



A thermally actuated 3D foldable bioelectronic interface for multimodal electrophysiological and cytokine profiling of human spinal cord organoids

Seungjun Lee^{a,b,1}, Sangmin Han^{c,d,1}, Hyogeun Shin^{a,e,f,*},
Ju-Hyun Lee^{c,g,**}, Namsun Chou^{b,h,***}

^a School of Electronic and Electrical Engineering, Kyungpook National University, Daegu, 41566, Republic of Korea

^b Emotion, Cognition & Behavior Research Group, Korea Brain Research Institute (KBRI), Daegu, 41062, Republic of Korea

^c Brain Science Institute, Korea Institute of Science and Technology (KIST), Seoul, 02792, Republic of Korea

^d Department of Biomedical Sciences, Korea University College of Medicine, Seoul, 02841, Republic of Korea

^e School of Electronics Engineering, College of IT Engineering, Kyungpook National University, Daegu, 41566, Republic of Korea

^f Brain Science and Engineering Institute, Kyungpook National University, Daegu, 41566, Republic of Korea

^g Division of Bio-Medical Science & Technology, KIST School, Korea University of Science and Technology (UST), Seoul, 02792, Republic of Korea

^h Graduate School, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu, 42988, Republic of Korea

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ABSTRACT

Three-dimensional (3D) organoids provide physiologically relevant models for studying human neural development and disease; however, stable bioelectronic interfacing with curved and dynamic organoid surfaces remains challenging. Here, we present a thermally actuated, shape-memory polymer (SMP)-based foldable bioelectronic interface for simultaneous electrophysiological recording and electrochemical cytokine sensing in human spinal cord organoids (hSCOs). Near physiological temperature (36–37 °C), the SMP substrate undergoes pronounced mechanical softening, enabling conformal 3D wrapping around organoids without external mechanical compression. The platform integrates gold microelectrodes for extracellular neural recording and antibody-functionalized sensing electrodes for label-free cytokine detection. Finite element simulations and experimental mechanical analyses confirmed temperature-dependent bending, enhanced conformal contact, low interfacial stress, and mechanically stable biointerfacing. Electrochemical characterization demonstrated linear cytokine sensing responses over 1–100 ng/ml, with calculated limits of detection (LOD) of 0.44 ng/ml for TNF- α and 0.41 ng/ml for IL-8. Using this multimodal platform, stable electrophysiological recordings and cytokine monitoring were simultaneously obtained from hSCOs under inflammatory stimulation. TNF- α treatment induced dose-dependent increases in neural firing activity together with corresponding electrochemical cytokine responses. Collectively, this thermally adaptive bioelectronic interface provides a minimally invasive platform for integrated electrical and biochemical profiling of intact organoids and offers a promising tool for neuro-inflammatory organoid studies.

1. Introduction

Neuroinflammation plays a central role in the pathogenesis of neurodegenerative disorders, autoimmune neurological diseases, and spinal cord injury. (Brambilla, 2019; Kwon and Koh, 2020; Zhang et al., 2023). Pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-8 (IL-8) regulate intracellular signaling

pathways, glial activation, and neural network remodeling, ultimately influencing electrophysiological excitability (Buffolo et al., 2021; Lee et al., 2023; Ramesh et al., 2013). Therefore, the ability to quantitatively assess inflammatory signaling in parallel with functional neural activity is essential for mechanistic studies and functional analysis of neuroinflammatory responses (Mac et al., 2026). In this context, human spinal cord organoids (hSCOs) have gained increasing attention as

* Corresponding author. School of Electronic and Electrical Engineering, Kyungpook National University, Daegu, 41566, Republic of Korea.

** Corresponding author. Brain Science Institute, Korea Institute of Science and Technology (KIST), Seoul, 02792, Republic of Korea.

*** Corresponding author. Emotion, Cognition & Behavior Research Group, Korea Brain Research Institute (KBRI), Daegu, 41062, Republic of Korea.

E-mail addresses: hyogeunshin@knu.ac.kr (H. Shin), nschou@kbri.re.kr (N. Chou).

¹ These authors contributed equally to this work.

advanced three-dimensional (3D) in vitro models that recapitulate key features of human neural circuit development and inflammatory responses (Huang et al., 2022; Lee et al., 2022; Stöberl et al., 2023). These systems provide organized cytoarchitecture and network-level functionality that are not achievable in conventional two-dimensional cultures. However, their spherical morphology and continuously evolving microenvironment make stable and reliable bioelectronic interfacing inherently challenging.

Most existing neural bioelectronic platforms have been developed for planar tissues or monolayer cultures (Kim et al., 2025; Li et al., 2021). Although recent advances in flexible and stretchable electronics have improved mechanical compliance, the majority of organoid-interfacing systems still rely on planar or partially wrapped configurations. As a result, they provide limited contact area and often exhibit unstable signal coupling when applied to curved 3D tissues (Abu Shihada et al., 2024; Boufidis et al., 2025; Wang et al., 2025). To overcome these limitations, recent work has introduced 3D microelectrode array (MEA) architectures capable of conformally covering organoid surfaces, enabling large-area electrophysiological recordings and more comprehensive analysis of network-level activity (Liu et al., 2026). However, these systems are typically fabricated with predefined geometries tailored to specific organoid shapes, which restrict their adaptability to organoids with heterogeneous morphologies. Moreover, rigid or geometrically constrained designs can introduce mechanical mismatch at the electrode–tissue interface, potentially compromising signal quality and increasing interfacial impedance (Lee and Liu, 2025; Xu et al., 2025). Importantly, most of these platforms are optimized for electrophysiological measurements alone, limiting their ability to simultaneously capture electrochemical signaling and to probe the interactions between electrical activity and neurochemical dynamics within organoid networks.

At the same time, neuroinflammatory assessment in organoid models largely relies on endpoint assays, including conventional histological and molecular analyses, as well as bulk cytokine measurements via ELISA (Jung et al., 2018; Recaladin et al., 2024). Although these approaches offer molecular specificity, they require destructive processing or indirect sampling and therefore cannot provide real-time, organoid-level correlations between cytokine release and electrophysiological activity.

To address these limitations, an effective bioelectronic interface for organoids must meet two key requirements: (i) it must achieve conformal integration with curved 3D tissues without imposing excessive mechanical stress, and (ii) it must support the simultaneous acquisition of electrical and biochemical signals within a single, stable platform.

Here, we present a thermally actuated, shape-memory polymer (SMP)-based 3D foldable bioelectronic interface designed to dynamically adapt its mechanical properties under physiological conditions. Conventional shape-memory polymers (SMPs) possess the ability to reversibly transform into predefined shapes in response to external stimuli such as temperature or environmental conditions. In this study, we exploited the thermally induced transition of the SMP from a rigid glassy state at lower temperatures to a softened state near physiological temperature, enabling stable conformal contact according to the geometry of the organoid surface. Near 36–37 °C, the SMP exhibits a pronounced reduction in storage modulus, enabling passive, conformal wrapping around organoids without the need for external mechanical compression (Liu et al., 2023). This temperature-responsive behavior promotes uniform surface contact and controlled stress distribution, thereby minimizing mechanical perturbation at the tissue–electrode interface.

The proposed interface integrates patterned gold microelectrodes for electrophysiological recording with antibody-functionalized sensing sites for label-free electrochemical detection of cytokines. Antibody-modified gold electrodes have been widely demonstrated to provide high sensitivity and specificity for cytokine detection (Cancelliere et al.,

2022; Hou et al., 2013). Finite element simulations, together with experimental validation, confirm the temperature-dependent deformation behavior of the device and its ability to maintain mechanically stable and safe conformal contact with organoid surfaces.

To evaluate the functionality of this multimodal platform, hSCOs were subjected to graded TNF- α stimulation. The system enabled simultaneous monitoring of network-level electrophysiological activity and cytokine-dependent electrochemical signals within the same intact organoid. By capturing these synchronized responses, the platform allows direct correlation between neural activity and inflammatory signaling, providing a robust and minimally invasive approach for high-fidelity neuroinflammatory studies in organoid systems.

2. Experimental section

2.1. Materials

A complete list of materials is provided in the Supporting Information.

2.2. Fabrication of the SMP-based foldable bioelectronic interfaces

The foldable bioelectronic interface was fabricated on an SMP substrate using standard photolithography and metal deposition processes (Fig. S1). Briefly, an SMP precursor solution was spin-coated onto a sacrificial copper layer and UV-cured to form the substrate. Ti/Au electrodes were subsequently patterned via photolithography and ICP-RIE etching, followed by encapsulation with an additional SMP insulation layer. Finally, the sacrificial layer was removed to release the foldable bioelectronic interface. Detailed fabrication procedures are provided in the Supporting Information.

2.3. Bioelectronic interface activation chamber development

A custom activation chamber was developed to support thermal actuation and stable electrophysiological measurements of the foldable bioelectronic interface. The chamber enabled controlled conformal interfacing between the device and organoids during recording experiments.

2.4. Electrochemical impedance characterization of the foldable bioelectronic interface

Electrochemical impedance spectroscopy (EIS) was performed to evaluate temperature-dependent electrode–electrolyte coupling before and after thermal actuation of the foldable interface. Impedance spectra were acquired over a frequency range of 0.1 Hz to 1 MHz in PBS electrolyte.

2.5. Thermomechanical characterization of the SMP foldable bioelectronic interface

Finite element simulations were performed using the Solid Mechanics module in MATLAB R2024a (MathWorks Inc., Natick, MA, USA) to investigate thermally induced deformation of the SMP-based foldable interface. For the finite element method (FEM) simulations, we employed DMA-derived storage modulus values as the approximate effective Young's modulus to comparatively represent the rigid and softened mechanical states of the SMP substrate. At room temperature (the rigid state), the SMP exhibited a low $\tan \delta$ value (<0.1), making the storage modulus a reasonable approximation of elastic stiffness under small deformation conditions. Conversely, near the physiological body temperature of 37 °C (the softened state), the $\tan \delta$ value increased to ~ 0.7 , indicating enhanced viscoelastic behavior. In this highly viscoelastic regime, the storage modulus measured at a physiologically relevant frequency (e.g., 1 Hz) was utilized. This dynamic modulus

under low-frequency oscillation provides a more accurate representation of the scaffold's *in vivo* mechanical stiffness against continuous biological micro-motions than a static Young's modulus. Ultimately, these simulations were designed to provide a simplified comparative thermomechanical analysis of the substrate rather than an absolute prediction of complex viscoelastic deformation. Additional image-based curvature and deformation analyses were performed to evaluate bending behavior and conformal mechanical compatibility under thermally actuated conditions. Detailed simulation parameters and analysis procedures are provided in the Supporting Information.

2.6. hSCO generation and TNF- α responsive neuroinflammation

hSCOs were generated from H9 human embryonic stem cells following a previously reported protocol. Organoids were sequentially differentiated using neural induction and caudalization factors, followed by maturation under suspension culture conditions. Detailed differentiation procedures and reagent compositions are provided in the Supporting Information.

2.7. Immunocytochemical staining and confocal imaging

Immunocytochemical staining was performed using antibodies against NF- κ B, GFAP, and C3, followed by confocal fluorescence imaging. Detailed staining procedures and antibody information are provided in the Supporting Information.

2.8. Quantitative analysis of inflammatory gene expression

Total RNA was extracted from hSCOs using TRIzol reagent according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized from 1 μ g of total RNA using a reverse transcription master mix. Quantitative PCR was performed using a real-time PCR system with a SYBR Green-based master mix and gene-specific primers (Supplementary Table 2). Gene expression levels were normalized to GAPDH and calculated using the $2^{-\Delta\Delta Ct}$ method. Results are presented as fold changes relative to the untreated control group (TNF- α 0 ng/ml).

2.9. Electrophysiological recording and signal analysis

Electrophysiological recordings were obtained using a multichannel Intan RHD2000 system following thermally induced conformal interfacing between the device and organoids. Signals were digitized and analyzed offline using MATLAB-based custom scripts.

2.10. Cytokine detection

Antibodies specific to TNF- α and IL-8 were directly immobilized onto exposed gold electrode pads via physical adsorption without additional coupling chemistry. Briefly, antibody solutions were applied to the exposed gold electrodes and incubated at 4 °C overnight, followed by PBS washing and BSA blocking to minimize nonspecific adsorption. Following TNF- α treatment, hSCOs were washed with PBS prior to electrochemical measurements. Cytokine detection was subsequently performed using cyclic voltammetry (CV) in a three-electrode configuration with antibody-functionalized gold electrodes. Electrochemical signals were analyzed based on normalized current changes relative to untreated controls. Detailed electrochemical measurement conditions are provided in the Supporting Information.

3. Results and discussion

3.1. Design and working principle of a 3D foldable bioelectronic interface

The thermally adaptive, 3D foldable bioelectronic interface is an integrated diagnostic platform designed for multimodal organoid

sensing (Fig. 1). This interface undergoes autonomous structural transformation to establish seamless contact with complex biological architectures. By exploiting the temperature-dependent mechanical transition of a SMP, the device achieves precise and conformal coupling, thereby overcoming the mechanical mismatch between conventional planar electrodes and 3D tissues. This functionality arises from its thermally driven mechanical adaptability. Upon exposure to physiological temperatures (35–37 °C), the storage modulus of the SMP substrate decreases by more than 10 times, enabling the interface to transition into a compliant, elastic state. This softening allows passive conformal wrapping around the curved organoid surface without the need for external force or anchoring structures. As a result, mechanical perturbation to the developing organoid is minimized while intimate electrode–tissue contact is established, leading to improved signal-to-noise ratio (SNR).

To enable dual sensing modalities within a single platform, the interface was fabricated using microelectromechanical systems (MEMS) processes that integrate recording electrodes, antibody-functionalized electrochemical sensors, foldable regions, and microscale drainage openings (Fig. 1; Fig. S1–S4). The MEA is configured to monitor electrophysiological activity, while the functionalized electrochemical sites detect inflammatory cytokines such as TNF- α and IL-8. The sensing region consists of eight recording electrodes, with four positioned on the bottom surface and four on the upper surface. This arrangement enables 3D electrophysiological recording once the interface conformally envelops the irregular organoid surface. To ensure stable organoid positioning and to prevent the accumulation of culture medium, drainage openings are incorporated around the sensing region. These apertures allow excess medium to escape during organoid placement, thereby enhancing mechanical stability and improving electrode–tissue contact. The integration of antibody-functionalized sensing sites alongside electrophysiological electrodes enables simultaneous monitoring of neural firing patterns and biochemical markers, including TNF- α and IL-8. By enabling real-time correlation between functional network dynamics and cytokine release, this multimodal interface provides a robust platform for investigating the mechanisms of neuroinflammation in 3D, human-relevant models.

3.2. Thermally induced conformal interfacing with 3D organoids

The thermomechanical properties of the proposed SMP were tuned to enable seamless conformal interfacing with organoids (Fig. 2A). The storage modulus exhibited a sharp decrease with increasing temperature, dropping from 1.06 GPa at 25 °C to 96.3 MPa at 37 °C. The loss factor ($\tan \delta$) showed a pronounced peak near 37–38 °C, corresponding to the glass transition temperature (T_g) of the SMP. This transition at physiological temperature (37 °C) allows the SMP-based substrate to soften and undergo structural deformation, thereby facilitating passive wrapping around the organoid (Fig. 2B; Fig. S5). At 25 °C, the scaffold remains relatively planar and establishes only partial contact with the organoid surface. In contrast, at 37 °C, the thermally softened SMP bends toward the organoid, enabling the sensing interface to conformally envelop the irregular 3D structure.

To verify conformal contact between the bioelectronic interface and the organoid, electrochemical impedance spectroscopy (EIS) was performed at both 25 °C and 37 °C (Fig. 2C–F). Channel-wise impedance mapping revealed clear temperature-dependent differences in contact behavior (Fig. 2C and D). At 25 °C, the bottom electrodes exhibited lower impedance than the top electrodes, primarily due to gravity-assisted contact with the organoid. In contrast, the top electrodes remained partially detached, resulting in higher impedance. At 37 °C, thermal softening of the SMP enabled the interface to wrap conformally around the organoid, leading to uniformly improved electrode–organoid contact across both top and bottom channels. Consequently, all channels showed substantially reduced impedance, indicating more homogeneous and efficient electrical coupling through conformal interfacing.

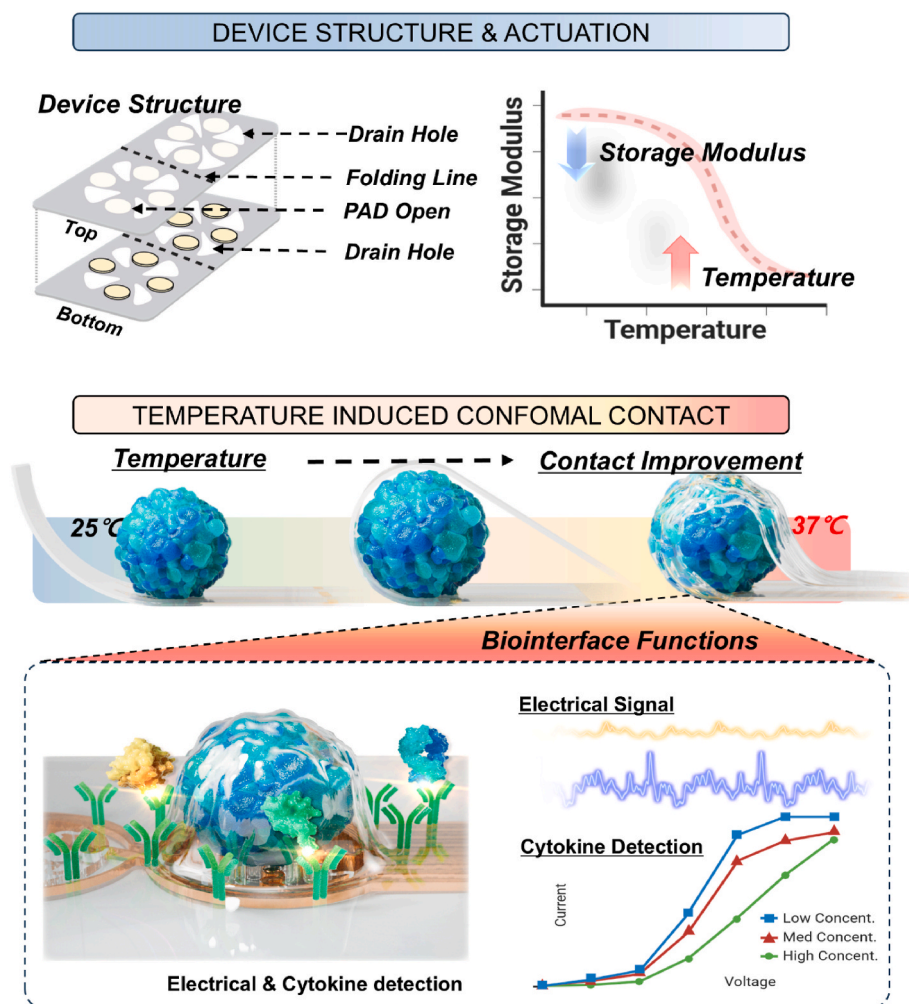


Fig. 1. Schematic illustration of a thermally adaptive, 3D foldable bioelectronic interface.

Representative frequency-dependent impedance and phase spectra further supported this temperature-dependent stabilization of the interface (Fig. 2E and F). At 25 °C, the device exhibited relatively high impedance across the measured frequency range, with a 1 kHz impedance of $9.42 \pm 0.68 \text{ k}\Omega$ (Fig. 2E). Following thermal activation at 37 °C, the impedance decreased markedly to $1.96 \pm 0.18 \text{ k}\Omega$ at 1 kHz (Fig. 2F), reflecting improved electrical contact and reduced interfacial resistance. Collectively, these results demonstrate that thermally induced conformal interfacing enhances electrode–organoid coupling and significantly improves electrical stability.

3.3. Simulation-based analysis of thermally induced deformation and contact behavior

To further elucidate the deformation mechanism underlying conformal organoid interfacing, finite element simulations were conducted using MATLAB to model the thermally induced bending behavior of the SMP-based structure. As illustrated in Fig. 3A, the interface was designed to bend along a predefined arc toward the curved organoid surface. The simulated deformation profile (Fig. 3B) revealed progressive out-of-plane bending with increasing temperature, closely reproducing the experimentally observed wrapping behavior. Cross-sectional simulations further illustrated the evolving conformal interaction between the organoid and the sensing substrate during deformation (Fig. S6).

To quantify the impact of deformation on physical interfacing, cross-sectional analyses were performed to examine the contact state between

the scaffold and the organoid at 25 °C and 37 °C. The adhesion map (Fig. 3C) indicates that, at 25 °C, the predicted contact region is limited to a small, localized area near the edge of the structure. In contrast, at 37 °C, the contact region expands substantially along the curved interface. This result suggests that thermal activation significantly increases the effective contact area between the device and the organoid.

The corresponding temperature distribution map (Fig. 3D) shows a relatively uniform thermal profile across the interfacing structure, with no evident localized hotspots. The simulated temperature rises from 25 °C to 37 °C throughout the activated region, indicating that deformation is driven by a broadly distributed thermal field rather than localized heating. The displacement map (Fig. 3E) further demonstrates a pronounced increase in structural deformation at 37 °C compared with 25 °C. At the lower temperature, displacement was restricted to a narrow peripheral region, whereas at 37 °C it extended across a much larger portion of the scaffold. This behavior is consistent with the experimentally observed transition from partial to fully conformal wrapping. Additional mechanical simulations corroborate the temperature-dependent increase in compliance of the sensing substrate.

Collectively, these simulations support experimental observations by demonstrating that thermal activation induces reproducible bending, expands the effective contact area, and enhances structural displacement of the interface. These modeling results provide a mechanistic basis for the improved conformal wrapping and reduced interfacial impedance observed experimentally, confirming that the SMP-based scaffold enables mechanically stable and electrically reliable bio-interfacing with 3D organoids. Additional quantitative mechanical

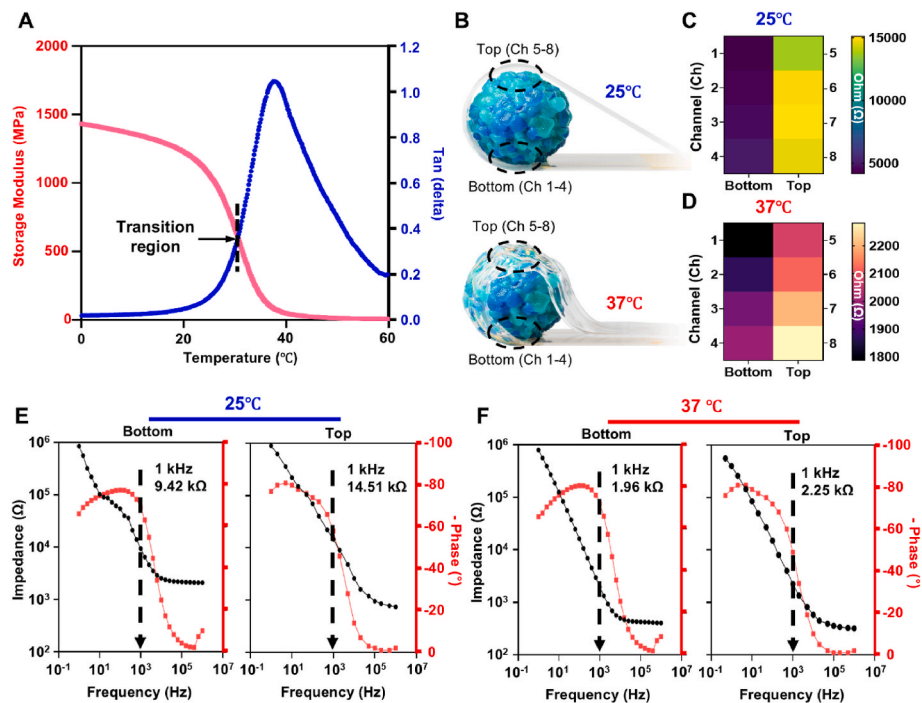


Fig. 2. Thermomechanical activation and impedance characteristics of the SMP-based foldable bioelectronic interface.

(A) Dynamic mechanical analysis of the SMP substrate showing temperature-dependent changes in storage modulus and $\tan \delta$. (B) Schematic illustration of thermally induced conformal wrapping of the bioelectronic interface around an organoid at 25 °C and 37 °C. (C, D) Channel-wise impedance heatmaps measured at 25 °C (C) and 37 °C (D). (E, F) Representative EIS profiles of the interface at 25 °C (E) and 37 °C (F). Data are presented as mean $\pm$ SD ($n = 4$ independent channels).

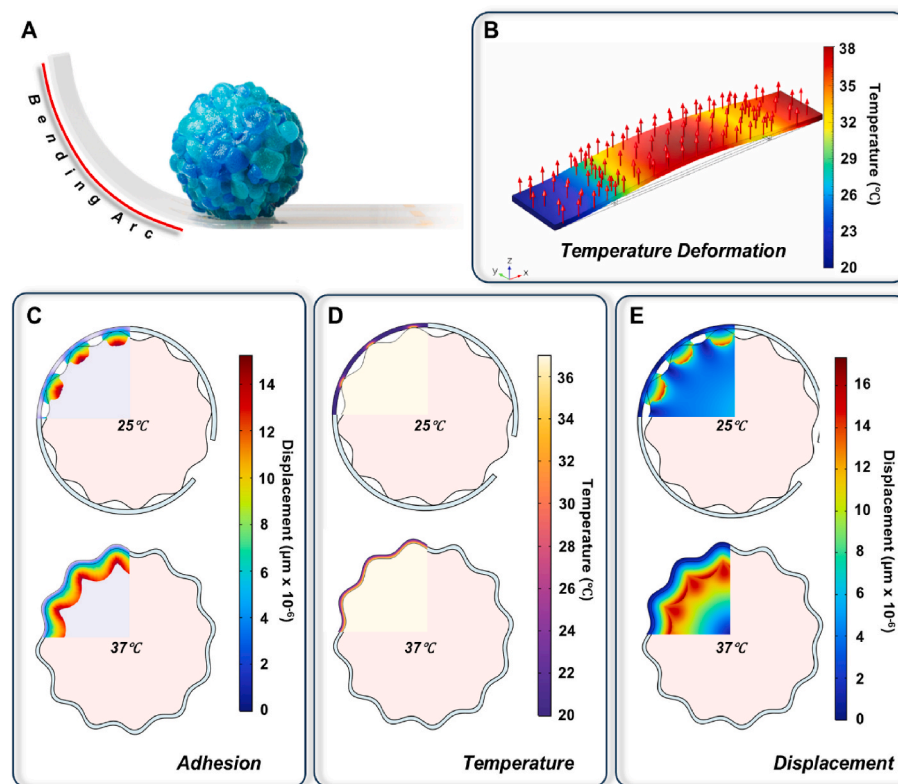


Fig. 3. Temperature-dependent mechanical adaptation of the SMP-based bioelectronic interface enabling conformal contact with a 3D organoid.

(A) Schematic illustration of thermally induced bending of the SMP scaffold toward the organoid surface. (B) Finite element simulation of temperature-dependent deformation of the foldable interface. (C) Simulated contact distribution between the interface and organoid under rigid and softened conditions. (D) Simulated temperature distribution across the interface during thermal activation. (E) Simulated displacement map of the foldable structure under thermal activation.

analyses were performed to validate the conformal mechanical compatibility of the foldable SMP interface with soft 3D organoid systems. A spherical bead model with diameters of approximately 1218 and 1071 μm was used to mimic the curvature of spinal cord organoids. Image-based curvature analysis demonstrated that the bending radius of the thermally actuated SMP interface decreased from $660 \pm 45 \mu\text{m}$ at 25°C to $550 \pm 38 \mu\text{m}$ at 37°C , approaching the average radius of the organoid-mimicking bead under physiological conditions. To indirectly evaluate interfacial stress and strain during conformal contact, a spherical bead positioned on the SMP device spanning a 3 mm opening was vertically displaced. Under the 37°C condition, the softened SMP interface formed intimate conformal contact with the bead surface, resulting in $\sim 0.2 \text{ mm}$ upward deformation of the device, whereas

negligible deformation was observed at 25°C . Based on the 3 mm unsupported span geometry, the resulting strain was estimated to be $\sim 0.88\%$. In addition, the measured force at 0.2 mm displacement ($\sim 2.8 \text{ mN}$) was converted into nominal interfacial stress using the projected bead contact area (0.785 mm^2 for a 1 mm bead), yielding an estimated stress of $\sim 3.57 \text{ kPa}$. Considering that neural organoid tissues typically exhibit elastic moduli in the kPa range ($\sim 5 \text{ kPa}$), the estimated stress suggests mechanically compliant interfacing without substantial mechanical loading. Furthermore, lateral displacement experiments demonstrated that the force required to move the bead by 1 mm was substantially greater under the 37°C condition than under the 25°C condition, indicating enhanced conformal adhesion and increased effective contact pressure between the softened SMP interface and the

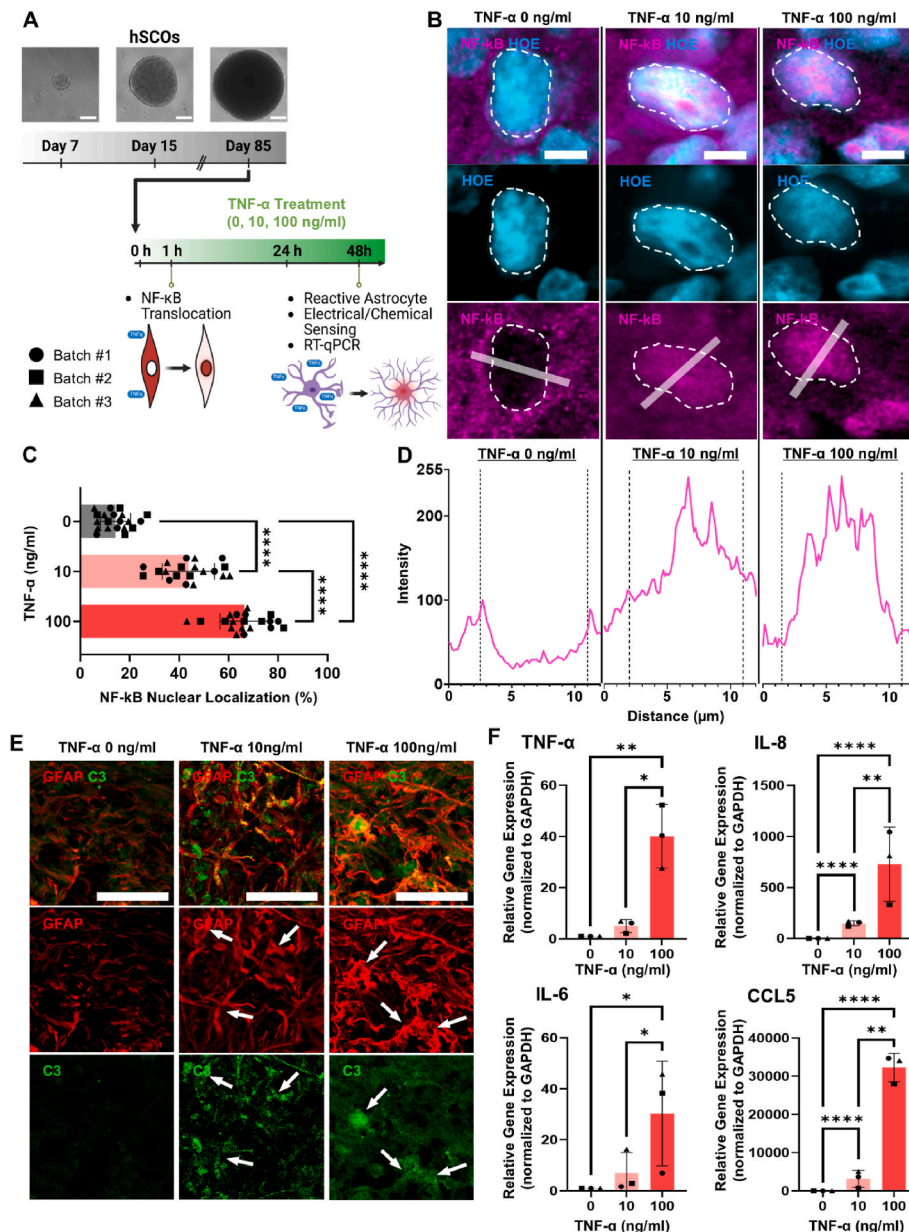


Fig. 4. TNF- α -induced neuroinflammatory responses in hSCOs.

(A) Experimental schematic of TNF- α treatment in hSCOs. Scale bar, 200 μm . (B) Representative immunofluorescence images of NF- κ B (magenta) and nuclei (cyan) following TNF- α treatment. Scale bar, 5 μm . (C) Quantification of NF- κ B nuclear translocation. (D) Line profile analysis of NF- κ B fluorescence intensity across the nucleus. (E) Representative immunofluorescence images of GFAP and C3 expression following TNF- α treatment. Scale bar, 50 μm . (F) Quantitative analysis of inflammatory gene expression following TNF- α stimulation. Data are presented as mean $\pm$ SD. Statistical significance was assessed using one-way ANOVA followed by Tukey's multiple-comparison test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

organoid-mimicking bead (Fig. S7).

3.4. Early detection of TNF- α induced neuroinflammation via NF- κ B nuclear translocation

Prior to multimodal sensing experiments, a TNF- α -induced neuroinflammation model was established in well-characterized hSCOs exhibiting spinal cord identity and progressive maturation (Figs. S8 and S9). To determine whether TNF- α stimulation rapidly activates neuroinflammatory signaling in hSCOs, we monitored the nuclear translocation of NF- κ B, a hallmark of the inflammatory cascade, 1 h after treatment (Fig. 4A). In untreated organoids (0 ng/ml TNF- α), NF- κ B was predominantly localized in the cytoplasm, with minimal nuclear signal. In response to increasing TNF- α concentrations, NF- κ B exhibited pronounced nuclear translocation, consistent with progressive activation of the NF- κ B pathway (Fig. 4B; Fig. S10). NF- κ B nuclear translocation was quantified from immunofluorescence images by calculating the ratio of nuclear to cytoplasmic NF- κ B intensity (Fig. 4C). This analysis revealed a significant, dose-dependent increase in nuclear localization, with nuclear fractions of $14.18 \pm 6.17\%$ at 0 ng/ml TNF- α , $43.79 \pm 10.5\%$ at 10 ng/ml TNF- α , and $66.46 \pm 9.89\%$ at 100 ng/ml TNF- α . Consistent with these findings, line profile analysis of NF- κ B fluorescence intensity further demonstrated progressive enrichment of the signal within the nucleus as TNF- α concentration increased (Fig. 4D). The corresponding intensity profiles exhibited a bell-shaped distribution, with peak intensity located within the nucleus. Moreover, absolute fluorescence intensity increased in a dose-dependent manner, indicating that higher TNF- α concentrations elicited stronger activation of NF- κ B signaling.

Collectively, these results demonstrate that TNF- α stimulation induces rapid, dose-dependent nuclear translocation of NF- κ B in hSCOs, reflecting early neuroinflammatory activation, consistent with previous reports. (Bagaev et al., 2019; Hyvärinen et al., 2019).

3.5. Glial and inflammatory marker expression induced by TNF- α

To determine whether early NF- κ B activation is followed by structural and transcriptional changes associated with neuroinflammation, hSCOs were exposed to TNF- α for 48 h and subsequently analyzed by immunostaining and gene expression profiling. Immunofluorescence analysis revealed a dose-dependent increase in the astrocytic marker GFAP and the reactive astrocyte-associated marker complement component C3 following TNF- α treatment, despite the absence of immune cells (Fig. 4E; Fig. S11). In addition to increased signal intensity, GFAP-positive cells displayed more pronounced filamentous structures and evident astrocytic hypertrophy, characterized by thickened cellular processes. In contrast, untreated organoids showed low, diffuse GFAP and C3 signals without noticeable morphological changes. Transcriptional analysis further corroborated these observations. The expression levels of pro-inflammatory cytokines (TNF- α and IL-6) and chemokines (IL-8 and CCL5) were significantly upregulated in a dose-dependent manner compared with untreated controls (Fig. 4F). These responses were consistently observed across independent organoid batches, indicating robust and reproducible induction of inflammatory gene expression following TNF- α stimulation. Taken together, these findings indicate that early NF- κ B nuclear translocation induced by TNF- α is followed by downstream astrocytic activation, reflected in both molecular and morphological changes, as well as coordinated upregulation of inflammatory genes. This progression supports the establishment of a sustained neuroinflammatory state in hSCOs.

3.6. Electrophysiological and electrochemical characterization of inflammatory organoids

Electrophysiological recordings were conducted to evaluate how inflammatory stimulation modulates spontaneous neural activity in hSCOs. The foldable bioelectronic interface enabled stable extracellular

recordings from the organoid surface under conformal contact conditions, while simultaneously supporting electrochemical cytokine sensing through antibody-functionalized electrodes (Fig. 5A). Representative extracellular traces showed spontaneous spike activity under all conditions, with clear alterations in temporal firing patterns following TNF- α treatment (Fig. 5B; Fig. S12). Quantitative analysis revealed a dose-dependent increase in neuronal excitability, with firing rates rising from 8.3 ± 3.46 spikes/s in untreated controls to 29.7 ± 3.08 spikes/s and 35.8 ± 3.19 spikes/s in organoids treated with 10 and 100 ng/ml TNF- α , respectively (Fig. 5C). SNRs remained consistently high across all groups (8.59, 8.55, and 9.52), confirming stable recording quality during inflammatory stimulation. These observations are consistent with previous reports indicating that TNF- α enhances excitatory synaptic transmission and neuronal responsiveness (Chen et al., 2015; Olmos and Lladó, 2014; Stellwagen and Malenka, 2006).

Analysis of spike timing further supported these findings. Time-resolved spike rate maps also revealed denser and more sustained firing activity following TNF- α exposure, particularly at 100 ng/ml. Together, these results indicate that inflammatory stimulation promotes network-level hyperexcitability while altering the temporal organization of neural activity, consistent with TNF- α -mediated disruption of excitation–inhibition balance reported in previous studies. In parallel, cytokine release from organoids was evaluated electrochemically using integrated antibody-functionalized gold electrodes. Prior to organoid measurements, antibody immobilization and sensing performance were systematically validated. Calibration experiments demonstrated dose-dependent signal attenuation over a range of 5–100 ng/ml for both TNF- α and IL-8 sensors (Fig. S13A). Linear fitting confirmed robust analytical performance, with distinct and reproducible responses across the tested range. Baseline electrochemical characterization further showed no significant difference between bare gold electrodes and antibody-functionalized electrodes prior to cytokine binding (Fig. S13B), indicating that antibody immobilization did not substantially alter the intrinsic electrochemical properties of the sensing surface. Consequently, subsequent signal changes were attributed primarily to target-induced interfacial blocking rather than nonspecific baseline drift.

Using the multimodal platform, cytokine levels were quantified in organoids subjected to graded TNF- α stimulation. Cyclic voltammetry (CV) measurements showed a progressive decrease in peak current amplitude with increasing inflammatory stimulation for both TNF- α and IL-8 sensing channels (Fig. S14). Quantitative analysis revealed that the TNF- α channel decreased from 1.50 ± 0.12 μ A under control conditions to 1.29 ± 0.01 μ A and 1.07 ± 0.02 μ A following treatment with 10 and 100 ng/ml TNF- α , respectively (Fig. 5D). Similarly, the IL-8 channel decreased from 1.49 ± 0.12 μ A to 1.22 ± 0.02 μ A and 0.99 ± 0.002 μ A under the same conditions (Fig. 5E). Using the calibration curves obtained from Fig. 5I, these current responses were further converted into calibration-derived apparent cytokine-equivalent concentrations. The 10 ng/ml TNF- α -treated group corresponded to approximately 30.7 ng/ml TNF- α and 31.2 ng/ml IL-8, whereas the 100 ng/ml TNF- α -treated group corresponded to approximately 100 ng/ml for both cytokine sensing channels. These reductions are consistent with increased steric hindrance and charge-transfer resistance resulting from cytokine binding to immobilized antibodies at the electrode interface.

For normalized comparison across conditions, current responses were converted to relative signal changes ($\Delta I/I_0$, %). The TNF- α sensing channel exhibited increases from $13.2 \pm 2.54\%$ at 10 ng/ml to $18.4 \pm 3.46\%$ at 100 ng/ml, whereas the IL-8 channel increased from $28.1 \pm 3.08\%$ to $34.0 \pm 3.19\%$ over the same range (Fig. 5F). Notably, the IL-8 channel showed a larger relative response than the TNF- α channel, suggesting robust secondary cytokine induction following TNF- α stimulation. Representative cyclic voltammetry (CV) responses further confirmed concentration-dependent electrochemical signal attenuation for both TNF- α and IL-8 sensing (Fig. 5G and H). Calibration analysis demonstrated linear sensor responses over 1–100 ng/ml, with calculated

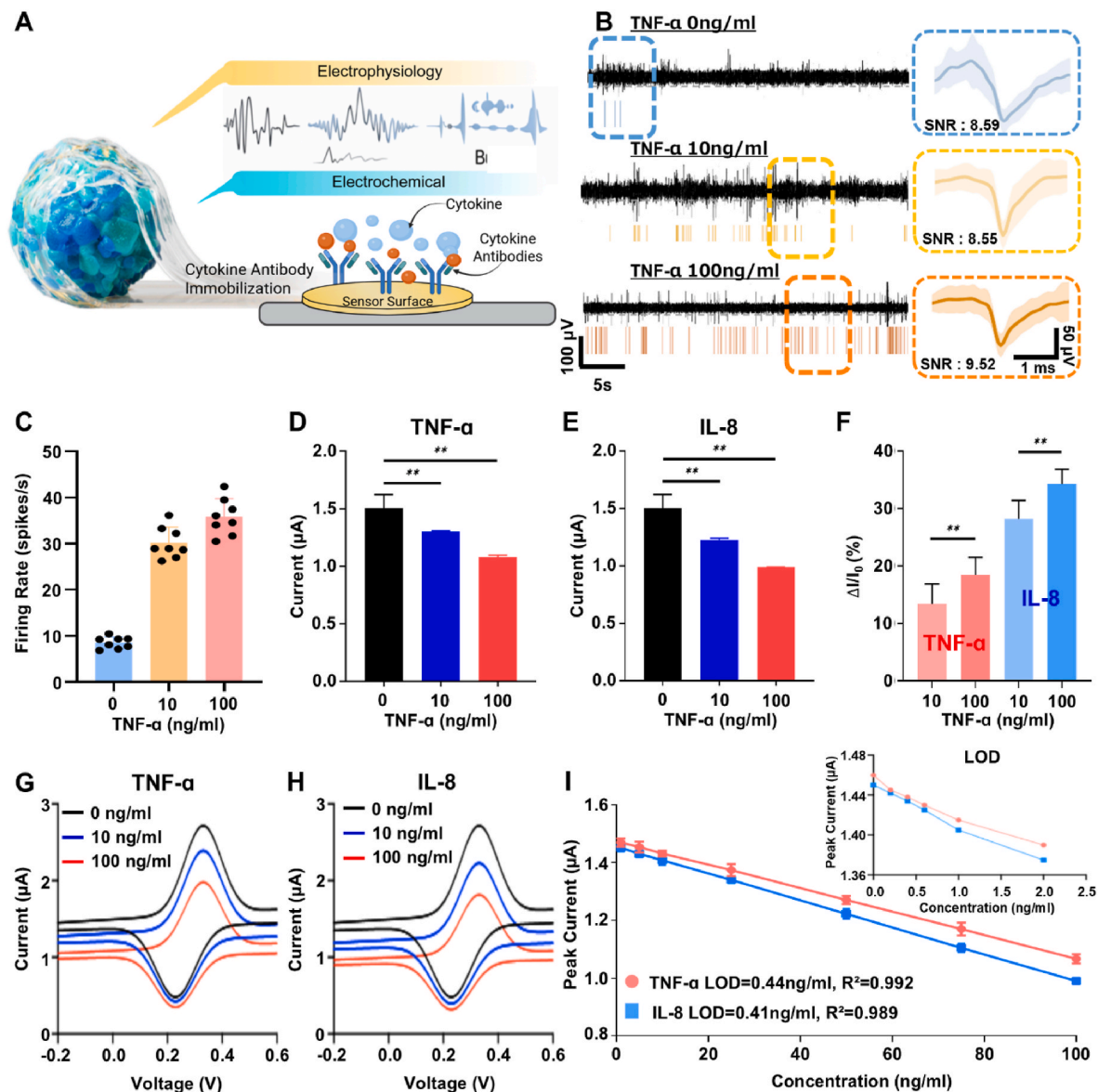


Fig. 5. Simultaneous electrophysiological and electrochemical characterization of TNF- α induced inflammatory responses in human spinal cord organoids.

(A) Schematic illustration of the multimodal bioelectronic interface enabling concurrent electrophysiological and electrochemical recordings from a 3D organoid. (B) Representative extracellular recordings from organoids treated with 0, 10, and 100 ng/ml TNF- α . Expanded spike waveforms and raster plots are shown on the right and bottom, respectively. (C) Quantified firing rates following TNF- α treatment. (D, E) Electrochemical current responses for TNF- α (D) and IL-8 (E) detection. (F) Relative signal changes ($\Delta I/I_0$, %) for TNF- α and IL-8 sensing. (G, H) Representative cyclic voltammetry (CV) responses of TNF- α (G) and IL-8 (H) sensing channels under different TNF- α stimulation conditions. (I) Calibration curves of TNF- α and IL-8 sensors showing linear concentration-dependent current changes over a range of 1–100 ng/ml, including calculated limits of detection (LOD) and corresponding R^2 values. Data are presented as mean $\pm$ SD ($n = 9$ independent measurements). Statistical significance was assessed using one-way ANOVA followed by Tukey's multiple-comparison test (* $p < 0.05$, ** $p < 0.01$).

LOD values of 0.44 ng/ml for TNF- α and 0.41 ng/ml for IL-8, and corresponding R^2 values of 0.992 and 0.989, respectively. Low-concentration validation showed that reliable current changes became clearly distinguishable from the blank/background response from 0.6 ng/ml, indicating a practical detection level of 0.6 ng/ml under the present experimental conditions (Fig. S1, Fig. S15).

Overall, inflammatory stimulation induced concurrent increases in neuronal excitability and cytokine release in hSCOs. TNF- α treated organoids exhibited elevated firing rates, shorter inter-spike intervals, and more sustained firing activity, while electrochemical measurements revealed dose-dependent increases in TNF- α , and IL-8-associated signals. These findings demonstrate that the foldable bioelectronic interface enables simultaneous and correlated monitoring of electrical and

biochemical responses, providing an integrated platform for investigating neuroinflammatory dynamics in human organoid models. Negligible signal crosstalk during simultaneous electrophysiological recording and electrochemical CV detection was further confirmed (Fig. S16).

4. Conclusion

This study presents a thermally adaptive bioelectronic interface for the multimodal acquisition of electrophysiological and electrochemical signals from hSCOs. By incorporating a SMP that softens at physiological temperature (36–37 $^{\circ}\text{C}$), the platform achieves conformal integration with complex 3D organoid geometries, thereby addressing key

interfacial limitations associated with conventional planar and curved bioelectronic systems.

The integration of electrophysiological recording electrodes with antibody-functionalized electrochemical sensors within a single device enables the simultaneous monitoring of neural network activity and cytokine release. Finite element simulations, together with experimental validation, confirm that temperature-dependent mechanical adaptation occurs within mechanically and thermally safe regimes, supporting stable and reliable biointerfacing. Experimentally, the platform enables consistent electrophysiological recordings under both basal and TNF- α induced inflammatory conditions. Organoids exposed to graded TNF- α stimulation exhibit distinct modulation of electrical activity, accompanied by dose-dependent electrochemical responses associated with TNF- α and IL-8 release.

Importantly, in contrast to conventional destructive endpoint assays that rely on sample fixation or lysis, the proposed interface enables non-destructive, real-time monitoring of both functional and biochemical signals. This capability enables repeated measurements from the same organoid while preserving tissue integrity, thereby reducing sample consumption and improving experimental efficiency. Rather than replacing established molecular assays, this platform serves as a complementary and minimally invasive approach for rapid functional screening.

Although the present study focuses on inflammatory modulation in hSCOs, additional work is needed to assess long-term operational stability and scalability under extended culture conditions. Future efforts will focus on integrating therapeutic intervention studies, including drug treatment and functional recovery assays, while expanding the range of detectable biomarkers and disease-specific organoid applications.

CRediT authorship contribution statement

Seungjun Lee: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Sangmin Han:** Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. **Hyogeun Shin:** Formal analysis, Project administration, Validation, Writing – review & editing. **Ju-Hyun Lee:** Investigation, Project administration, Validation, Writing – review & editing. **Namsun Chou:** Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix. ASupplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bios.2026.118950>.

Data availability

Data will be made available on request.

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