frontiers | Frontiers in Nanotechnology

TYPE Mini Review
PUBLISHED 24 July 2025
DOI 10.3389/fnano.2025.1652480

OPEN ACCESS

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RECEIVED 23 June 2025
ACCEPTED 18 July 2025
PUBLISHED 24 July 2025

CITATION
Son YH and Jeong G-J (2025) Nanobiosensors for monitoring of stem-cell differentiation and organoids.
Front. Nanotechnol. 7:1652480.
doi: 10.3389/fnano.2025.1652480

Nanobiosensors for monitoring of stem-cell differentiation and organoids

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Nanobiosensors now allow continuous, nondestructive tracking of stem cell differentiation and organoid maturation. Classical assays such as immunostaining and polymerase chain reaction are invasive snapshots that overlook fast molecular events guiding lineage choice. Nanoscale probes operate inside living constructs, translating genetic, metabolic, and mechanical signals into optical, electrical, or magnetic readouts while leaving viability intact. This review arranges recent progress by cell type. In pluripotent systems CRISPR Cas13a fluorescence resonance energy transfer beacons, single layer molybdenum disulfide nanopores, and dCas9 SunTag reporters reveal minute scale waves of microRNA and transcription factor activity, addressing teratoma risk. Mesenchymal stromal cells use locked nucleic acid beacons, piezoelectric scaffolds, and magnetic tracers to quantify Notch signaling, mechano sensing, and engraftment. Brain, cardiac, and vascular organoids adopt microneedle electrode arrays, stretchable optical membranes, and impedance chips to monitor deep electrophysiology, contractility, and barrier integrity, while quantum dots and metal organic frameworks combine delivery and sensing across other organoid models. Key hurdles remain, including lack of fabrication standards, uncertain probe occupancy limits, and unclear regulatory pathways. Multimodal chips, artificial intelligence driven analytics, and biodegradable sensor substrates offer potential solutions, moving nanobiosensors closer to routine clinical use.

KEYWORDS

nano biosensor, stem cell, organoid, differentiation, sensor

1 Introduction

In regenerative medicine, stem cells and organoids have rapidly become a triple-driving force that powers modeling, therapies, and cell manufacturing all at once (Pazzin et al., 2024). Patient-specific induced pluripotent stem cells (iPSCs) can supply an inexhaustible, genetically matched cell source, while CRISPR-based editing corrects or installs disease mutations with single-nucleotide precision, yielding human-relevant models that outclass traditional animals (Torizal et al., 2024; Yao et al., 2024; Wiley et al., 2015). Nevertheless, the rapid progress in stem cell and organoids has outpaced the tools that are typically used to research them (Kang et al., 2023). Traditional analytical methods such as immunocytochemistry, polymerase chain reaction, and Western blot analysis present significant limitations, being invasive, time-consuming, and destructive to the cellular systems under investigation. These conventional approaches often require cell fixation and staining procedures that preclude real-time monitoring of dynamic biological processes.

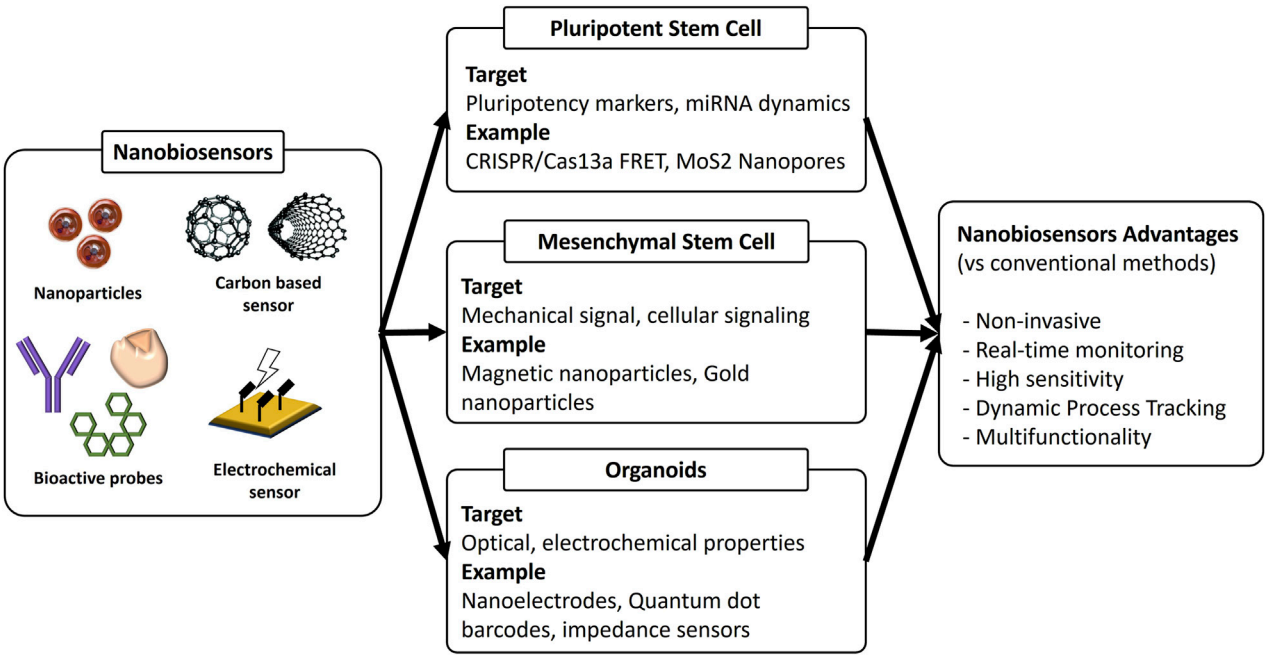

FIGURE 1
Summary of nanobiosensors for stem cell and organoids.

Consequently, there has been an urgent demand for non-invasive, non-destructive, and label-free sensing techniques capable of providing continuous, real-time monitoring of stem cell differentiation and organoid function.

Nanotechnology has emerged as a paradigm-shifting solution to the challenges of monitoring stem cell differentiation and organoid development, offering highly precise control over cellular fate and enabling ultrasensitive detection of molecular and phenotypic changes in real time (Jarrige et al., 2021). Nanoparticle-based biosensors, due to their physicochemical properties like high surface-area-to-volume ratios, tunable surface chemistry, and the ability to interact with biomolecules at the nanoscale, can be integrated into biological systems to monitor key biomarkers and cellular processes during differentiation. Unlike conventional sensing methods that are often destructive, endpoint-based, or lack spatial and temporal resolution, nanoparticle biosensors can mimic the structural and biochemical features of the extracellular matrix, providing both dynamic guidance cues and real-time feedback to cells (Ferrari, 2023). For example, gold nanorod-based molecular beacons can track lineage-specific miRNA expression in neural stem cells over several days, while carbon quantum dots and MOF nanoparticles can be engineered to deliver differentiation factors and simultaneously report on cellular responses (Hou et al., 2025). Furthermore, these nanostructures can be functionalized to respond to mechanical, electrical, or biochemical stimulus, creating closed-loop systems that not only sense but also actively modulate the stem cell microenvironment (Lin et al., 2024; Jhunjhunwala et al., 2023). This integration of sensing and actuation at the nanoscale is paving the way for advanced, automated, and personalized approaches in regenerative medicine, enabling unprecedented insight into the dynamics of stem cell fate decisions and organoid maturation.

This mini review examines the current state of nanoparticle-based biosensor technologies for monitoring stem cell differentiation and organoid development. Rather than focusing on sensor technology classifications, this review adopts a biologically centered approach, organizing the discussion around specific stem cell types and organoid systems (Figure 1). This perspective provides a more clinically relevant understanding of how nanoparticle biosensors are being applied to advanced regenerative medicine applications.

2 Pluripotent stem cells

The clinical translation of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) faces a critical safety bottleneck: teratoma formation risk from residual undifferentiated cells. Even minute subpopulations of pluripotent cells can initiate tumor formation, as demonstrated by studies showing that cells retaining pluripotency characteristics remain present following differentiation protocols and can recover their tumorigenic potential under appropriate culture conditions (Ramirez et al., 2007). Traditional pluripotency markers including OCT4, NANOG, and SOX2 provide only static, endpoint snapshots of cellular state, fundamentally failing to capture the dynamic micro-RNA oscillations and transcription factor fluctuations that preceded and control germ layer commitment (Wang W. et al., 2020). This limitation creates a dangerous gap in safety monitoring, as classical markers miss the rapid molecular transitions occurring at sub-hour timescales during differentiation initiation (Fu et al., 2017). Recent evidence demonstrates that pluripotency maintenance involves complex regulatory networks where factors like Gas5 long non-coding

RNA form positive feedback loops with Sox2, Oct4, and Nanog, creating dynamic expression patterns that conventional assays cannot detect (Gonzales et al., 2021).

2.1 CRISPR/Cas13a FRET sensors

The development of CRISPR/Cas13a-based fluorescence resonance energy transfer (FRET) beacons represents a paradigm shift in real-time microRNA detection within differentiating stem cells (Kutsche et al., 2018; Papadimitriou et al., 2023). This groundbreaking proof-of-principle study employed cytoplasmically expressed Cas13a coupled with exogenous guide RNA, though the initial signal-to-background ratio remained modest and required further optimization for clinical applications (Wang Y. et al., 2020). Recently, two significant advancements were made that substantially improved the sensitivity and temporal resolution of CRISPR-based biosensors (Wang Y. et al., 2020; Montagud-Martínez et al., 2023). First, the guide RNA (gRNA) was strategically pre-assembled with a quencher-labeled single-stranded DNA (ssDNA) reporter, creating a sophisticated detection mechanism where target binding unleashed Cas13a′s collateral cleavage activity, thereby releasing the fluorophore and significantly magnifying the detection signal (Montagud-Martínez et al., 2023; Iwasaki and Batey, 2020). Second, microfluidic integration technology confined reagents to picolitre volumes, compressing reaction time to under 15 min while maintaining high sensitivity and specificity (Degliangeli et al., 2014; Xiao et al., 2018).

The integration of CRISPR/Cas13a systems with stem cell monitoring has revolutionized understanding of microRNA dynamics during differentiation processes (Ishikawa et al., 2020). These systems enable unprecedented single-cell resolution tracking of lineage commitment, capturing the rapid molecular transitions that occur at sub-hour timescales during differentiation initiation (Lu and Yoo, 2018). The ability to detect miRNA-124, a critical neuronal differentiation marker, with such high temporal resolution has opened new avenues for studying neural stem cell fate decisions and optimizing differentiation protocols (Papadimitriou et al., 2023; Ishikawa et al., 2020).

2.2 Monolayer nanopores

While CRISPR beacons excel at detecting relative microRNA ratios, solid-state nanopores address the critical need for measuring absolute transcription dynamics with single-molecule precision (Li et al., 2021; Ren et al., 2025). Transition-metal dichalcogenides such as molybdenum disulfide (MoS_2) uniquely combine atomic thickness, exceptional mechanical robustness, and highly tunable electronic properties that make them ideal for nanopore applications (Xiao et al., 2018; Li et al., 2021). The sophisticated ability to temporal-stamp each individual blockade event enabled these researchers to reconstruct detailed bursty transcription kinetics in differentiating embryoid bodies with unprecedented resolution (Wheat et al., 2020). Solid-state nanopores, especially MoS_2 nanopores, allow single-molecule discrimination of transcript isoforms and real-time measurement of transcriptional bursting. Although large arrays remain in development, proof-of-concept studies demonstrate detection of individual mRNA molecules from live cell extracts, offering direct observation of stochastic gene expression that underlies iPSC fate decisions (Zhong et al., 2023). This capability reveals burst frequency and amplitude for key pluripotency genes (e.g., OCT4, SOX2) at the single-cell level.

2.3 Optical nanobiosensors

A complementary non-destructive approach utilizes dCas9-SunTag scaffolds strategically fused to promoters of interest, providing an alternative method for monitoring transcriptional activity (Kumar et al., 2023; Papikian et al., 2019; Pflueger et al., 2018). The fluorescence signal is dramatically amplified through a sophisticated tandem array of antibody-binding peptides, with each peptide recruiting ten dye-conjugated single-chain variable fragment (scFv) components (Pflueger et al., 2018). Another research reported dCas9-CRISPR system, functioned as programmable transcriptional biosensors in ESCs, enabling real-time functional validation of microRNA promoter activity by targeting genomic regions with sgRNAs and detecting resultant changes in miRNA expression. In mouse ESCs, these tools confirmed that miR-335 expression is exclusively regulated by the host gene *Mest*'s promoter, demonstrating their utility for mapping lineage-specific regulatory elements in pluripotent systems (Kumar et al., 2023).

3 Mesenchymal stem cells

Mesenchymal stromal cells (MSCs) function as the mechanosensitive engines of regenerative orthopedics, supporting modern approaches to bone grafting, cartilage resurfacing and tendon reconstruction (Lee et al., 2014; Kang et al., 2024). Late in the lineage-commitment timeline researchers can confirm differentiation by measuring RUNX2 for osteoblasts, PPARγ for adipocytes and COL2A1 for chondrocytes, but these transcripts emerge only after the decisive cues have already nudged the cell toward a specific fate. In fact, the earliest choices unfold over milliseconds to hours and are dictated by biophysical stimuli such as plasma-membrane stretch, cytoskeletal tension, integrin clustering, transient calcium influx and shifts in membrane potential (An et al., 2015). Substrate stiffness, nanoscale surface texture and fluid shear convert into these intracellular events, activating mechanosensitive ion channels together with RhoA and ROCK signaling well before any osteogenic or chondrogenic gene is detectable. For that reason, a lineage-centric biosensor suited to MSC culture must capture mechanical or electro-mechanical parameters directly and preferably do so without external wiring so that scaffold designers and bioreactor operators can intervene while cell identity is still flexible rather than after the transcriptional trajectory has become locked in (Bagnaninchi and Drummond, 2011; Ding et al., 2024; Lee et al., 2024).

3.1 Osteogenic differentiation tracking

Mesenchymal stem cell osteogenic differentiation represents a cornerstone process in tissue engineering and regenerative medicine

applications. Double-stranded locked nucleic acid (LNA)/DNA nanosensors enable real-time monitoring of Notch1-Dll4 signaling pathway roles at the single mesenchymal stem cell level (Zhao et al., 2022). These sensors track Dll4 mRNA expression dynamics during osteogenic differentiation, allowing observation of differentiation process heterogeneity (Zhao et al., 2022). Barium titanate nanoparticles (BT NPs) combined with alginate polymers provide a novel biocompatible three-dimensional scaffold approach for inducing osteogenic stem cell differentiation (Amaral et al., 2019). BT NP/alginate 3D scaffolds demonstrate osteogenic differentiation induction potential without requiring osteogenic supplements, with BMP-2 and ALP mRNA significantly upregulated after 21 days. The scaffolds exhibit highly interconnected pores and surface nano topography favorable for mesenchymal stem cell differentiation, with mineralization nodules formation and morphological changes from spindle to cuboid shape. Another study reported a dual-color nano sensor in which PLGA nanoparticles co-encapsulate molecular beacons for GAPDH (housekeeping control) and ALP (early osteogenic marker), enabling live, non-destructive read-out of MSC differentiation (Wiraja et al., 2016). Because the particles slowly degrade intracellularly, ALP mRNA dynamics could be followed continuously for 18 days, and the ratio metric fluorescence closely matched conventional RT-qPCR data, validating both accuracy and viability preservation. Applied in standard 2-D culture and on tricalcium-phosphate-loaded PCL films, the sensor revealed an earlier ALP peak on the osteo-inductive scaffold, demonstrating its utility for rapid screening of next-generation bone-graft materials.

3.2 Magnetic nanoparticle-enhanced delivery systems

Magnetic nanoparticle-enhanced extracellular vesicles (GMNPE-EVs) derived from bone marrow mesenchymal stem cells provide an effective therapeutic strategy for diabetic osteoporosis through miR-15b-5p delivery. These systems transfer miR-15b-5p to osteoclasts, downregulating GFAP expression and inhibiting osteoclast differentiation (Xu et al., 2024). *In vitro* experiments demonstrate that GMNPE-EVs effectively deliver miR-15b-5p to osteoclasts, while *in vivo* tests confirm therapeutic potential in alleviating diabetic osteoporosis. Gold nanoparticles have proven effective for mesenchymal stem cell tracking in tumor-targeted delivery systems (Xu et al., 2023). Mesenchymal stem cell-mediated gold nanoparticle delivery showed 2.4- to 9.3-fold enhanced performance in tumor accumulation and penetration compared to conventional enhanced permeability and retention (EPR) effect-based delivery.

3.3 Superparamagnetic iron oxide nanoparticle tracking

Superparamagnetic iron oxide nanoparticles (SPIOs) serve as powerful tools for stem cell tracking applications. Cubic iron oxide nanoparticles with 22 nm edge length (CIONs-22) are optimized for magnetic particle imaging (MPI), showing superior MPI performance compared to commercialized tracers like Vivotrax (Wang Q. et al., 2020). These magnetic properties ensure high sensitivity and resolution for MPI application-ns, with efficient cellular uptake enabling real-time and prolonged monitoring of stem cells transplanted into hindlimb ischemia mice.

4 Organoids

Organoids are self-organizing, three-dimensional mini tissues that recapitulate key aspects of native architecture, multicellular heterogeneity, and dynamic physiology far better than two-dimensional cultures (Septiana and Pawitan, 2024; Thangam et al., 2024). Their complex luminal structures, steep oxygen and nutrient gradients, and millimeter-scale thickness make conventional end-point assays, fixation, sectioning, bulk PCR, both invasive and poorly representative of spatiotemporal variation. Nanobiosensors meet these challenges by embedding ultrasensitive optical, electrical or magnetic probes directly within the organoid matrix, where they can track local gene expression, metabolic flux and mechanical cues without disrupting growth. Because nanoparticles can be functionalized to respond to specific biomolecules or physical forces, they translate otherwise invisible events, such as hypoxic shifts in a liver organoid core or calcium sparks in cerebral organoids, into real-time signals. This continuous, non-destructive feedback is essential for validating developmental fidelity, optimizing culture conditions and, ultimately, qualifying organoids for personalized drug testing and regenerative-medicine applications.

4.1 Brain organoids

Electrophysiological and metabolic read-out of brain organoids has advanced far beyond occasional calcium-imaging snapshots (Yousuf et al., 2025; Park et al., 2024). Zips et al. used aerosol-jet and ink-jet additive printing to fabricate high-aspect-ratio microneedle electrode arrays composed of the conductive polymer poly (3,4-ethylenedioxythiophene):polystyrene sulfonate blended with multi-walled carbon nanotubes, creating nano-electrodes able to penetrate 400–600 μm brain spheroids and cerebral organoids (Zips et al., 2023). The microneedles had 20 μm tips, centimeter scale mechanical flexibility, and week-long electrochemical stability transform them into a practical biosensing platform that captures deep extracellular signals unreachable by conventional planar microelectrode arrays. By delivering reliable recordings from both engineered neutrosphere cultures and self-assembled organoids, the work closes a critical depth-sensing gap and illustrates how additive-manufactured nano-electrodes can advance three-dimensional neural-tissue electrophysiology. Multimodal "Phase-Zero" platforms have been reported to go a step further by threading a broadband vis/NIR fiber through the same chamber, turning the setup into a combined spectrophotometer and electrophysiology rig (Dutta et al., 2020). Local-field-potential spectral exponents in the 30–50 Hz band are logged in parallel with the optical redox read-out of cytochrome-c oxidase; together, these metrics chart excitation–inhibition balance and mitochondrial health during neurodevelopment or drug exposure in a way that single-modality systems cannot.

4.2 Cardiac organoids

Mechanical, biochemical, and electrical cues in cardiac organoids require similarly integrated monitoring (Son et al., 2025). Stretchable PDMS wave-guide membranes patterned with micro-optical structures record sub-percent changes in organoid length as shifts in transmitted light intensity, enabling real-time quantification of systolic and diastolic strain under physiologically relevant after-load (Sannino et al., 2023). When human PSC-derived constructs on these membranes receive low-dose doxorubicin, contractility drops are detected hours before visible beating irregularities emerge, providing an early-warning screen for cardiotoxic compounds. On-chip electrochemical sensors add biochemical depth: aptamer-coated gold microelectrodes incorporated into the same heart-on-a-chip quantify femtomolar surges of creatine-kinase-MB and troponin-T, correlating biomarker release with beat-rate slow-down and viability loss (Devarasetty et al., 2017). At the sub-cellular scale, genetically encoded biosensors that dock a Förster-pair to the ryanodine receptor let investigators visualize cAMP transients exactly where excitation–contraction coupling begins. In β-adrenergic stimulation experiments, these reporters reveal sub-second cAMP spikes that foreshadow maladaptive remodeling, showing how optical genetics, electrochemistry, and mechanics can converge on a single micro-tissue platform.

4.3 Vascular organoids

Adding vasculature introduces new sensing demands that nanotechnology is beginning to meet (Kim J-E. et al., 2025). Electric cell–substrate impedance sensing, miniaturized onto transparent indium-tin-oxide grids, now follows tight-junction maturation in endothelial sheets derived from vascular organoids, delivering second-by-second barrier-resistance curves while leaving the epithelium available for fluorescence or trans-endothelial electric-potential imaging (Ouahoud et al., 2024; Eshetie et al., 2023; Schoon et al., 2025). Perfusable organoid-on-chip devices extend this principle: parallel microchannels lined with HUVECs are fluidically coupled to developing kidney or liver organoids, whose endogenous endothelial buds anastomose with the channel to form macro vessels (Kim Y. et al., 2025). A vascularized tumor-organoid chip grows its own micro-vessels and lets nanosensors watch endothelial sprouting, cancer cell escapes and Notch signaling live (Du et al., 2025). The open channels accept quantum-dot barcodes or plasmonic gold probes, so oxygen use, and cytokine bursts can be measured during drug tests without harming the tissue. A companion smart vascular graft places a laser-written graphene strain lattice inside flexible silicone, sensing blood-flow strain down to three ten-thousandths of a percent through more than thirty-two thousand cycles (Ma et al., 2025). The porous graphene acts both as a strain gauge and a high-area surface ready for electrochemical coatings, streaming wireless data that can flag clots or early wall thickening. Together these platforms show how nanosensors can give continuous, patient-specific feedback from both *in-vitro* disease models and implanted vascular devices.

4.4 Other organoids

Beyond brain, cardiac, and vascular organoids, nanobiosensors are increasingly being applied to other organoid types, most notably kidney, liver, and intestinal organoids, to advance disease modeling, drug screening, and tissue engineering. In kidney organoids, biosensors such as ATP/ADP ratio sensors and impedance-based devices have been integrated to enable real-time, non-invasive assessment of nephrotoxicity, transporter function, and barrier integrity, providing a sensitive platform for evaluating drug-induced injury and organoid maturation *in vitro* (Susa et al., 2023; Tabibzadeh and Morizane, 2024; Tabibzadeh et al., 2023). High-throughput, automated imaging systems combined with label-free biosensor algorithms further facilitate large-scale toxicity screening and functional analysis of personalized kidney organoids, achieving accuracy comparable to conventional biological assays. Organ-on-a-chip technologies have also been successfully merged with kidney and liver organoids, incorporating microfluidic flow and multisensor arrays to monitor biochemical, mechanical, and electrophysiological parameters continuously, thereby enhancing organoid maturation and physiological relevance for regenerative medicine applications (Ferrari et al., 2020; Zhang et al., 2017). Additionally, nanomaterial-based biosensors are being explored across diverse organoid models, including intestinal and tumor organoids, to monitor cell differentiation, tissue barrier function, and responses to mechanical or chemical stimuli, underscoring the broad utility of nanobiosensors in advancing 3D organoid research and translational biomedicine (Yousafzai and Hammer, 2023; Shen et al., 2023).

5 Discussion

Broad adoption of nanobiosensors for stem cell and organoid research is slowed by three interconnected challenges. First, little standardization exists; particle size, surface chemistry, and readout protocols vary from laboratory to laboratory, making results hard to reproduce and to compare. Second, safety guidance is vague. Investigators have yet to agree on a probe occupancy limit that defines how much RNA or protein a sensor can bind before it perturbs differentiation, and the long-term fate of many nanomaterials in living tissue remains uncertain. Third, the regulatory path is unclear; moving a prototype into a fully compliant Good Manufacturing Practice workflow demands costly retooling and exhaustive documentation that most academic centers cannot support.

Next-generation sensor platforms now merge optical, electrochemical, magnetic, and mechanical readouts on a single chip to capture the full complexity of lineage choice. Artificial intelligence tools sift these high dimensional data streams, uncovering faint molecular patterns that predict fate decisions and automatically compensating for sensor drift. Emerging systems even pair real time sensing with on board actuators such as microfluidic pumps or drug releasing nanoparticles, allowing the culture environment to adjust itself in response to live feedback and thereby reducing batch variability while enabling truly personalized cell products.

The coming years are likely to bring biodegradable sensor substrates, gene circuit smart beacons, and wireless implantable networks that extend monitoring from the dish into the patient. Success will depend on community driven benchmarks covering device specifications, data formats, and analysis pipelines. International working groups must align quality metrics, while explainable artificial intelligence frameworks will help regulators trust machine guided judgements. Should these pieces align, nanobiosensors will finally offer regenerative medicine precise and continuous control over cell fate and tissue function from the bioreactor all the way to clinical application.

Author contributions

YS: Conceptualization, Writing – original draft, Writing – review and editing. G-JJ: Conceptualization, Funding acquisition, Writing – original draft, Writing – review and editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (Grant No. RS-2023-00237493) and the Ministry of Trade, Industry and Energy (MOTIE), Republic of Korea (Grant No. RS-2024-00424600 and RS-2024-00433255).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

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