so oxidase redox-based electrochemical, luminescent, and colorimetric biosensors were utilized. However, the advances in material engineering and packaging technology brought real-time monitoring, miniaturization of electrode fabrication, enhanced enzyme encapsulation, and reliable sensor format to the cell culture monitoring system. These approaches can prevent the potential contamination of the batch process and minimize the sampling volume. However, in the laboratory's small working volume culture, several cell media assay methods have been proposed to determine the exact quantity of nutrients in the medium and deduce the cell consumption rate for detailed identification of cell functionality. In the case of cell-on-chips, an electrochemical sensor quantifies the glucose using the enzymatic or catalytic reaction on the chip. It is available for sub-milliliter volume-scaled conditions.^[88] Enzymatic

assays are usually associated with redox mediators such as hydrogen peroxide and other electron transfers.^[89] Due to the redox reaction on the enzyme, electrodes on the in situ, in vitro cell chip can conduct potentiometric analysis.^[90] On the other hand, the optical biosensing method is accompanied by colorimetric, turbidimetric, absorbent, and luminescent reactions to quantify the light intensity to the optical receivers.^[91] For the colorimetric sensor, colorimetric dyes are chemically modified by the redox cycle with enzymes and are increased in accordance with the target molecules' catalytic reactions. Thus, platforms like disposable fluidic and paper-based microfluidic sensors can utilize colorimetric or absorbance changes.^[92–94] However, these approaches were not frequently used in culture monitoring due to the need for more sensitivity and reliability. In addition, luminescent and fluorescent dyes can be employed as signal indicators in the case of the luminescent response. As the oxidase-based enzymatic reaction commonly produces hydrogen peroxide, fluorescent dyes can generate light signals or modify chemical structures.^[95] Representative optical and electrical sensing approaches regarding molecular metabolites are discussed in the following sections, and details are listed in Table 3.

4.1 | Nutrient sensing

4.1.1 | Optical sensing techniques

Probe-based instruments can provide real-time information without adversely affecting the microenvironment. However, these assays often need more specificity and provide quantitative information. As a result, efforts have been made to exploit the selectivity of chromatographic methods for small-scale cell culture and bioprocess to enable online or inline monitoring. Based on the fact that spectroscopic analysis is the fastest, most reliable method to detect molecular profiles. Thus, it has become a gold standard method in nutrients, metabolites, and amino acids. The most frequently used instruments are liquid chromatography-mass spectroscopy (LC-MS), which could help form the basis of omics technology. However, due to the bulkiness of the device, sample preparation is conducted by bypassing flow from the reactor system, which could not immediately reflect the exact condition of the microenvironment. Therefore, owing to overcome the sampling limitation, NIR or Raman spectroscopic probe-based online monitoring has become an alternative method in the bioprocessing and manufacturing field.^[105,106] Despite the bulkiness being resolved, the miniaturization of probe-type sensing devices is standard. In a current study, Domján et al. reported that Raman

TABLE 3 Sensor preparation methods for cellular metabolites and contaminants.

No.	Targets	Sensing source	Culture format	Sensor performance	Sensing surface shape & material	Chip/sensing surface preparation method with the principle	Ref.
1	Glucose (Glu), lactate (lac)	Raman spectroscopy	Culture flask	<Glu/Lac> LOD: 0.986 mM, 0.998 mM, response time: real-time	Raman probe (Kaiser optical systems)	- Chemometric model development-based glucose and lactate sensing	[5]
		Fluorescence-based biosensor	Culture flask	<Glu> LOD: 0.45 mM, range: 0–20 mM, response time: <10 min	GOx layer-integrated oxygen sensor (luminescent optical fiber)	- Adhesion of polymeric enzyme layer on commercial oxygen sensor (PreSens)	[6, 96]
		Fluorescence-based biosensor	Petri dish	<Glu> LOD: 11.1 mM, response time: N.A.	Glucose-responsive FRET dyes in hydrogel	- 3d printable ink (FRET dye-encapsulated hydrogel) on culture ware	[4]
		Amperometry	3d microtissue spheroid culture network	<Glu/Lac> LOD: 1.84, 7.07 mM, response time: N.A.	Enzyme membrane (GOx, albumin, glutaraldehyde)	- Enzyme membrane applied microfabricated Pt electrode on glass	[97, 98]
2	Reactive oxygen species	Amperometry	N.A.	LOD: 10 μ M, response time: N.A.	MnO ₂ -electrochemically reduced graphene oxide (ERGO) electrode	- Electrochemical deposition of MnO ₂ nanowire on a reduced graphene sheet	[99]
3	Protein biomarker	Electrochemical impedance spectroscopy	Organ-on-chip	<CK-MB> LOD: 0.0024 nM, response time: N.A.	Streptavidin-functionalized SAM on Au electrode	- Serial deposition of PtNP, PEDOT: PSS on 3d PDMS scaffold	[101]
5	Bacteria and bacterial nucleic acid	In situ microscopy	Bioreactor	Detection starts from 10 ⁵ cell/mL, response time: real-time	Sterilizable microscope	- Size-dependent microorganism detection algorithm in image processing	[38]
		Fluorescence-based biosensor	Versatile (offline only)	Detection range 10 ¹ –10 ⁷ cells/mL, response time: 30 min	Luminescent spectrophotometer	- Primer inserted PAM (red fluorescent) - Selectively amplified products can emit the red fluorescence	[102]
6	Endotoxin (LPS)	Differential pulse voltammetry	Culture flask (offline only)	Detected conc. 10 ⁵ cell/mL, response time: 1 h	Luminescent spectrometer	- Fluorescent enzyme activity degradation-based bacteria detection	[103]
		Differential pulse voltammetry	Culture flask (offline only)	LOD: 1.8 ng/mL, response time: 1 h	Capture molecule on a conductive sensing surface	- Affinity capture peptide, polymyxin B, immobilized on nanochannel, electron mediator-based DPV	[104]

Abbreviations: DPV, differential pulse voltammetry; LOD, limit of detection; LPS, lipopolysaccharide; PEDOT: PSS, poly(3,4-ethylenedioxythiophene) polystyrene sulfonate.

spectroscopy utilized a glucose monitoring system for automatic nutrient feeding in Chinese hamster ovary (CHO) cells.^[5] As a detection strategy, the chemometric model-based Raman spectrum of glucose, lactate, and amino acids was obtained from a culture medium. Along with the profiling of the calibration of various glucose concentrations of the medium, quantification results were found to be eligible for label-free detection. The root mean square error of prediction for glucose and lactate was 1.64 and 4.51 mM, respectively. It is worth noting that Raman spectroscopy coupled with chemometrics has a limitation—the calibration dataset must be trained specifically for the system in question. This study used Raman spectroscopy for real-time culture monitoring and nutrient levels to predict nutrient consumption.

Fluorescent dye-associated glucose monitoring techniques are developed to ensure sensitivity and reliability. Among the various applications from fluorescent signaling methods to glucose biosensors, Krstic et al. reported a fluorescence resonance energy transfer-based glucose sensor that tracks glucose-level changes in human embryonic kidney (HEK) cell culture.^[4] Notably, this study's fluorescent dyes for FRET phenomena were encapsulated in polyvinyl alcohol (PVA) hydrogel bead and implemented over 10 days of HEK cell monitoring glucose consumption. FRET glucose sensors were prepared as sensing ink that was printable on the petri dish with UV curing (Figure 5a). Despite the potential instability from UV curing and reliability issues for long-term monitoring, the authors deduced the calibration curve between 0.2 and 2.0 g/L. Other approaches to luminescent glucose biosensors were reported by Tric et al.^[6] and Lederle et al.^[96] The authors integrated a commercial optical fiber oxygen sensor, discussed above in Section 3.1.1 (PreSens), with a glucose oxidase layer to monitor glucose levels inside the cell culture flask (Figure 5b). While oxygen generates from the redox reaction of glucose oxidase, oxygen-responsive fluorescent dyes on optical fiber sensors can capture the emission signals that are in accordance with the glucose oxidase kinetics. Resultingly, the integrated sensor acquired oxygen level interpreted as glucose quantity.

4.1.2 | Electrical sensing

Developing an electrochemical glucose sensor is a significant history in the biosensor. Well-known strategies involving enzymes and catalysts have myriad variations and applications. Therefore, the examples do not address newly discovered principles but exhibit new platform approaches in the last few years. One of the most active approaches to advance on electrochemical glucose sensors

is encapsulated enzyme or catalytic nanostructure-integration on composite electrodes.^[97] Following this trend, commercially available glucose sensors for bioreactor systems also employ enzymatic membranes on a thin metal electrode, such as Biosensor IV4, for Glucose measurement (Innovative sensor technology, Ebnat-Kappel). 2d thin film type sensor has the advantage of minimum contact on the culture medium. From this trend, Misun et al. integrated an enzyme hydrogel-based glucose sensor into the 3d microtissue spheroid hanging-drop network device to monitor nutrient consumption in real time^[98] (Figure 5c). Microfabrication on the glass substrate showed a cylindrical recess and ring-shaped electrode on the enzyme hydrogel structure. A layer for sensing glucose was created on the substrate by depositing a Pt electrode and applying glucose oxidase, bovine serum albumin, and glutaraldehyde crosslinker. The authors demonstrated the real-time glucose-level tracking performance that simultaneously monitors incremental nutrient levels and decremental waste levels during the medium culture renewal step. This sensing platform allows about 24 h of performances that are demonstrated to provide detailed nutrient data in the formation of 3d spheroid formation. Panjan et al. proposed a cost-effective and straightforward method for assembling components on a microfabrication-based chip platform. Their approach facilitates micro-bioprocess control and enables online glucose sensing in a 3d-printed microbioreactor.^[107] The integration chip for the biosensor has a flow channel where the sample flows toward a disposable glucose sensor (Figure 5d). The cultivation method selected *Saccharomyces cerevisiae* with an 8-h fermentation period. The glucose sensor uses a polymeric enzyme composite on a screen-printed carbon electrode (ItalSens). The monitoring device construction is achieved through the purchase and assembly of most components, excluding the housing. The sterilization process and the diffusion-limiting membrane in the inline sampling structure ensure minimal contamination risk in the incubator. The assembly process is simple and easy to follow. Nutrition quantification from the cell culture medium is essential for predicting the cell culture yield. In this context, accurate and reliable methods were suggested for probe and sensor types using enzymatic or catalytic reactions from catalytic components. The electrical or electrochemical analysis of nutrition might be the oldest and widest application in biosensing history. From the first oxidase and related enzymatic reaction, nutrition sensing has been implemented in all biological areas, including biological specimens (e.g., blood, urine, sweat, saliva, and tear), food, and environmental sensing. However, specific requirements for cell monitoring might be non-invasive without residues. Because conventional large-scale devices are available for continuous, accurate, fast, and

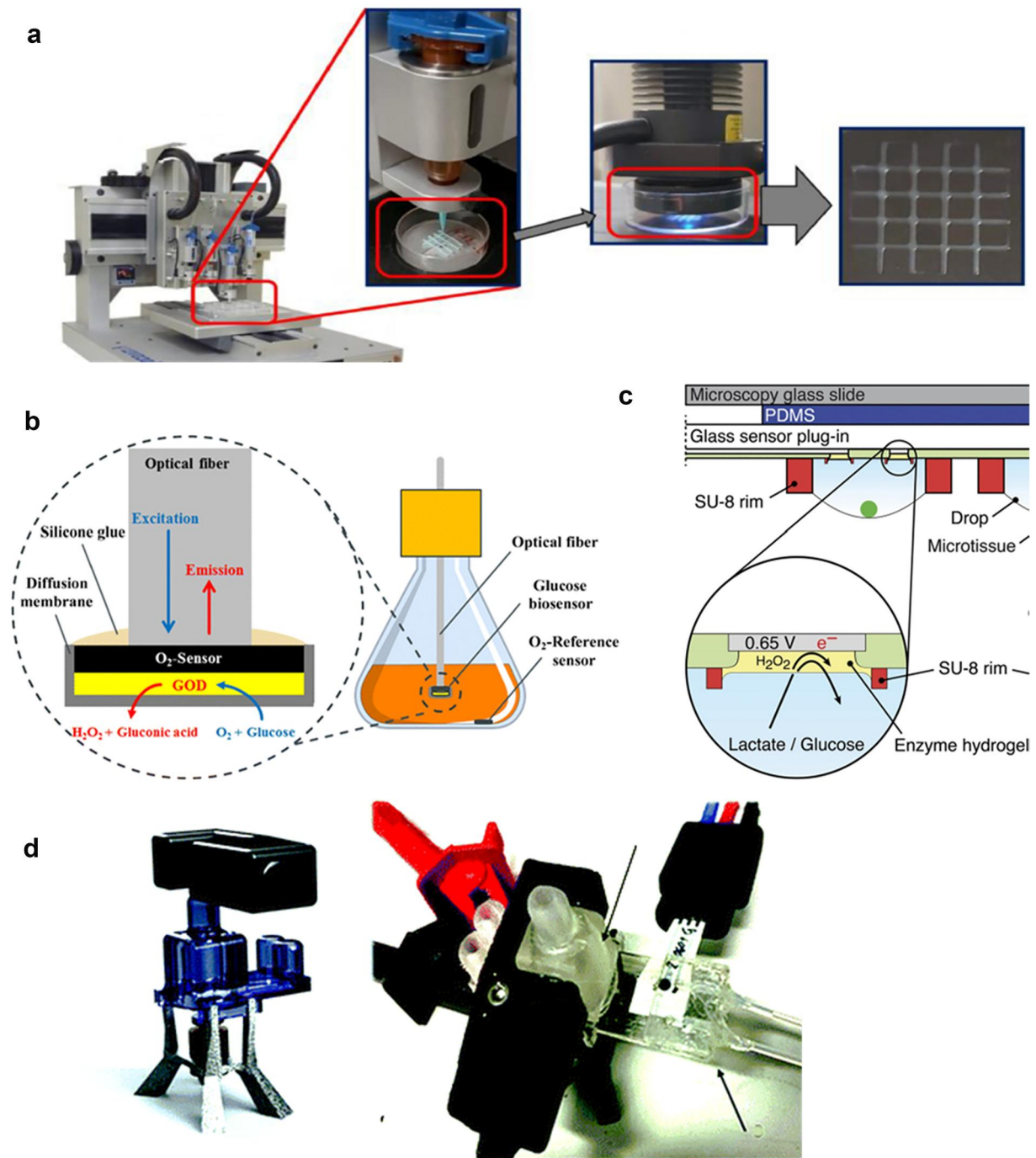


FIGURE 5 Application for monitoring cellular metabolite, including nutrients and byproducts. (a) Glucose sensing ink preparation using a 3d bioprinter (first on the left). The printing procedure comprises sensor ink printing onto a plasma-treated petri dish (second on the left), and the subsequent UV curing process (third on the left). Resulting magnified image for the printed structure (fourth on the left). (b) Schematic view of glucose biosensor using enzyme layer integrated with optical fiber oxygen sensor. Catalytic reaction from the glucose oxidase layer induces changing of oxygen level from the diffusion membrane and is reflected on the oxygen sensor. (c) Configuration of the glucose and lactate sensor chip with a cross-sectional view in the microfluidic hanging-drop network device. (d) Schematic of glucose sensor-integrated reactor with SLA 3d printed micro-bioreactor platform. Rights and permission (a) Reprinted with permission. Copyright 2023 from Elsevier.^[4] (b) Reprinted with permission. Copyright 2017 from Springer Nature.^[6] (c) Reprinted with permission. Copyright 2016 from Springer Nature.^[98] Creative Commons CC-BY license. (d) Reprinted with permission. Copyright 2018 from Royal Society of Chemistry.^[107]

multitarget monitoring, microfluidic or chip format monitoring devices must be achieved in small shapes along with continuous detection.

4.2 | Other metabolite and biomarkers

There are myriad metabolites except for nutrients and waste in terms of energy and signal pathways in cellular biology. However, in this section, we will describe cell-secreted chemicals and biomarkers that come from stress induction and cell functionality, respectively. In the cell response monitoring from the stimuli, immune cells such as macrophages have an intrinsic nature of releasing hydrogen peroxide to peripheral environments. Dong et al. developed MnO₂ nanowires on graphene paper electrodes to track the hydrogen peroxide secretion from live macrophages.^[99] The sensor fabrication was MnO₂ nanowire graphene composite film with ultrathin (ca. 5 μm) and flexible properties. The 10 μM N-formyl-methionyl-leucyl-phenylalanine (fMLP) stimulation detected macrophage-secreted hydrogen peroxide within 2 min. The sensor response was linear to H₂O₂ concentrations in the range from 0.1 to 45.4 mM, with a detection limit of 10 μM. One of the indicators of cancer cells responding to drugs is the generation of reactive oxygen species (ROS). Zhang et al. reported 3d culture to the scaffold with a conductive polymer coating capable of drug screening possibilities with amperometry sensing of ROS.^[100] The calculated ROS sensitivity was 460 nA/μM, while the detection limit was 76 nM. To enable sensitivity, the authors designed and fabricated the 3d scaffold comprised PDMS frame coating with a conductive material layer of PEDOT and platinum nanoparticles (PtNPs). PEDOT: PSS was treated on sodium carboxymethyl cellulose reacted PDMS surface. After then, the PtNPs electrodeposition finalized the scaffold. Due to the conductive nature of coatings on scaffold surfaces, model cells (MCF, HeLa, HUVEC) can adhere and be 3d-cultured. Since the materials for the scaffold have elastomeric properties, advances in flexible and stretchable properties are expected. Monitoring the quantified information of biomarkers secreted from target cancer cells can be utilized for drug screening. Zhang et al. demonstrated the potential approaches for organ-on-chips to be conducted in situ monitoring of organoid-secreted biomarkers (albumin, glutathione S-transferase α [GST-α], and creatine kinase-MB(CK-MB)).^[101] This was done through the immunoaffinity reaction-based impedimetric analysis that can detect the quantified data production from the multi-channeled organ-on-chip platform. Sensor modules were fabricated on a PMMA substrate deposited

with two Au and one Ag surface as working counter and reference electrodes, respectively. After that, the Au electrodes were functionalized by 11-mercapto undecanoic acid through a self-assembled monolayer to capture the protein capture molecular set. Redox reaction of ferricyanide [Fe (CN)₆]⁴⁻ was employed to facilitate electron transfer toward the electrode. From this approach, linear ranges and limit of detection are calculated as albumin (0.1–100 ng mL⁻¹, 0.09 ng mL⁻¹), GST-α (0.1–100 ng mL⁻¹, 0.09 ng mL⁻¹), and CK-MB (0.01–10 ng mL⁻¹, 0.0024 ng mL⁻¹), respectively.

5 | CONTAMINANT

Bacteria, viruses, and fungi are the critical infectious microorganisms that lead to the failure of the cell culture. It threatens the original cell's proliferation, metabolic pathway, cell-to-cell signaling, and damaging physiological properties. Consequently, this can lead to the generation of spurious data and pose potential risks to biological safety.^[108,109] Therefore, most cell culture medium contains anti-bacterial agents to prevent infection. Except for the expansion of target bacteria or viruses, typical animal cell culture conducted the bacteria culture or inoculation to verify the infection or molecular diagnostic kit, including polymerase chain reaction (PCR), is registered as a mandated guideline for quality assessment. In the case of the pharmaceutical biomanufacturing process includes several contaminants detection. Adventitious agents inadvertently infiltrate biological systems and comprise a variety of life forms. Such examples are bacteria, fungi, viruses, and *mycoplasma*, which pose considerable challenges to biopharmaceutical manufacturing. For example, mycoplasmas are 0.1–0.3 μm sized bacteria without cell walls capable of penetrating the typical 0.1–0.22 μm sterilized filters used in research and biomanufacturing.^[110] Previously, an incidence rate was reported for *mycoplasma* incidence on cell banks, which showed the significance of the potential threat to the research and process field.^[108,111] Some bacteria release toxic chemicals on the outer membrane of Gram-negative bacteria, a highly toxic chemical called endotoxin exists as a potential contaminant of many bioprocesses. This chemical is released during different phases of bacterial growth, and even in small amounts, can cause various health issues such as septic shock, inflammation, fever, and organ dysfunction.^[112,113] Thus, this section will focus on two distinctive adventitious agents, such as *mycoplasma* and endotoxin. Current contaminants detection methodology can be divided into biological detection and biosensing techniques for

classification. Biological detection techniques include rabbit pyrogen test, Limulus amoebocyte lysate (LAL) assay,^[114] and bovine whole blood assay, which use natural methods of endotoxin detection and are still in use today.^[115] Biosensor techniques rely more on recent scientific achievements rather than traditional biological processes. These techniques represent the up-and-coming detection methods along with the advances in target sensing strategies. Various techniques can be categorized into three types: electrochemical, optical, and mass spectrum based. There are enormous kinds of infection candidates in culture environments. However, this section will describe representative assays or monitoring methods for *mycoplasma* and endotoxins, which are discussed in the following sections and listed in Table 3.

5.1 | Optical sensor

Unlike most, contamination assessments are conducted as offline sampling and significant laboratory procedures to achieve the result, in situ microscopic online detection of contamination methods has been desired.^[39] One of the attempts to meet this requirement is reported by Gustavsson et al., which can be described as an in situ microscopic determination of contaminant cells using image processing algorithms.^[38] Figure 6a depicts the in situ microscope device for the online connection to the bioreactor. The authors successfully distinguished yeasts in mammalian cell culture. By using time-lapse imaging from the initial stage of the bioprocess, early detection of contaminant cell presence can be expected.

Despite the ideal detection method for bacterial contamination should be simple to perform, sensitive, specific, rapid, inexpensive, and can be used to routinely test various cell cultures simultaneously, conventional microbial sample culture, inoculation, and PCR-based detection assays were taking long assay time with the sophisticated and complicated procedure. However, new approaches have been developed to target contaminants through optical biosensing techniques. In the molecular diagnostic field, emerging research activity regarding CRISPR-based technology, which is a highly accurate and feasible nucleotide editing technique, has been utilized for various pathogenic monitoring and diagnostics.^[116–118] For example, Wang et al. suggested CRISPR/Cas12a-based *mycoplasma* detection method.^[102] The authors focus on negating the potential aerosol contamination process during the assay procedure to ensure the accuracy of the result. Therefore, the recombinase polymerase amplification and Cas12a-mediated cleavage reactions are integrated into a single reaction system. The optimization achieved a 10 aM DNA concentration limit of

detection from *mycoplasma* (Figure 6b). Mycoplasma-infected samples are determined by developed sensing techniques within 30 min as an applicability test. Another example, except for the nucleic acid amplification test (NAAT) approach, is enzymatic activity profiling-based bacterial infection monitoring. Degeling et al. proposed *mycoplasma* biosensing techniques based on luminescence biosensors by monitoring Gasussia luciferase (Gluc) activity.^[103] Design and evaluation of the *mycoplasma* test begin with the expression of Gluc in various types of animal cells and subsequent infection with *mycoplasma*. After that, the authors verified the correlation between *mycoplasma* infection and expressed Gluc degradation.

Moreover, owing to achieving a cell-free assay, a test sample was prepared from mycoplasma-infected HeLa cell medium. Then, recombinant Gluc was applied to quantify the luminescent degradation on a commercial luminometer. Optimized test time was 24 h from the start of the assessment with a detection limit of 1600-fold diluted medium samples. Conventionally, optical sensing strategies for contaminants heavily relied on the NAAT-based novel nucleic acid amplification approaches using a fluorescent spectroscope to ensure accuracy and reliability. However, in the transition era of the manual to an automated process, there are substantial requirements for altering the offline assay to the continuous sensing method.

5.2 | Electrical sensor

Most electrochemical sensing strategies for infectious pathogens and endotoxins rely on affinity reactions that mediate the capturing molecules on the sensing surface. A capturing molecule that has an affinity towards the bacteria and endotoxin molecules can be an antibody, aptamer, or peptide sequence. However, capturing bacteria in the early stage of cell culture is quite challenging due to their low abundance in the large working volume in culture. Therefore, the NAAT-based screening method is more effective than the direct capture of bacteria. However, in the case of endotoxin, there are chances to identify it in the early stage or at the beginning of the culture process due to its sources being predictable. Endotoxin presence is a reliable indicator of bacterial contamination in cell culture ecosystems, including water, sera, medium additives, and glass/plastic ware. Endotoxin may occur in altering cell growth, differentiation, contractility, and protein production. Among various endotoxins, lipopolysaccharide (LPS) is the most common molecule from bacteria and is found in cell culture systems.^[72,104,119–121] Some affinity sensing-based

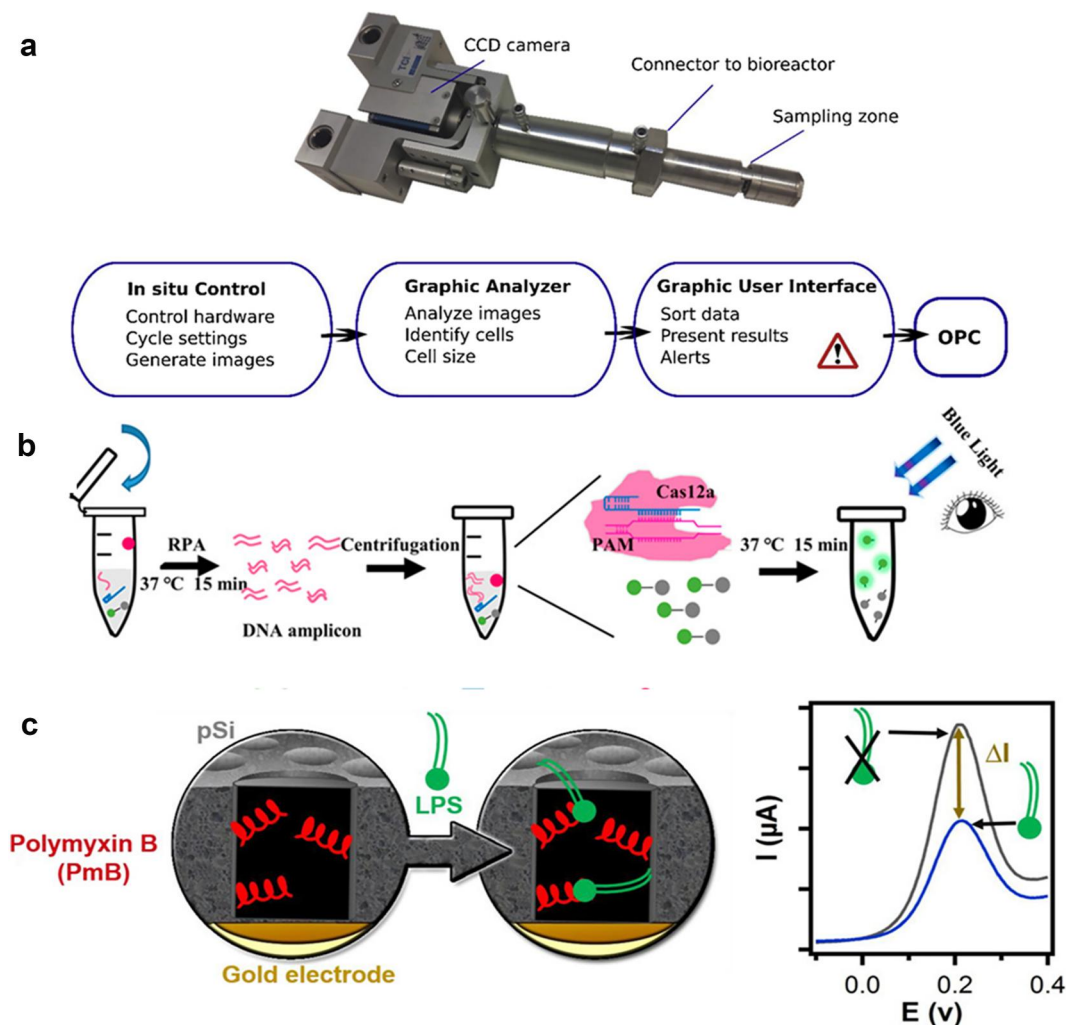


FIGURE 6 Application for detecting contaminants, including bacteria infection and endotoxin. (a) In situ microscopic approaches with image processing tool for distinguishing bacteria in culture medium. The constructed microscopic device can be directly inserted into the bioreactor (above). Workflow of image processing for bacteria identification in cell culture medium (below). (b) Schematic of Cas12aVDet for visual nucleic acid detection of *mycoplasma*. After 15 min of RPA reaction, the Cas12a enzyme was centrifuged into the reaction mix for target cleavage for an additional 15 min. The fluorescent signals can be observed by the naked eye under blue light. (c) Schematic of label-free lipopolysaccharide detection using a silicon nanochannel sensor with differential pulse voltammetry. Rights and permission (a) Reprinted with permission. Copyright 2023 from Elsevier.^[38] (b) Reprinted with permission. Copyright 2019 from American Chemical Society.^[102] (c) Reprinted with permission. Copyright 2019 from American Chemical Society.^[104]

electrochemical biosensing strategies identify the endotoxin in the sample using EIS and voltammetry. To determine the potential LPS presence among cell culture components, the authors conducted water tests as an example for quality assessment before cell culture. Reta et al. reported a label-free LPS detection from water supplies using a silicon nanochannel sensor.^[104] The LPS detection process involves measuring the alterations in the diffusion of the redox probe which is included in the solution. Diffusion yield alteration occurs due to the binding of LPS to the immobilized polymyxin B (PmB), an antimicrobial peptide with a solid affinity to LPS, and the subsequent channel obstruction. Differential pulse

voltammetry was utilized to measure the diffusive nature of the redox probe during sensing (Figure 6c) with the capability to detect LPS molecules in buffer and gram-negative bacteria in culture medium at concentrations as low as 1.8 ng/mL and 1 cfu/mL, respectively. Early detection has proven to be an effective warning system for potential contamination events. Current electrical sensing strategies for contaminants face a significant hurdle when attempting a nucleic acid detection. The main challenge lies in the low abundance of the target gene within a sample, making reliable detection without amplification and enrichment difficult. Consequently, obtaining a precise quantitative or qualitative analysis of

target molecules and genes from untreated samples remains unreliable. Although various methods employing chemicals, proteins, and polynucleotides with aptamers and other capturing molecules on the sensing surface have been explored, there is a need for groundbreaking technologies to enable continuous sensing and achieve real-time detection.

6 | PERSPECTIVE

New techniques for cell culture monitoring have been developed using optical and electrical biosensors, different from traditional methods that use tabletop analytical instruments such as ultraviolet-visible/infrared spectroscopy, mass spectrometry, and gas/liquid chromatography. Despite their excellent sensitivity, accuracy, and specificity, these tools are limited in continuous monitoring, portability, and compatibility with bioreactors. In order to address these limitations, researchers have developed various biosensors with distinctive advantages. They comprise the bioreceptor, reacting with specific molecules or detecting physical phenomena, and the signal transducing part, transducing optical or electrical signals to inform the quantitative and qualitative information from the specimen. They can be fabricated in smaller shapes than benchtop devices and continuously processed from single to multiple targets on the desired location within the cell culture platform. Especially in the fabrication aspect, technological improvements are assisted by microfabrication, novel optical and conductive materials, sensitive signaling probes, and microfluidic chip packaging, enabling tailor-made applications from the conventional plastic cultureware to the disposable bioreactor. Biosensing devices offer numerous advantages due to their flexibility in creating micro/nanoscale sensors that can replicate cellular microenvironments with acceptable sensitivity, accuracy, compatibility, and cost-effectiveness. However, several technical challenges need to be considered for implementing next-generation miniaturized culture platforms and multi-target monitoring devices for cell culture.

Optical biosensors are good candidates to obtain information in terms of sensitivity due to their innate high signal-to-noise ratio property. However, challenges should be addressed to ensure specific properties of long-term stability. In the case of the disposable luminescent sensor for pH monitoring, the patch-type luminescent dye becomes unreliable within a month with gradual luminescent degradation driven by oxidative quenching effect, which is only applicable to time-limited fed-batch culture but continuous culture system. This specific property is representative of challenges across

luminescent or fluorescent dyes for the monitoring cell. To prevent the quenching effect caused by oxidation or water molecules during a cell culture experiment lasting more than a month, modifying the structure of luminescent or fluorescent dyes should be necessary for long-term stability. Optical sensors are commonly required to equip the optical fiber and signal transducing devices, such as a benchtop spectrophotometer. However, changing its form factor would significantly assist the signal transducing device to be miniaturized and wireless on the bioreactor or culture platform. Prior work has yet to demonstrate this. Creating a lightweight, wireless optical biosensor with a transducing device for reactors will not encompass replicating conventional modular spectrophotometers. As a critical optical component, a highly integrated device consisting of a light-emitting diode, optical filters, and photodiode is required. Further, the soft electronics emphasized optical probe may facilitate the ease of the interconnection to the transducer. Similar miniaturization approaches for optical transducers have been suggested on the handheld scale using smartphones and optical components; still, recent work has yet to be conducted and reach the level of commercial integration for cell monitoring devices. The industrial and research fields will guide to this end, but additional considerations for optical pathway, filtration, shielding yield for emitting light, and noise-controlling post-processing will also be addressed. Consequently, it will be overcome by following the trends in the integration of optical components and will be expected to realize the concept of the miniaturized optical transducer for continuous optical biosensing applications.

The microfabrication patterning and packaging technologies empower electrical and electrochemical sensors on cell culture chips. Nano-microfabrication patterning enables the control of the surface area-to-volume ratio, enhancing the active surface area and resulting in high sensitivity on the sensing surface. Especially, the catalytic properties of metallic derivatives (e.g., PtNP, platinum oxide derivatives, iridium oxides) and conductive polymeric composites are broadly employed in microscale electrode fabrication on the cell culture platform. As this review has shown, the current accuracy and sensitivity for metabolite sensing performance in a short period are acceptable. However, ensuring reproducibility and stability for long-term sensor operation remains a future task—specifically, the lack of chemical stability and biological adsorption on the sensing electrode surface. The novel catalytic electrode with 3D nanostructures is currently the most common approach for electrode fabrication in the microscale cell culture method. However, there is a potential risk that the continuous charging on the electrode accelerates the corrosion of the metallic

substrate, leading to signal registration failure. Thus, replacing the catalytic substrate with novel material or modifying the packaging procedure to prepare a protective membrane or sacrificial layer will be required. To prevent cells from attaching directly and proteins from being adsorbed on the sensing surface, we need to consider modification strategies for the surface. Molecular spacers or surfactants can be used to create an anti-fouling surface. The anti-fouling surface can withstand the fouling impact caused by amino acids, nutrients, and proteins in the culture medium's serum.

Nonetheless, controlling the surface chemistry of electrical sensors by implementing an anti-fouling and catalytic sensor modification can aid in refining the fundamentals for monitoring device development. Modular cell culture and sensing chips are expected to serve as multiplex cell culture platforms for controlling and monitoring cell parameters. In addition, a wide range of monitoring options available through optical and electrical biosensing suggests possibilities for facilitating the understanding of optimal cell culture more efficiently. Collectively, a combination of novel approaches in fabricating/printing techniques for 3D microstructures, enhanced biocompatibility of materials, improved packaging techniques, and image processing with machine learning will be employed to make substantial strides in developing an integrated platform for monitoring cell cultures.

7 | CONCLUSION

Cell culture monitoring technologies are crucial in various biotechnological research and industrial applications. These technologies are transitioning and evolving to meet the demands of the next generation cell culture techniques. Key advancements include real-time monitoring, compatibility with various cell culture formats, and multiplex target monitoring. These efforts have been implemented to assess and assure cell production quality and yield by sensing the parameters from physiological, environmental, and target molecules, including metabolites and contaminants. This review categorizes cell culture monitoring parameters into four types: cellular physiology, cellular environments, cellular metabolites, and contaminants. Cellular physiology monitoring involves cellular behavior, growth, and survival characteristics. Initially reliant on visual perception, this parameter has been developed to provide quantitative measurement results using image processing, increasing reliability. Additionally, electrical sensors, particularly impedance mapping on electrode arrays, can measure cell presence, condition, and motion. Cellular environment monitoring is traditionally controlled by the incubator. However, in the case of experiments requiring specific cell culture formats like

microreactors, electrical sensors with novel catalytic nanomaterials on precise and intricate micropatterns offer accurate and sensitive measurements. Plus, target-responsive optical signaling probe benefits non-invasive detection. Metabolite sensing heavily relies on conventional stationary analytical instruments such as infrared spectroscopy, mass spectrophotometers, and gas/liquid chromatography in the industry. Nevertheless, enzymatic electrochemical sensors with fabrication and packaging technologies enable versatile sensing applications in various cell culture formats. Contamination monitoring is an offline operation that requires sample separation and assay preparation, such as NATT-based fluorescent detection. However, an image processing algorithm for size specification empowers visualization-based in situ bacteria monitoring, implying the possibility of automated real-time monitoring. Additionally, electrical sensing based on the affinity reaction with endotoxin benefits from the surface modification with capture molecules on nanomaterials. Along with the advances in highly integrated analytical modules in optical and electrical analysis, luminescent dyes, microfabrication techniques, and composite materials have enabled the microscale sensing modules and highly sensitive profiling of cell culture environment. Overall, complicated and precise catalytic surface modification-enabled electrical sensors and high-sensitive optical systems are up-and-coming tools for accurately detecting specific parameters for cellular physiology, environment, metabolites and contaminants, and continuous cell monitoring interfaces. Consequently, these advancements have paved the way for more comprehensive and precise cell culture methodologies, holding significant promise for future developments in the field of biotechnology and healthcare.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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