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NOTE

Organotypic human brain slice cultures as a translational testing platform for novel neuromodulation devices

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Abstract

Objective. To establish organotypic human brain slice cultures (hBSCs) as a translational screening platform for evaluating novel neuromodulation devices and to demonstrate the feasibility of the model using magnetoelectric nanoparticles (MENPs) as a representative neurostimulation modality. *Approach.* Viable hBSCs were prepared from resected cortical tissue of epilepsy surgery patients and GCaMP-based calcium imaging, multi-electrode array recordings, and immunohistochemical staining for c-Fos were conducted. The MENPs were injected into the hBSCs and stimulated with an alternating magnetic field to assess their neuromodulatory effects. *Main Results.* GCaMP transduction enables the real-time visualization of MENP-induced neuronal activity. Electrophysiological signals, including spiking and local field potentials, were observed in fresh, but not cultured, slices. c-Fos immunostaining revealed a significant increase in c-Fos expression in stimulated MENP-injected cultures compared to sham-treated controls. This protocol yielded reproducible tissue viability and consistent results across patient-derived samples. *Significance.* This technical note demonstrates that hBSCs represent a reproducible and ethically preferable translational model suitable for screening applications in neurotechnology research. The platform enables early-stage functional evaluation of neuromodulatory devices, particularly those with a higher risk of failure *in vivo* or curiosity-driven early-phase concepts in a setting superior to traditional *in vitro* approaches. This platform may help reduce reliance on animal models in neurotechnology development.

1. Introduction

With 9237 542 animals used for scientific purposes in 2022 in EU member states and Norway (EU 2022), the reliance of the scientific community on animal models cannot be underestimated (Keifer and Summers 2016). This reliance also holds for neuroscience, as it relies heavily on animal models, particularly rodents, to investigate complex brain functions and behavior (Keifer and Summers 2016, Moulin *et al* 2021). From an ethical perspective, the use of animals in scientific research can be justified (Machan 2002). However, according to the 4R's framework (Reduction, Refinement, Replacement, and Responsibility) the capacity of animals to experience pain and suffering continues to present an ethical conflict in their use (Kiani *et al* 2022).

Several *in vitro* and *ex vivo* methodologies have been proposed as alternative platforms for testing of neural implants, particularly as screening tools for

assessing tissue responses before progressing to *in vivo* studies (Gulino *et al* 2019). Conventional cell culture models, including human-induced pluripotent stem cells and immortalized cell lines, have been identified as valuable tools for central nervous system-related testing platforms (Nikolakopoulou *et al* 2020). However, they lack the structural and cellular complexity of native brain tissue. Brain-on-a-chip systems and organoids offer advanced alternatives; however, they still face challenges in replicating the intricate cytoarchitecture and functional connectivity of the human brain (Cassotta *et al* 2022).

Nanomaterials have been tested in organotypic brain slice cultures (BSCs) across different species, demonstrating their utility in studying neuroinflammation, tissue responses, and neurodegeneration (Kristensen *et al* 2001, Huuskonen *et al* 2005, Ereifej *et al* 2013, Usmani *et al* 2016). Despite these advancements, widely accepted testing tools for assessing nanomaterial toxicity in neural implants remain scarce (Chen *et al* 2020). Human BSCs (hBSCs) are advantageous because they preserve the human-specific cytoarchitecture, cellular diversity, extracellular matrix composition, and neuronal connectivity (Noraberg 2004, Schwarz *et al* 2019, Barth *et al* 2021). These attributes make hBSCs a compelling model for evaluating novel nanomaterials for neuromodulation, particularly during preliminary screening (Gulino *et al* 2019). This work introduces hBSCs as a translational testing platform for evaluating novel implantable devices for deep-brain neuromodulation. In the early stages of neurostimulation material development, various screening studies are required to assess stimulation parameters and material biocompatibility before progressing to *in vivo* experiments.

As magnetoelectric nanoparticles (MENPs) have already been tested in rodents, showing their potential to change neurophysiological activity and behavior (Kozielski *et al* 2021, Kumari *et al* 2024, Dominguez-Paredes *et al* 2025), this study establishes a pipeline for hBSC-based testing of MENPs, validating tissue viability, and employing techniques such as calcium imaging, electrophysiology, and immunohistochemistry (IHC) for c-Fos to assess their potential for neuromodulation research in a reverse validation approach. c-Fos was selected as a marker of neuronal activity because of its well-established and widespread use in neuroscience research, and straightforward quantification (Lara Aparicio *et al* 2022).

2. Method

2.1. Brain tissue and cerebrospinal fluid collection

Eleven patients undergoing anterior temporal lobectomy (maximal temporal lobe resection in one case) for drug-resistant epilepsy between January 2023 and September 2024 at the Department of Neurosurgery of the Maastricht University Medical Center + (MUMC+) were asked to provide consent (METC 2024-0344) to collect resected brain tissue and human cerebrospinal fluid (hCSF) during their presurgical visit to the outpatient clinic.

After obtaining written informed consent, resected brain tissue was collected in the operating theatre immediately after resection. Furthermore, the hCSF removed by the surgeon as part of the standard procedure was collected in a tube and placed on ice (figure 1(A)). As part of general anesthesia, drugs such as propofol, norepinephrine, sufentanil, and dexamethasone were administered at typical doses. Following anterior temporal lobectomy, 1 × 1.5 cm brain tissue samples were taken from the posterior lateral region of the excised temporal neocortex specimen. The remaining tissue was used for histopathological analysis as part of standard medical process.

2.2. Magneto-electric nanoparticles

The MENPs consist of magnetostrictive $CoFe_2O_4$ nanoparticles coated with piezoelectric $BaTiO_3$. When exposed to a direct (DC) magnetic field overlaid with an alternating (AC) field, the magnetostrictive core is deformed and strain is applied to the piezoelectric coating, thus eliciting an electric polarization. MENPs contained 36.1 ± 0.6% $BaTiO_3$ and 63.9 ± 0.6% $CoFe_2O_4$, and the average diameter was 277 ± 18 nm with a zeta potential of −6.7 ± 0.5 mV in artificial cerebrospinal fluid (aCSF) as measured in detail in a previous study of our group (Kozielski *et al* 2021). Before application, the MENPs were diluted in Milli-Q water (100 μg ml^{-1}) and vortexed thoroughly.

2.3. hBSCs preparation

The organotypic human brain slice protocol was adopted from a previous work by Bak *et al* 2024. In summary, the neocortical brain tissue samples were immediately submerged in ice-cold, carbogenated (95% O_2 5% CO_2) slicing-aCSF (s-aCSF; figure 1, A). s-aCSF was composed of 110 mM choline chloride, 26 mM $NaHCO_3$, 1.25 mM $Na_2HPO_4{}^*2H_2O$, 11.6 mM sodium ascorbate, 3.1 mM sodium pyruvate, 7 mM $MgCl_2$, 0.5 mM $CaCl_2$, 2.5 mM KCl, 10 mM glucose dissolved in MilliQ, for incubation 1% penicillin/ streptomycin/ amphotericin B was added. Neuronal activity was blocked by choline, which replaces sodium, to mitigate the expected slicing stress. hCSF was centrifuged (4 °C, 2000 g) and sterile filtered to remove blood and either stored at −80 °C or used immediately.

After cleaning and trimming (figure 1(B)), brain slices with an area of approximately 0.75 × 0.75 cm and a thickness of 300 μm were produced using a vibratome (Leica VT1200S, Wetzlar,Germany; figures 1(C) and (D)). Throughout the procedure, the tissue was submerged in ice-cold carbogenated

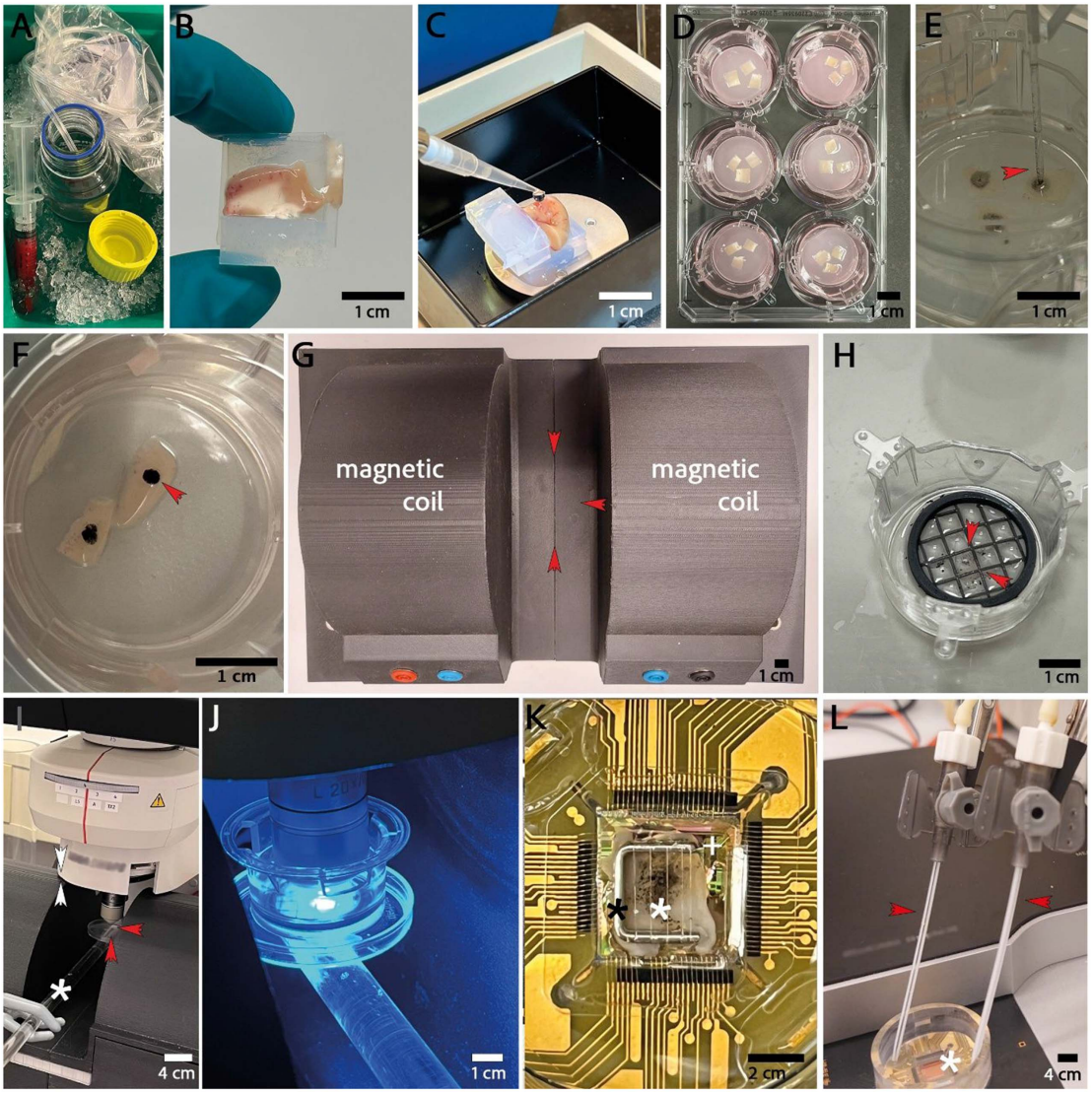

Figure 1. (A) Brain tissue acquisition and transport in ice-cold, oxygenated s-aCSF. (B, C) Preparation of organotypic cultures: a trimmed block of brain tissue is glued to agarose and sectioned in a vibratome chamber under continuous perfusion with ice-cold, oxygenated s-aCSF. (D) Cultures placed on an air–liquid interface membrane insert. (E), (F) injection of MENPs into the hBSC. (G) Custom-built stimulation coil used for magnetic actuation, with highlighted stimulation chamber. (H) MENP-injected hBSC fixated beneath a custom resin net grid prior to immersion for calcium imaging to prevent floating. (I) *Custom made acrylic well holder, red arrows point at well holder within the stimulation chamber, white arrows indicate a gap between the coil and the microscope as to prevent any vibration being transmitted to the microscope. (J) Active calcium imaging set-up (acrylic well holder as seen in (I) now holding well/ hBSC/ resin net under stimulation and ongoing fluorescent microscopy). (K) Multi-electrode array (MEA) with a net-fixated MENP injected hBSC (*(black) harp slice grid, *(white) hBSC with visible MENP aggregates, +MEA chip). (L) *Zoomed out MEA chamber with arrows pointing at CSF-perfusion system.

s-aCSF. Afterwards, the brain slices were kept at 37 °C in an air–liquid interface, including an intermediate step of HEPES medium (23.5 ml DMEM/F-12, 23.5 ml Neurobasal medium, 1 ml B27 supplement, 0.5 ml N2 supplement, 0.5 mL GlutaMAX and 0.5 ml non-essential amino acids are mixed to yield 50 ml) for 60 min. For cultivation, slices were transferred to a well plate with semi-permeable (0.4 μm pore size) net inserts (Corning® Transwell® 24 mm Transwell with 0.4 μm pore polyester membrane insert, TC-treated) serving as an air–liquid interface of hCSF and aCSF in a 1:1 ratio (125 mM NaCl, 25 mM $NaHCO_3$, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 1 mM $MgCl_2$*6 H_2O, 2 mM $CaCl_2$, 25 mM glucose diluted in MilliQ) interface and incubated for at least 48 h (37 °C, CO_2 5%, O_2 95% in humidified environment; figure 1(D)).

2.4. Viral transduction

The cultures were then transduced using adeno associated retrograde viral vector (AAV) derived from pGP-AAV-syn-jGCaMP7f-WPRE (Addgene, Massachusetts, USA). The AAV was applied by bringing a 2 μl drop into contact with the surface of the hBSC. Brain slices were cultured for 3, 7 and 10 d, during which 0.75 ml of aCSF was replaced in well plates every other day to optimize transduction efficiency. On the 10th day post-transduction, the experiments were conducted.

2.5. Stimulation and fluorescence image acquisition

hBSCs were randomly assigned into two groups: MENP and stimulation (M+/S+) and MENP and no stimulation (M+/S−). Both groups received 2 μl injections of MENP suspension (∼3 μg of MENPs), followed by incubation to allow MENP diffusion (figures 1(E) and (F)). After injection, the M+/S+ group was stimulated with a custom coil system (figure 1(G)) at 220 mT DC; 6 mT AC, 140 Hz. The stimulation parameters were based on our recent *in vivo* and in silico studies, through which we have developed and tested MENP-based neurostimulation technology (Kozielski *et al* 2021, Kumari *et al* 2024, Dominguez-Paredes *et al* 2025). The stimulation was performed under a Leica FS fixed-stage microscope (Leica Microsystems GmbH, Wetzlar, Germany) equipped with a fluorescence illumination system and a 20×/0.50 numerical aperture (NA) objective water-dipping lens (figures 1(H)–(J)). Slices in M+/S− group was handled in the same manner, but with the coil turned off such that the tissue was only exposed to the DC magnetic field. Throughout the calcium imaging data acquisition, hBSCs were submerged in carbogenated aCSF and mechanically stabilized using a custom-made net grid (figure 1(H)). Low-intensity fluorophore light and reduced exposure time were used to minimize phototoxicity and photobleaching. Vibration issues were addressed using linear stack alignment with SIFT in ImageJ software [version 1.8; National Institutes of Health, Bethesda, USA] to ensure precise image alignment. Additionally, background subtraction was performed to correct for photobleaching effects and enhance the accuracy and clarity of the resulting images. Two hours after calcium imaging, M+/S+ and M+/S− groups received DC/AC and DC stimulation respectively, followed by 2 h of incubation and fixation paraformaldehyde for cFos IHC.

2.6. Multi-electrode array recordings

For electrophysiological recordings with multi-electrode array (MEA), aCSF was prepared by dissolving the following in 500 ml of Milli-Q water: 3.68 g NaCl (126 mM), 0.112 g KCl (3 mM), 1.008 g $NaHCO_3$ (24 mM), 0.991 g glucose (11 mM), 0.147 g $CaCl_2$ (2.65 mM), 0.154 g $MgSO_4$ (2.56 mM), and 0.078 g NaH_2PO_4 (1.3 mM). The pH of the solution was adjusted to 7.4. Brain slices were incubated in carbogenated aCSF for 1.5 h prior to recording, which was performed using the CorePlate™ Single-Well HD-MEAs, a high-density MEA system (3Brain AG, Switzerland; figures 1(K) and (L)). hBSCs were stimulated in the coil prior to recording.

2.7. Immunohistochemical staining

After fixation in paraformaldehyde for 24 h, the tissue slices were incubated in 20% sucrose for another 24 h, then snap-frozen, sectioned into 20 μm slices, and stored at −80 °C until staining. c-Fos staining was used to assess neuronal activity (Morgan and Curran 1986). Tissue sections were incubated overnight with polyclonal c-Fos (rabbit 1:1000; Santa Cruz Biotechnology Inc.; sc-253), followed by biotinylated donkey anti-rabbit secondary antibody (1:400; Jackson ImmunoResearch Laboratories Inc.; 711065152) and avidin–biotin peroxidase complex (1:800; Elite ABC kit, Vector Laboratories; PK-6100). Staining was visualized using 3,3′-diaminobenzidine combined with NiCl2 intensification.

2.8. Bright-field image acquisition and quantification of c-Fos positive neurons

Bright-field images were acquired using an Olympus AX 70 microscope (Olympus, Zoeterwoude, The Netherlands). With a 10×/0.30 NA objective and CellP software (version 6.1). The lamp intensity stabilized at 6.8 V, and the exposure time was set to 80 ms in the monochrome mode for all samples. A daily calibration of pixel-to-distance (1 pixel = 0.49 μm) was conducted using a stage micrometer. For each section, three non-overlapping 1 mm^2 cortical regions were systematically imaged from the medial to the lateral edge. Image analysis was carried out using FIJI (ImageJ v1.54) by an evaluator blinded to the experimental grouping and conditions: Renyi entropy auto-thresholding followed by manual adjustment (±5%), ROI delineation, and multi-point counting of Ni-DAB-labeled c-Fos positive nuclei. Cell counts were normalized to area and averaged across 3–5 sections per slice, and statistical analysis was performed using GraphPad Prism 10.1.2 (GraphPad Software, San Diego, California, USA).

3. Results

3.1. Study population

Eleven experiments were conducted using neocortical tissue samples obtained from 11 patients who underwent anterior temporal lobectomy with amygdalohippocampectomy for epilepsy treatment at Maastricht University Medical Center between January 2023 and September 2024 (table 1).

3.2. Viral transduction and calcium imaging

During the study, viral transduction was halted on days 3, 7, and 10 to perform fluorescence imaging, with the optimal results observed on day 10 (figures 2(A)–(F)). Optimal' refers to the combination of reproducible, robust GCaMP fluorescence, preserved neuronal morphology, and visible spontaneous calcium transients across multiple fields of view, indicating mature viral expression and functional neuronal activity suitable for subsequent stimulation experiments. hBSCs were submerged in aCSF and held in place using a 3D-printed net (figure 1(H)) to allow for accurate image acquisition. The coil had to be detached from the microscope to avoid

Table 1. Overview of surgical procedures and histopathological findings in patients undergoing anterior temporal lobectomy for epilepsy. The table summarizes the type and side of surgery performed and neuropathology of the neocortex.

Exp	Age	Sex	Side	Histopathological analysis neocortex
1	61	M	Right	Minor gliotic changes
2	55	F	Left	Mild gliotic changes
3	46	F	Right	Minor gliotic changes in the temporal lobe, severe hippocampal sclerosis
4	60	M	Right	Minor hemorrhagic changes
5	32	M	Left	Minor gliotic changes
6	41	M	Left	Minor gliotic changes
7	27	F	Right	Minor gliotic changes
8	32	M	Left	Minor gliotic changes.
9	44	M	Left	Minor gliotic changes
10	57	F	Left	Minor gliotic changes
11	39	M	Left	Minor to moderate gliotic changes

vibration (figure 1(I)). The image was adjusted by carefully moving the custom-made hBSC-well holder (figure 1(J)). Fluorescence stacks were analyzed using Fiji (Schindelin *et al* 2012), where background subtraction was applied to mitigate the effects of photobleaching and temporal noise. To further stabilize the images and prevent flickering artifacts, the 'Linear Stack Alignment with SIFT' plugin was employed.

3.3. Multi electrode array recordings

MEA recordings were performed in a 1:1 mixture of hCSF and aCSF. Fresh, carbogenated aCSF was continuously perfused through the MEA chamber throughout the recordings (figure 1(L)). Because the magnetic field of the coil impaired the performance of the device, stimulation had to take place before placing the hBSC on the MEA. Notably, spikes and local field potentials (LFPs) were only detected in carbogenated aCSF supplemented with 7.5 mM KCl or 500 μM 4-aminopyridine. Recordings were attempted on cultured human brain slices immediately after preparation and on days 2, 5, and 7. However, LFPs and spikes were exclusively observed on the day of sectioning. Consequently, electrophysiological recordings were conducted immediately after slicing the brain tissue to a thickness of 300 μm prior to culturing.

3.4. IHC and c-Fos quantification

c-Fos expression was clearly detectable within the tissue (figures 3(A) and (B)) along with visible aggregates of MENPs (figure 3(C)). In addition to the intended nuclear staining, cytoplasmic c-Fos signals were observed; however, only cells with clearly distinguishable nuclear staining were included in the analysis. Although not consistently observed, group-dependent differences in c-Fos expression were at times discernible by qualitative assessment (figures 3(D)–(G)).

Pooled c-Fos counts from the respective experimental conditions were assessed for normality using the Shapiro–Wilk test, and the data violated assumptions of normality ($p < 0.0001$). c-Fos-positive cell counts were normalized within each experimental group, and group differences in fold change were subsequently analyzed using the non-parametric two-tailed Mann–Whitney test. A significantly higher fold-change in c-Fos-positive cells was observed in the M+/S+ group than in the M+/S− group ($p = <0.001$) (figure 3(H)).

4. Discussion

This study aimed to evaluate whether hBSCs represent a translational testing platform for assessing the neuromodulatory potential of novel technologies using MENPs as a representative example. We successfully prepared hBSCs, performed viral transduction, and modulated neuronal activity by using MENPs. Neuronal modulation and culture viability were confirmed using a dual readout setup for calcium imaging and IHC staining for c-Fos. The visible expression of c-Fos in all experimental groups implied tissue viability at the time of fixation (Lara Aparicio *et al* 2022), whereas significant differences in expression levels between groups showed the ability of MENPs to modulate neuronal activity.

4.1. Customization of BSCs setup

Several adaptations were made to optimize the procedure for specific needs, improve reproducibility, and address the technical challenges encountered during implementation. The protocol described by Bak *et al* was both thorough and adaptable. Effective communication with the surgical team was crucial, and we found it essential to be present in the operation room prior to opening the dura mater to collect hCSF for incubation. When feasible, instead of suctioning, the surgeon aspirated excess hCSF using a syringe, particularly after accessing the subdural space, allowing immediate cooling on ice. Regarding tissue preparation, we removed the pia mater only when it was clearly identifiable or naturally detached,

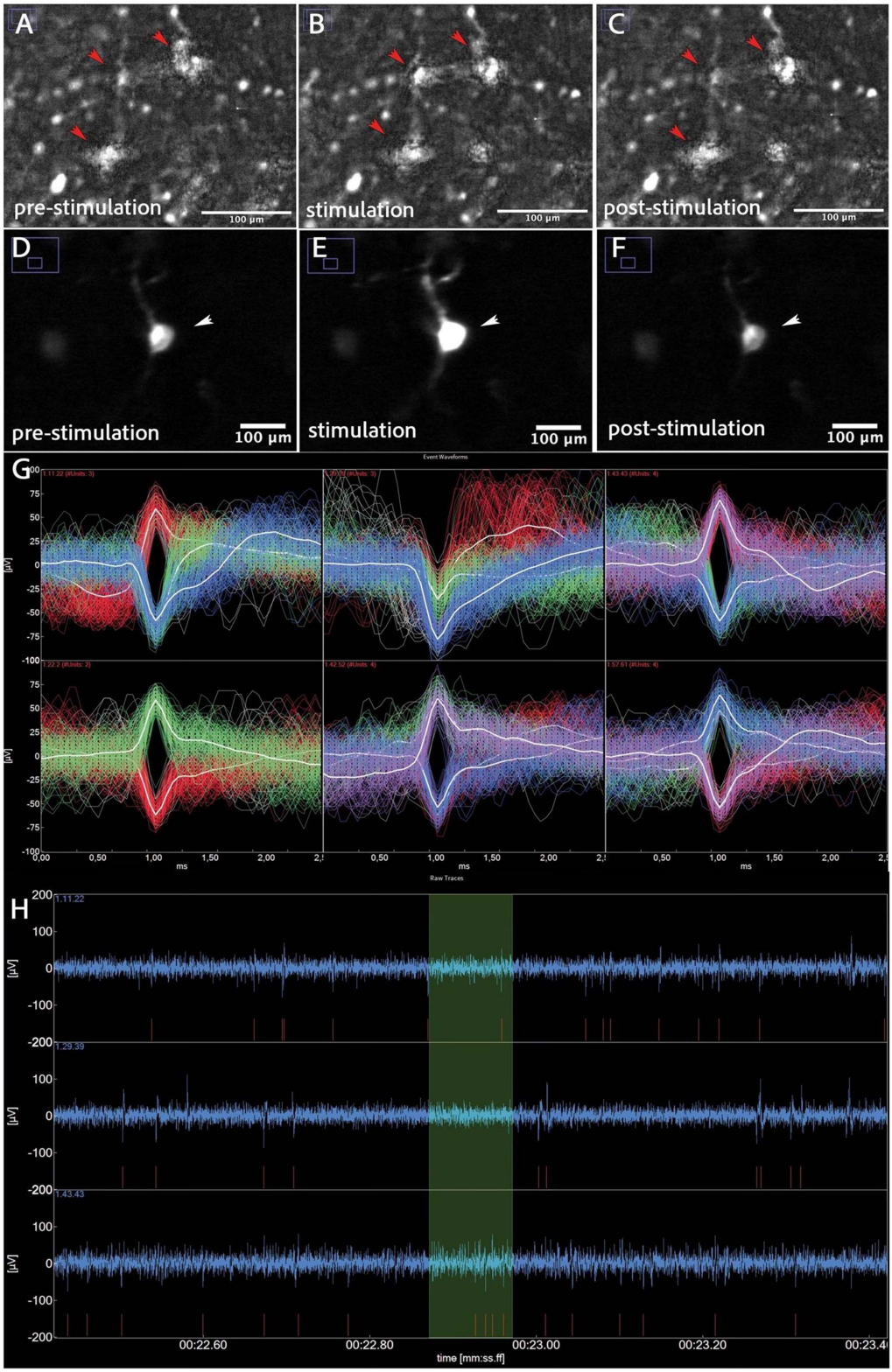

Figure 2. (A) GCaMP positive neurons pre-stimulation (B) GCaMP positive neurons showing network activity (arrows) whilst under stimulation. (C) GCaMP positive neurons, post-stimulation. (D) Single GCaMP positive neuron (arrow), pre-stimulation. (E) Single GCaMP positive neuron (arrow) showing calcium influx under stimulation. (F) Single GCaMP positive neuron (arrow) with reduced calcium influx, post-stimulation. (G) Spike waveforms. Each panel corresponds to a different electrode channel, displaying multiple event-aligned waveforms over a 2–3 ms window. The superimposed traces (in red, white, green, and blue) reflect individual neuronal spikes and/or population responses, demonstrating variability in amplitude and shape across electrodes. (H) Raw electrophysiological trace recording. The blue waveform shows extracellular voltage over time, reflecting spontaneous neuronal activity. Red markings beneath the trace indicate individual spike detection events (putative action potentials). Green shaded regions highlight intervals of interest (burst detection windows). The relatively stable baseline and discrete spike events suggest viable neuronal health and clear signal-to-noise separation.

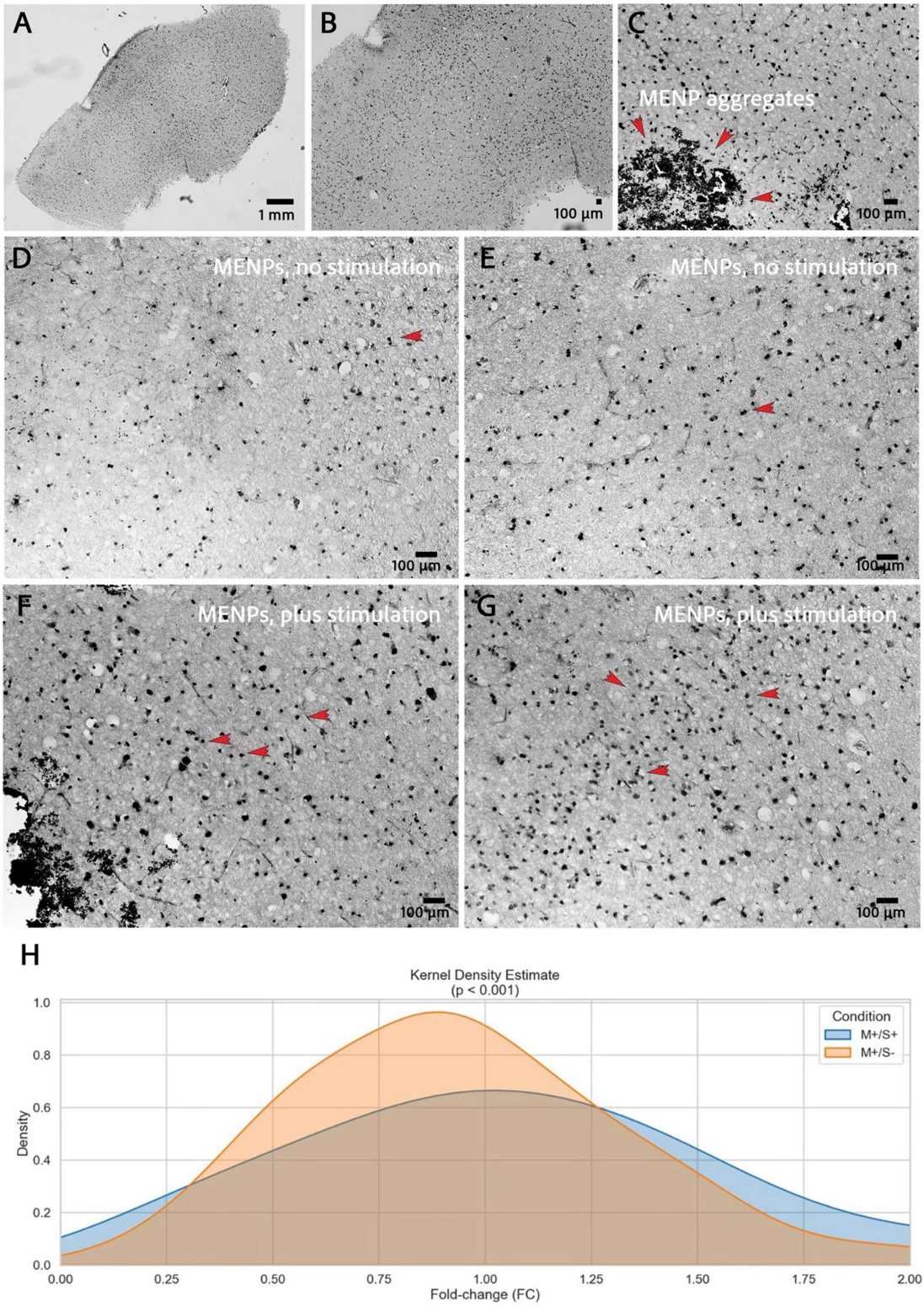

Figure 3. (A) Overview image of a whole hBSC-slice, stained for c-Fos. (B) Visible dense c-Fos expression. (C) Magnification used to count c-Fos positive nuclei and exemplary MENP aggregates. (D/E) IHC images of hBSCs from the MENP no-stimulation group showing visibly less c-Fos expression (red arrows indicate exemplary c-Fos-positive nuclei). (F/G) IHC images of hBSCs from the MENP stimulation group showing visibly increased c-Fos expression density (red arrows indicate exemplary c-Fos-positive nuclei). (H) c-Fos expression of M+/S+ vs M+/S− groups fold change, visualized by Kernel Density estimate (Mann–Whitney $p = <0.001$).

as manual dissection often caused cortical damage. Notably, vibratome sectioning was unaffected by its presence. For transferring tissue slices, a blunt spatula or probe minimized mechanical stress. After trimming and positioning hBSCs on net inserts, excess liquid was removed using a 1000 μl pipette. If hBSCs

overlapped, folded, or were mispositioned, the precise application of small aCSF droplets resolved these issues. Depending on the amount of brain tissue obtained, we produced 18, 36, or 44 hBSCs, with each well containing three slices. In theory, MENP injection could occur at any time between production and at least 2 h prior to stimulation. However, immediate injection resulted in visible particle migration from the hBSCs to the net insert, which was reduced when injection was performed 2 h prior to stimulation. We tested two injection setups: a microinjector and a 2 μl pipette. While the microinjector allowed for precise spatial approximation, its static tubing system led to particle settling, clogging, or uneven distribution. The 10 μl pipette proved more practical, allowing agitation of the MENP suspension immediately before injection. We first formed a droplet at the pipette tip, brought it into contact with the tissue until the surface tension broke, and injected the remaining volume. The insert was removed from the plate and placed in a single well for stimulation. The wells were removed from the incubator for stimulation and returned. The experimental groups were assigned to the individual wells. All groups were handled identically to prevent detection of c-Fos expression due to mechanical stress.

The staining protocol largely followed standard procedures; however, special attention was required during tissue mounting because of fragility of the slices. We used a cut-off pipette to transfer the slices onto glass slides, removing excess liquid with tissue paper, and a 10 μl pipette. Following overnight drying, we recommend extending the oven incubation period from one to three hours to improve adhesion and prevent tissue detachment during dehydration.

4.2. Evaluation of hBSCs as a translational model

We aimed to determine whether hBSCs, as prepared and maintained in this system, can serve as a robust and scalable platform for functional studies and screening tools. This evaluation is central to our overarching motivation to reduce dependence on animal experiments by advancing human *ex vivo* models.

Depending on the experimental design, several cultures could have been produced for testing, for example, different stimulation regimes, particle loads, or readouts such as electrophysiological recordings. When applied to animal experiments, this would necessitate multiple ethical reviews and significant investments in time and money. Furthermore, as there are significant differences in gene expression and cell morphology between rodents and humans (Hodge *et al* 2019), the observability of any effects or mechanisms in hBSCs should be mandatory before proceeding to *in vivo* experiments, thus highlighting its capability as a translational tool for promising new approaches. A significant challenge when working with this model is the heterogeneity in patient characteristics, such as age, sex, medication (antiepileptic drugs that directly influence neuronal activity), peri-surgical conditions, and morphology of the retrieved tissue. Although this can be tackled by either including a multitude of cases and showing an overall significant difference, as we did, or by treating every experiment as standing alone and performing analysis only within hBSCs drawn from one case. The choice of readout method introduces additional challenges. For example, c-Fos is commonly used as a marker for neuronal activity because it is relatively easy to detect and is widely recognized for this purpose. However, these limitations should also be considered. c-Fos is not exclusively expressed by neurons but also by other central nervous system cells, such as glial cells. Furthermore, mechanical stimuli can induce c-Fos expression in glia and neurons (Jaworski *et al* 2018). These mechanical stimuli pose practical challenges because handling cultures during experiments exerts mechanical stress that may trigger c-Fos expression. It is crucial to handle cultures from different conditions as consistently as possible and to minimize false-positive detections by waiting at least two hours after injecting cultures with MENPs before assessing c-Fos expression. In contrast, electrical stimuli exclusively induce c-Fos expression in neurons (Hisanaga *et al* 1990, Cruz-Mendoza *et al* 2022). Additionally, c-Fos expression reaches its maximum earlier in astrocytes, within 20–45 min after stimulation (Cruz-Mendoza *et al* 2022), whereas peak expression in neurons occurs at approximately two hours (Lara Aparicio *et al* 2022). Therefore, the differences quantified at the selected time point are expected to reflect neuronal activation in response to MENP stimulation. To further characterize the c-Fos-positive cells, double immunofluorescence labeling can be performed depending on the study design. Spontaneous epileptiform activity originating from the resected tissue may lead to false-positive electrophysiological recordings. Although this concern is not strongly supported by existing literature, it warrants acknowledgment. Spontaneous interictal or ictal activity at the single-cell level is a rare phenomenon (Levinson *et al* 2020). Notably, a study employing a similar MEA-based experimental setup, co-culturing an epileptic human neocortical biopsy with hiPSC-derived cortical neurons, also demonstrated that epileptiform network activity emerged only in the hybrid co-culture, whereas neither the biopsy nor the neuronal layer alone showed seizure-like discharges (Hu *et al* 2023).

5. Conclusion

The hBSC model demonstrated the feasibility of studying novel neurostimulation devices in

functional human neuronal networks. This approach provides an ethically advantageous and translational testing platform that can be used to pre-screen promising technologies before testing in animal studies, especially those with a higher risk of failure *in vivo* or curiosity-driven early-phase products. Identifying effective interventions in human tissue may reduce the number of required animal experiments while increasing the relevance and impact of subsequent *in vivo* research. As ethical and funding frameworks increasingly limit the use of animal models, human-based systems like hBSCs are well-positioned to become essential tools in translational neuroscience. Future work will focus on additional experiments and refining the described readouts, further enhancing the platform for systematic evaluation of emerging nanoscale and bioelectronic neuromodulation technologies.

Data availability statement

The data cannot be made publicly available upon publication because no suitable repository exists for hosting data in this field of study. The data that support the findings of this study are available upon reasonable request from the authors.

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Conflict of interest

Franz A M Eggert acknowledges the support from the Thiemann Foundation through a doctoral scholarship. The foundation had no role in the study design, data collection, analysis, decision to publish, or manuscript preparation. All other authors declare no competing interests.

Ethics statement

Human brain tissue and cerebrospinal fluid (CSF) were obtained from patients undergoing anterior temporal lobectomy for drug-resistant epilepsy at the Maastricht University Medical Center (MUMC+). All procedures involving human participants were conducted in accordance with the Declaration of Helsinki and were approved by the local medical ethics committee (METC 2022-3539 and 2024-0344). Written informed consent was obtained from all participants prior to inclusion in the study. Tissue collection was performed in compliance with the institutional and national guidelines for research involving human biological materials.

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