

A Review of Real-time Electrochemical Sensing and Detection Technologies for Organoid Metabolites

Jiaqi Wang

School of Mechanical Engineering, University of Shanghai for Science and Technology, Shanghai 200093, China.

How to cite this paper: Jiaqi Wang. (2026) A Review of Real-time Electrochemical Sensing and Detection Technologies for Organoid Metabolites. *Engineering Advances*, 6(1), 45-49.
DOI: 10.26855/ea.2026.03.010

Received: January 29, 2026
Accepted: February 28, 2026
Published: March 31, 2026

***Corresponding author:** Jiaqi Wang, School of Mechanical Engineering, University of Shanghai for Science and Technology, Shanghai 200093, China.

Abstract

Real-time electrochemical monitoring of organoid metabolites is a critical technology for studying dynamic metabolism and functional states in in vitro organ models. This review focuses on electrochemical detection of two representative biomarkers—glutamate (Glu) and alanine aminotransferase (ALT)—in brain and liver organoid research. First, the enzymatic detection principles for both analytes are systematically introduced, including the evolution from first- to third-generation biosensors. Second, enzyme-based and non-enzymatic electrochemical methods are compared and evaluated regarding sensitivity, selectivity, linear detection range, and long-term stability. Third, the roles of nanomaterial-modified electrodes, including graphene, MXene, carbon nanotubes, and metal nanoparticles, in improving signal-to-noise ratio and overall detection performance are discussed. Fourth, recent advances in microelectrode array technology with innovative three-dimensional designs specifically developed for organoid applications are analyzed in detail. Finally, future development directions are prospected, including the construction of intelligent integrated sensing platforms, organ-on-a-chip integration, and multimodal data fusion analysis. Despite remaining technical challenges in system integration and long-term biocompatibility, these innovative technologies are expected to provide more standardized characterization tools for organoid-based basic research, drug screening, and personalized medicine.

Keywords

Organoids; Electrochemical biosensors; Microelectrode array; Metabolic monitoring; Glutamate; Alanine aminotransferase

1. Introduction

In vitro detection of metabolic secretions is indispensable for assessing physiological functions in organoid models [1]. Glutamate (Glu), the principal excitatory neurotransmitter, regulates neural transmission and synaptic plasticity, with abnormal levels linked to Alzheimer's and Parkinson's diseases [2]. Alanine aminotransferase (ALT), a key liver function biomarker, reflects hepatocellular damage and is widely used in clinical diagnosis of liver diseases [3]. With organoid technology advancing rapidly in disease modeling and personalized medicine, highly sensitive real-time detection methods are urgently needed [4].

Electrochemical biosensors are preferred for real-time metabolite monitoring due to rapid response, high sensitivity, and ease of miniaturization [5]. Two core technologies drive progress: nanomaterial-based electrode modifications that improve conductivity and catalytic activity, and microelectrode array (MEA) technology enabling simultaneous multi-parameter detection [6]. Combined Glu and ALT detection holds clinical value for diseases such as

hepatic encephalopathy [7].

This review covers: (i) electrochemical detection principles for Glu and ALT; (ii) enzyme-based and non-enzymatic sensing methods; (iii) nanomaterial and MEA technologies; and (iv) future perspectives, as outlined in Figure 1.

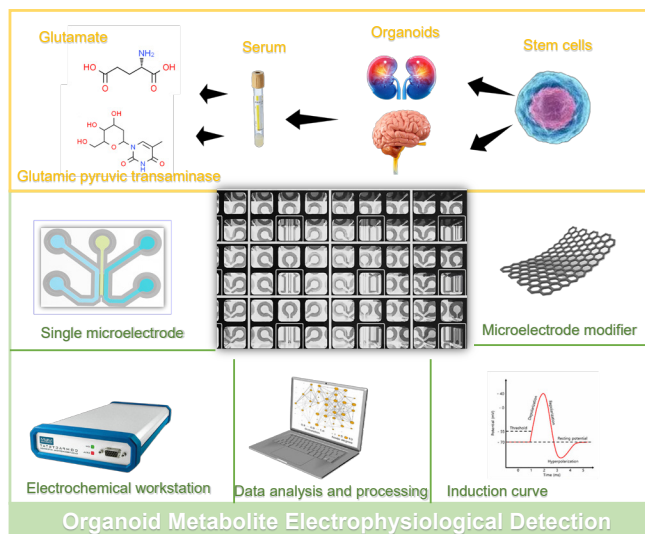
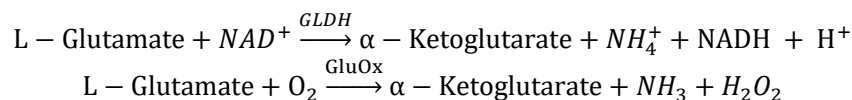


Figure 1. The process and key technologies of electrophysiological detection for organoid metabolites (from organoid culture through single microelectrode/modifier design to electrochemical workstation data acquisition).

2. Electrochemical Detection Principles

2.1 Glutamate Detection

Enzyme-based Glu sensors rely on redox reactions catalyzed by L-glutamate dehydrogenase (GLDH) or L-glutamate oxidase (GluOx) [8]. GLDH converts L-glutamate to α -ketoglutarate with NAD^+ reduced to NADH. GluOx oxidizes Glu using O_2 , generating electrochemically detectable H_2O_2 :



As shown in Figure 2, first-generation sensors detected H_2O_2 directly but suffered from interference at high potentials and oxygen dependence [9]. Second-generation sensors introduced redox mediators. Third-generation sensors achieve direct enzyme-electrode electron transfer, improving sensitivity and selectivity. Non-enzymatic approaches use metal oxide-modified electrodes (e.g., NiO_x), exploiting electrostatic interactions with glutamate anions and Ni^{3+}/Ni^{2+} catalyzed oxidation [10].

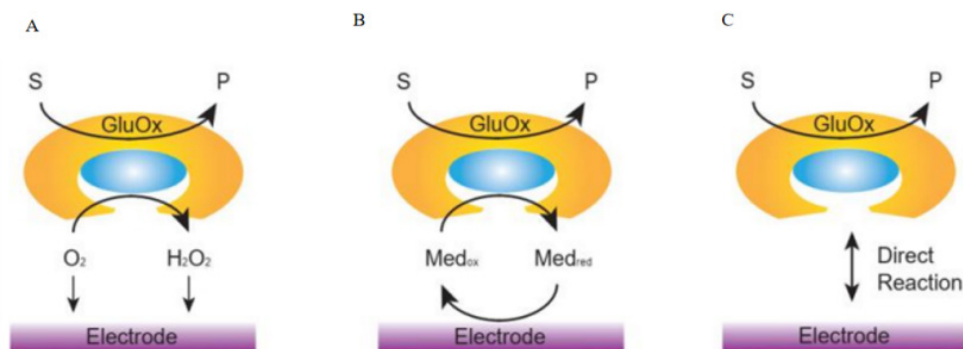


Figure 2. Schematic of enzyme-based glutamate sensor generations: (A) First-generation using H_2O_2 ; (B) Second-generation with redox mediators; (C) Third-generation with direct electron transfer.

2.2 ALT Detection

ALT catalyzes the transamination of L-alanine and α -ketoglutarate to pyruvate and L-glutamate [11]. The L-glutamate product is oxidized by GluOx, generating H_2O_2 for amperometric detection. Alternatively, pyruvate reacts with NADH under lactate dehydrogenase catalysis, and ALT activity is determined by monitoring NADH oxidation current decay. Normal serum ALT is 5–40 U/L; severe liver injury causes >50-fold elevations.

3. Electrochemical Sensing Methods

3.1 Enzyme-based Methods

Sol-gel embedding preserves enzyme activity better than glutaraldehyde cross-linking [12]. Martínez-Perinán et al. developed AA-CDs modified electrodes as NADH electrocatalysts (LOD: 3.3 μ M) [13]. Nasr et al. fabricated Cr/Pt microelectrodes with GluOx for brain organoid Glu detection (LOD: 5.6 μ M) [14]. Rajarathinam et al. constructed GluOx/Prussian blue biosensors with a linear range of 3.25–250 μ M and LOD 0.96 μ M, applied to ex vivo cortical models [15].

For ALT, Wang et al. used Mo_2C -modified electrodes, achieving RSD < 6% with good specificity [16]. Quan et al. developed graphene@MXene sensors with ALT recovery rates of 96.89–103.93% in serum [17]. Muratore et al. achieved real-time ALT monitoring using SiNW-FET platforms at sub-micromolar sensitivity [18].

3.2 Non-enzymatic Methods

Kim et al. used NiO_x /APTES-modified electrodes for glutamate detection via charge interaction. Xu et al. prepared Ni@NC from MOF precursors, achieving LOD of 1.67×10^{-3} μ M [19]. Ali et al. developed CuO/MWCNT sensors with a sensitivity of 8500 $\mu A \cdot mM^{-1} \cdot cm^{-2}$ [20]. Non-enzymatic sensors offer stability and oxygen independence but generally lower selectivity than enzymatic counterparts [21].

3.3 Comparison

Table 1 summarizes representative sensor performances. Enzymatic sensors excel in selectivity and biocompatibility but are limited by oxygen dependence. Non-enzymatic sensors offer superior stability and cost-effectiveness.

Table 1. Comparison of representative electrochemical Glu and ALT biosensors

Electrode/Modifier	Target	Type	Linear Range	LOD
AA-CDs/SPCE	Glu	Enzymatic	11–125 μ M	3.3 μ M
Cr/Pt/m-PD	Glu	Enzymatic	5–500 μ M	5.6 μ M
GluOx/PB	Glu	Enzymatic	3.25–250 μ M	0.96 μ M
Ni@NC/GCE	Glu	Non-enzymatic	0.005–500 μ M	0.00167 μ M
CuO/MWCNTs/SPCE	Glu	Non-enzymatic	20–200 μ M	17.5 μ M
PB/ Mo_2C /WE3	ALT	Enzymatic	5–200 U/L	2.76 U/L
Graphene@MXene/GCE	ALT	Enzymatic	0.5–400 U/L	0.16 U/L

4. Nanomaterials and Microelectrode Arrays

4.1 Nanomaterial Applications

Microelectrode miniaturization causes high impedance and low SNR, demanding conductive nanomaterial modifications [22]. Carbon nanomaterials (graphene, CNTs) provide abundant reactive sites and fast electron transport [23]. Three-dimensional MXene-graphene composite films with tunable porous structures enhance enzyme immobilization and electrocatalytic performance [24]. Noble metal nanoparticles reduce reaction activation energy and amplify signals [25]. However, nanomaterial stability and biocompatibility in biological environments require careful consideration, as protein corona formation and oxidation susceptibility may affect long-term performance [26].

4.2 Microelectrode Arrays

MEAs offer advantages over macroelectrodes, including high mass transfer, low ohmic drop, and spatially resolved detection [27]. For organoid applications, Huang et al. designed self-folding SU8/PEDOT: PSS shell MEAs adaptable to 400-600 μm brain organoids. McDonald et al. fabricated polyimide/gold mesh MEAs with >95% functional yield and impedance <100 k Ω . Phouphetlinthong et al. developed protruding cantilever arrays penetrating organoid interiors. Multi-analyte detection is achieved by independently modifying each electrode for specific targets.

5. Conclusion

This review summarizes advances in real-time electrochemical detection of Glu and ALT in organoids. Enzymatic sensors provide high sensitivity and selectivity, while non-enzymatic approaches offer complementary stability advantages. Nanomaterial modifications and 3D MEA architectures significantly enhance detection performance and enable spatially resolved multi-parameter monitoring. Future research should focus on: (1) biomimetic microelectrode interfaces for improved biocompatibility; (2) organ-on-a-chip integration for multi-organ metabolic studies; (3) deep learning-based intelligent metabolic profiling; and (4) addressing system integration, including microfluidic coupling and wireless transmission. These advances will provide standardized tools for organoid-based precision medicine and disease research.

References

- [1] Murphy SE, Sweedler JV. Metabolomics-based mass spectrometry methods to analyze the chemical content of 3D organoid models. *Analyst*. 2022;147(13):2918-29. <https://doi.org/10.1039/D2AN00533E>
- [2] Greenamyre JT, Penney JB, Young AB, D'Amato CJ, Hicks SP, Shoulson I. Glutamate transmission and toxicity in Alzheimer's disease. *Prog Neuropsychopharmacol Biol Psychiatry*. 1988;12(4):421-30. [https://doi.org/10.1016/0278-5846\(88\)90105-8](https://doi.org/10.1016/0278-5846(88)90105-8)
- [3] Jamal M, Xu J, Razeed KM. A stable and selective electrochemical biosensor for the liver enzyme alanine aminotransferase (ALT). *Biosens Bioelectron*. 2009;24(9):2926-30. <https://doi.org/10.1016/j.bios.2009.02.037>
- [4] Chung WG, Kang MS, Cho SH, Hong YT, Choi N, Lee SH. Recent advances in electrophysiological recording platforms for brain and heart organoids. *Adv NanoBiomed Res*. 2022;2(12):2200081. <https://doi.org/10.1002/anbr.202200081>
- [5] Li S, Li C, Chen S, Chen J, Li J. Electrochemical biosensors for whole blood analysis: recent progress, challenges, and future perspectives. *Chem Rev*. 2023;123(12):7953-8039. <https://doi.org/10.1021/acs.chemrev.2c00873>
- [6] Xue Z, Zhao J. Bioelectric interface technologies in cells and organoids. *Adv Mater Interfaces*. 2023;10(36):2300550. <https://doi.org/10.1002/admi.202300550>
- [7] Wei M, Qiao Y, Zhao H, Liang J, Li T, Luo Y, et al. Electrochemical non-enzymatic glucose sensors: recent progress and perspectives. *Chem Commun*. 2020;56(93):14553-69. <https://doi.org/10.1039/D0CC05650B>
- [8] Schultz J, Uddin Z, Singh G, Howlader MMR. Glutamate sensing in biofluids: recent advances and research challenges of electrochemical sensors. *Analyst*. 2020;145(2):321-47. <https://doi.org/10.1039/C9AN01609K>
- [9] Pu W, Zhou L, Wang H, Zhang W, Zhang S, Wang P, et al. Advances of development and application amino acid biosensors. *Sheng Wu Gong Cheng Xue Bao*. 2023;39(6):2485-501. <https://doi.org/10.13345/j.cjb.221029>
- [10] Kim EJ, Kang CM, Han JH. Nickel oxide electroplating and electrode surface modification for electrochemical detection of glutamate. *Electrochem Commun*. 2024;162:107701. <https://doi.org/10.1016/j.elecom.2024.107701>
- [11] Huang XJ, O'Mahony AM, Compton RG. Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. *Sensors*. 2006;6(7):756-82. <https://doi.org/10.3390/s6070756>
- [12] Matos-Peralta Y, Antuch M, Llano M, Páez M. Glutamate dehydrogenase-based electrochemical biosensors: the immobilization method defines sensor selectivity. *J Electrochem Soc*. 2019;166(13):B1146-51. <https://doi.org/10.1149/2.0071913jes>
- [13] Martínez-Perinán E, Bravo I, Revenga-Parra M, Pariente F, Lorenzo E. Azure A embedded in carbon dots as NADH electrocatalyst: development of a glutamate electrochemical biosensor. *Sens Actuators B Chem*. 2023;374:132761. <https://doi.org/10.1016/j.snb.2022.132761>
- [14] Nasr B, Chatterton R, Yong JQH, Mathews R, Iyer KS, Brett DJ, et al. Self-organized nanostructure modified microelectrode for sensitive electrochemical glutamate detection in stem cells-derived brain organoids. *Biosensors*. 2018;8(1):14. <https://doi.org/10.3390/bios8010014>
- [15] Rajarathinam T, Pandiaraj M, Madasamy T, Rajalakshmi M, Muthuramamoorthy M, Kumaravel A, et al. Glutamate oxidase sheets-Prussian blue grafted amperometric biosensor for the real time monitoring of glutamate release from primary cortical neurons. *Int J Biol Macromol*. 2024;254(Pt 2):127903. <https://doi.org/10.1016/j.ijbiomac.2023.127903>

- [16] Wang H, Liu Y, Zhang H, Wang J, Zhang F, Wang P, et al. Construction of a novel semiautomated electrochemical sensor array platform and its application in multiplexed monitoring of antibiotic therapy. *ACS Sens.* 2024;9(3):1349-58. <https://doi.org/10.1021/acssensors.3c02284>
- [17] Quan C, Liu H, Li J, He M, Wang F, Gao B, et al. Alanine aminotransferase electrochemical sensor based on graphene@MXene composite nanomaterials. *Microchim Acta.* 2024;191(1):45. <https://doi.org/10.1007/s00604-023-06121-2>
- [18] Muratore KA, Roper MG, Weber RJ. Alanine aminotransferase assay biosensor platform using silicon nanowire field effect transistors. *Commun Eng.* 2023;2(1):8. <https://doi.org/10.1038/s44172-023-00057-4>
- [19] Xu Y, Zhou J, Zhou J, Zhang J, Li L, Zhang J. Electrochemical detection of glutamate by metal-organic frameworks-derived Ni@NC electrocatalysts. *Microchem J.* 2022;175:107229. <https://doi.org/10.1016/j.microc.2021.107229>
- [20] Ali MY, Knight D, Howlader MMR. Nonenzymatic electrochemical glutamate sensor using copper oxide nanomaterials and multiwall carbon nanotubes. *Biosensors.* 2023;13(2):237. <https://doi.org/10.3390/bios13020237>
- [21] Hascup KN, Rutherford EC, Quintero JE, Day BK, Nickell JR, Pomerleau F, et al. Second-by-second measures of L-glutamate and other neurotransmitters using enzyme-based microelectrode arrays. In: Michael AC, Borland LM, editors. *Electrochemical Methods for Neuroscience*. Boca Raton (FL): CRC Press/Taylor & Francis; 2007. <https://www.ncbi.nlm.nih.gov/books/NBK2541/>
- [22] Ghane-Motlagh B, Sawan M. A review of microelectrode array technologies: design and implementation challenges. In: *Proceedings of the 2013 2nd International Conference on Advances in Biomedical Engineering*; 2013 Sep 11-13; Tripoli, Lebanon. IEEE; 2013. p. 61-4. <https://doi.org/10.1109/ICABME.2013.6648854>
- [23] Meskher H, Ragdi T, Thakur AK, Ha S, Khelifaoui I, Sathyamurthy R, et al. A review on CNTs-based electrochemical sensors and biosensors: unique properties and potential applications. *Crit Rev Anal Chem.* 2023;53(7):1631-52. <https://doi.org/10.1080/10408347.2022.2036971>
- [24] Gu H, Xing Y, Xiong P, Tang H, Li C, Chen S, et al. Three-dimensional porous Ti3C2Tx MXene-graphene hybrid films for glucose biosensing. *ACS Appl Nano Mater.* 2019;2(10):6537-45. <https://doi.org/10.1021/acsanm.9b01465>
- [25] Rasheed PA, Sandhyarani N. Electrochemical DNA sensors based on the use of gold nanoparticles: a review on recent developments. *Microchim Acta.* 2017;184(4):981-1000. <https://doi.org/10.1007/s00604-017-2143-1>
- [26] Xu L, Liang HW, Yang Y, Yu SH. Stability and reactivity: positive and negative aspects for nanoparticle processing. *Chem Rev.* 2018;118(7):3209-3250. <https://doi.org/10.1021/acs.chemrev.7b00208>
- [27] He C, Huang J, Li M, Yu Y, Zhang Y, Liu J, et al. Microelectrode-based electrochemical sensing technology for in vivo detection of dopamine: recent developments and future prospects. *Crit Rev Anal Chem.* 2022;52(3):544. <https://doi.org/10.1080/10408347.2020.1811946>