



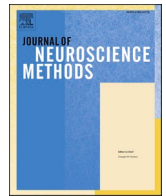
A platform for repeated electric field stimulation of organotypic spinal cord slice cultures enables comparison of myelin basic protein signal across

Downloaded from: <https://research.chalmers.se>, 2026-10-10 22:08 UTC

Citation for the original published paper (version of record):

Matter, L., Kellaway, S., McCaughey-Chapman, A. et al (2027). A platform for repeated electric field stimulation of organotypic spinal cord slice cultures enables comparison of myelin basic protein signal across stimulation paradigms. *Journal of Neuroscience Methods*, 437. <http://dx.doi.org/10.1016/j.jneumeth.2026.110908>

N.B. When citing this work, cite the original published paper.



A platform for repeated electric field stimulation of organotypic spinal cord slice cultures enables comparison of myelin basic protein signal across stimulation paradigms

L. Matter^{a,*}, S.C. Kellaway^b, A. McCaughey-Chapman^c, S. Lopez^b, B. Hawker^c, B. Harland^b, D. Svirskis^b, M. Asplund^{a,d}

^a Department of Microtechnology and Nanoscience, Chalmers University of Technology, Gothenburg, Sweden

^b School of Pharmacy, Cure Programme, University of Auckland, Auckland, New Zealand

^c Department of Pharmacology and Clinical Pharmacology, Cure Programme, Centre for Brain Research, School of Medical Science, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

^d Science for Life Laboratory (SciLifeLab), Gothenburg, Sweden

ARTICLE INFO

Keywords:

Electric stimulation
Organotypic spinal cord slice culture
Myelination

ABSTRACT

Background: Electric field (EF) stimulation is a promising strategy to promote repair in the central nervous system (CNS). The cellular mechanisms underlying CNS repair induced by multi-day subthreshold EF stimulation remain poorly understood, in part because systematic exploration of stimulation parameter space is challenging in animal models. Rat organotypic spinal cord slice cultures (OSCs) provide a physiologically relevant *ex vivo* model that preserves cell-to-cell interactions and tissue architecture, making them well suited for studying parameter-dependent EF effects. However, a method enabling controlled, repeatable, and biocompatible EF stimulation of OSCs has not yet been established.

New method: We developed and validated a stimulation platform and protocol for repeated EF stimulation of OSCs. The platform generated a spatially uniform transverse EF across submerged OSCs while maintaining electrochemical biocompatibility. To demonstrate biological applicability, we applied the method to lyssolecithin (LPC)-induced demyelinated OSCs.

Results: OSCs remained viable during repeated daily 40-minute submersion and stimulation over five days. EF stimulation influenced myelin basic protein, with higher-intensity or longer-duration stimulation producing a pattern consistent with partial recovery.

Comparison with existing methods: Previous platforms have demonstrated the feasibility of applying EFs to organotypic slice cultures for short durations, but whether OSCs can tolerate repeated submersion and EF stimulation without compromised viability remains unresolved. This method addresses that gap by enabling repeated, biocompatible EF stimulation of OSCs.

Conclusions: These findings establish a method for controlled EF stimulation of OSCs and highlight its utility for systematically studying EF-mediated CNS repair mechanisms.

1. Introduction

Demyelination disrupts signal transmission in the central nervous system (CNS) and underlies functional impairment in disorders such as multiple sclerosis as well as traumatic injuries including spinal cord injury (SCI) (Alizadeh et al., 2015; Dobson and Giovannoni, 2019; Franklin, 2002; Diaz and Morales, 2016). Although endogenous remyelination occurs, it is often incomplete, delayed, and results in

poorly formed myelin, leaving nerves dysfunctional (Alizadeh et al., 2015; Karimi-Abdolrezaee et al., 2004). Experimental platforms that enable controlled investigation of CNS repair mechanisms are critical for developing effective therapies. *In vitro* two-dimensional cell cultures offer experimental control and are well suited to explore specific molecular and cellular pathways, but they lack the complex tissue organization and multicellular interactions present *in vivo* (Ebert and Svendsen, 2010; Liu et al., 2022). Three-dimensional culture systems

* Corresponding author.

E-mail address: Lukas.matter@chalmers.se (L. Matter).

<https://doi.org/10.1016/j.jneumeth.2026.110908>

Received 25 June 2026; Received in revised form 5 August 2026; Accepted 10 September 2026

Available online 13 September 2026

0165-0270/© 2026 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

and organoids are more physiologically relevant and are often human-derived, yet they are limited by a lack of vascularization, incomplete recapitulation of native tissue organization, and variability between batches (Ogura et al., 2018; Ao et al., 2022; Andersen et al., 2020; Yildirim et al., 2019). In this context, *ex vivo* models are promising because they preserve cytoarchitecture, anatomical organization, and cellular interactions while avoiding many of the costs, ethical concerns, and technical complexities associated with three-dimensional *in vitro* and *in vivo* models (Pandamooz et al., 2016). Among these, organotypic spinal cord slice cultures (OSCs) are particularly interesting to explore demyelination and repair after SCI. Using an established *ex vivo* model of lysolecithin (LPC)-induced demyelination, based on rat OSCs (Hawker et al., 2024), the objective of this work is to (1) establish a platform for repeated electric field (EF) stimulation and (2) use this model to explore the effect of this treatment on remyelination.

Many pro-remyelination medications have been investigated or are in early phase clinical trials (Plemel et al., 2017). EF stimulation is an attractive complementary therapeutic strategy because it can simultaneously influence multiple biological processes involved in CNS repair (Jack et al., 2020; McCaig and Rajniecek, 1991; Yao and Li, 2016). With respect to remyelination, repeated EF stimulation over days using different stimulation paradigms has been shown to enhance proliferation and differentiation of oligodendrocyte progenitor cells (OPCs) in animal models (Li et al., 2010, 2020; Jing et al., 2015; Bacova et al., 2022; Fang et al., 2021; Zhang et al., 2014; Becker et al., 2010). Oligodendrocytes are the cells responsible for forming and maintaining the myelin sheath surrounding axons (Simons and Nave, 2016). Some studies have reported enhanced myelination in uninjured and SCI animals using short-duration (μ s) pulses that evoke muscle contraction and action potentials (Li et al., 2010, 2020; Becker et al., 2010), whereas others have observed beneficial effects after SCI using long-duration (min), subthreshold pulses (Jing et al., 2015; Bacova et al., 2022; Fang et al., 2021; Zhang et al., 2014). This suggests that the biological response to EF stimulation is robust to variations in stimulation parameters and may depend on the cumulative dose, such as a threshold time-integrated EF intensity (McCaig and Rajniecek, 1991; Tang-Schomer, 2018; Patel and Poo, 1984). In addition, EF stimulation has been shown to influence other aspects of CNS repair, including directed cell migration (Meng et al., 2011; Yao et al., 2008, 2009; Liu et al., 2015), increased neural excitability (Barra et al., 2022; Balaguer et al., 2025), and axonal guidance and growth (Jaffe and Poo, 1979; Patel and Poo, 1982; Borgens et al., 1986; Borgens, 1999; Hinkle et al., 1981).

Electrical stimulation of acute CNS slices is well-established and is typically implemented using parallel electrode setups, in which tissue slices are positioned between electrodes either at the air-liquid interface, fully submerged, or within a microfluidic chamber. For review, see Lu et al. (2023). Importantly, EF uniformity has been shown to be highest when slices are fully submerged in a confined microfluidic volume (Lu et al., 2023). Achieving a uniform field is critical for investigating dose-dependent EF effects, as non-uniform fields can lead to spatially heterogeneous EF, effectively exposing different volumes of the tissue to different EF doses. The short lifespan of acute slices restricts both analyzing the effects of repeated EF stimulation and evaluation of delayed effects in the tissue response. In contrast, organotypic slice cultures, which are commonly maintained at the air-liquid interface, can be studied over weeks, making them well suited for investigating longer-term effects such as myelination. Despite this advantage, only a limited number of studies have applied electrical stimulation to organotypic slice cultures. Meng et al. (2011) demonstrated electrotaxis of transplanted neural progenitor cells in OSCs using a microfluidic chamber during electrical stimulation lasting several hours. Shaner et al. (2023) developed a microfluidic platform for applying EFs to submerged organotypic brain slice cultures; however, repeated stimulation, while technically feasible, was not implemented. Johnson and Buonamano

(2009) developed a method for chronic stimulation of organotypic brain slice cultures using implanted microelectrodes, enabling local electrical stimulation over days. Heidemann et al. (2015) cultured OSCs on microelectrode arrays using the roller-tube method and performed electrophysiological recordings but did not apply stimulation. Together, these studies demonstrate the feasibility of applying EFs to organotypic slice cultures but leave unresolved whether OSCs can tolerate repeated submersion and EF stimulation without compromising viability, for example due to insufficient perfusion.

In this study, we establish and validate a platform and protocol for repeated EF stimulation of submerged rat OSCs, with a focus on practical handling and maintenance. The setup generates a spatially uniform transverse EF across the OSCs while maintaining electrochemical biocompatibility using conducting hydrogel electrodes with large bulk capacitance, allowing hybrid electronic/ionic charge transfer (Leal et al., 2023). Biocompatibility was validated both electrochemically, by confirming that electrode polarization remained within the water window, and biologically, by demonstrating that OSCs tolerated repeated daily 40-minute submersion and EF stimulation over five consecutive days without compromised cell viability. The experimental and stimulation durations were guided by *in vivo* studies on SCI, where prolonged electrical stimulation over weeks was found to be associated with promoted myelination (Jing et al., 2015; Bacova et al., 2022; Fang et al., 2021; Zhang et al., 2014). To provide a demonstration of the platform's biological applicability, we applied EF stimulation to OSCs after LPC-induced demyelination and quantified myelin basic protein (MBP). Higher-intensity or longer-duration stimulation resulted in MBP levels that were not significantly different from native slices; however, they also did not differ significantly from LPC-treated controls, suggesting possible partial recovery but not a reversal of the LPC-induced demyelination. Together, this work validates the platform and protocol for repeated EF stimulation of OSCs, establishes OSCs as an experimentally accessible model for controlled EF stimulation, and provides a foundation for systematically investigating EF-mediated CNS repair.

2. Results

2.1. Platform for uniform and biocompatible EF stimulation of OSCs

The platform was designed to address three requirements for repeated OSC stimulation: spatially uniform EF generation, electrochemical biocompatibility, and practical handling of culture inserts across multiple stimulation sessions. To accurately compare dose-response effects of EF, it is essential to generate a spatiotemporally uniform EF across the OSC. Previous studies have shown that achieving field uniformity requires tissue slices to be fully submerged, ensuring similar conductivity of the surrounding medium (Lu et al., 2023; Shaner et al., 2023). To this end, we developed a stimulation platform consisting of two non-metallic supercapacitive laser-induced graphene (LIG) electrodes coated with electropolymerized PEDOT:PSS to enhance their capacity to deliver longer current pulses while minimizing electrochemical side-effects (Fig. 1A) (Leal et al., 2023; Shaner et al., 2022; Matter et al., 2024). Such side effects include faradaic reactions leading to pH shifts, generation of reactive oxygen species, electrode corrosion or delamination, and the release of potentially cytotoxic by-products (Matter et al., 2024; Brummer and Turner, 1977; Schopf et al., 2016; Harland et al., 2025; Ehlich et al., 2022, 2024; Negi et al., 2010; Cogan, 2008). A common method to minimize these side effects is to maintain electrode polarization within the water window, *i.e.*, the thermodynamic limits for water electrolysis, beyond which hydrogen and oxygen evolution can induce rapid pH changes and gas formation that may damage both the electrode and the tissue. The electrodes were furthermore coated with agar salt bridges, which delay the diffusion of electrochemical stimulation by-products into the culture medium (Schopf et al., 2016). The electrodes were positioned above and below the culture insert. For electrical stimulation, the insert was transferred to the

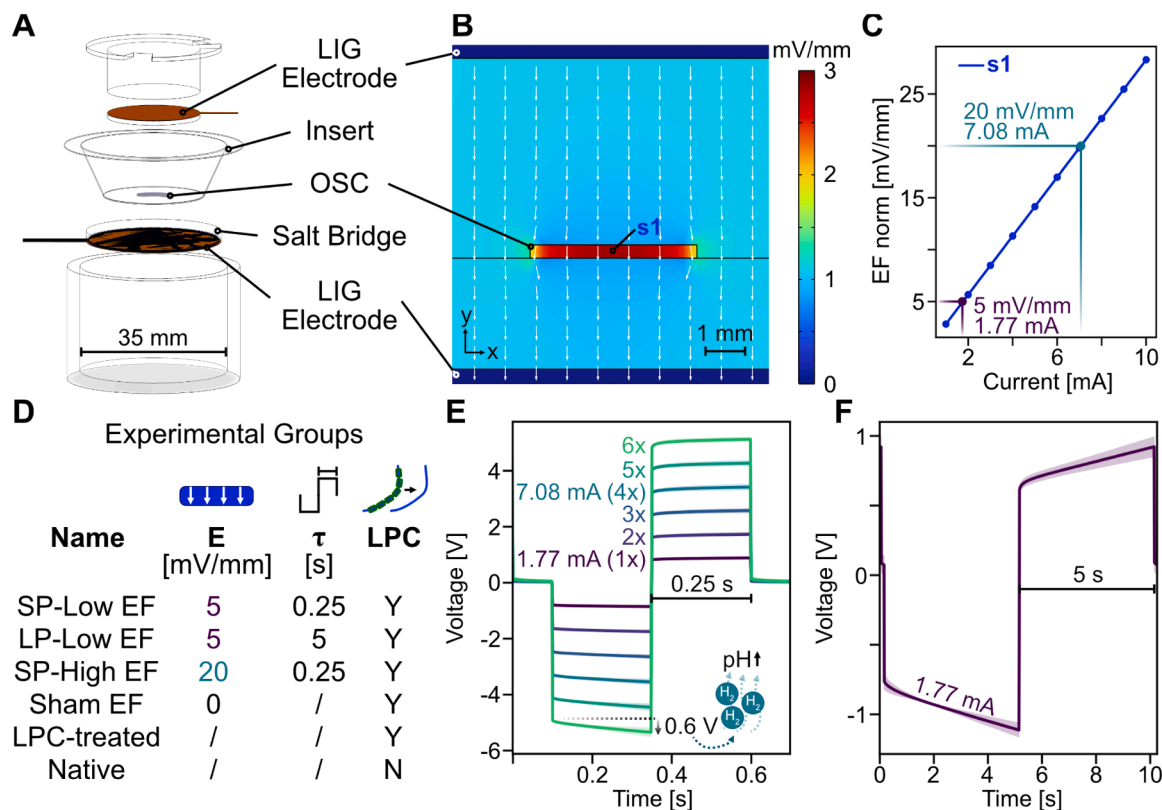


Fig. 1. A) The stimulation platform consists of two LIG electrodes coated with electropolymerized PEDOT:PSS and agar salt bridges. For EF stimulation, culture inserts were transferred to the platform, and medium was added to fully submerge the OSC (Fig. S1). B) FEA simulation at an applied current of 1 mA demonstrating spatially uniform EF across the modeled OSC. The white arrows show the direction of the EF. The x,y-plane is at the OSC mid-plane. C) EF magnitude in the center of the OSC at different applied currents. D) OSCs were exposed to LPC to induce demyelination. EF stimulation was then applied using varying parameters, including short-pulse (SP, 250 ms), long-pulse (LP, 5 s), low EF strength (5 mV/mm), high EF strength (20 mV/mm), and sham EF (0 mV/mm) conditions. Control groups were cultured under standard conditions and included LPC-treated only and native OSCs. E, F) VTs showing that electrode polarization remains within the water window (−0.6–0.9 V) for all tested stimulation parameters. The dashed line in E indicates the cathodic water window limit, which was reached at 10.62 mA, above the currents used for stimulation. Stimulation outside of the water window can lead, among other electrochemical side effects, to hydrogen evolution and rapid pH changes. Solid lines in E,F are mean values. Shaded areas represent SD (n = 3).

stimulation platform and medium was added on top to submerge the OSC (Fig. S1). Before use, the impedance at 10 Hz and 1 kHz varied by approximately 20% across the manually assembled stimulation platforms (Fig. S2A).

Finite element analysis (FEA) simulation confirmed that the electrode configuration generates a spatially uniform transverse EF within the modeled OSC (Fig. 1B, details in Fig. S3 and Supplementary Note: FEA simulation). An EF strength of approximately 5 mV/mm has been proposed as an effective target for CNS repair, based on observations of enhanced cell-to-cell interactions as well as improved axonal guidance and growth (McCaig and Rajniecek, 1991; Hernández-Labrado et al., 2011; Heo et al., 2011). Based on the FEA, achieving 5 mV/mm within the OSC corresponded to a stimulation current of 1.77 mA (Fig. 1C). Stimulation parameters were selected to reflect those previously applied in animals (Harland et al., 2025) while also accounting for the biocompatible charge delivery limits of the electrodes, as established in previous works (Shaner et al., 2023; Matter et al., 2024). Specifically, 250 ms biphasic pulses have been shown to improve locomotor function in a rat SCI model. In addition to this short-pulse (250 ms biphasic), low-EF (5 mV/mm) group (SP-Low EF), we included a long-pulse (5 s biphasic), low-EF (LP-Low EF), a short-pulse, high-EF (20 mV/mm) (SP-High EF), and a sham EF (0 mV/mm) treatment group (Fig. 1D). All treatment groups (SP-Low EF, LP-Low EF, SP-High EF, Sham EF) were submerged for 40 min daily. In addition, an LPC-treated and a native group maintained under standard culture conditions were included as control groups. Electrode voltage transient (VT) measurements indicated that the electrode polarization of the LIG PEDOT:PSS electrodes

remained within the water window (−0.6–0.9 V) at currents up to six times higher than that required to achieve 5 mV/mm with a 250 ms biphasic pulse width. This range also includes the 20 mV/mm group, which required a four times higher current than the 5 mV/mm group, confirming that all planned stimulation would remain within electrochemical safety boundaries (Fig. 1E,F) (Cogan, 2008).

After slice generation (experimental timeline, Fig. 2), OSCs were cultured for 23 days at the air-liquid interface to allow stable integration with the inserts' membrane to prevent detachment during subsequent submersion. Demyelination was then induced using LPC by supplementing the culture medium with 0.5 mg/mL LPC for 17 h according to a previously established protocol (Hawker et al., 2024). LPC acts as a glial toxin that induces demyelination while preserving axons under

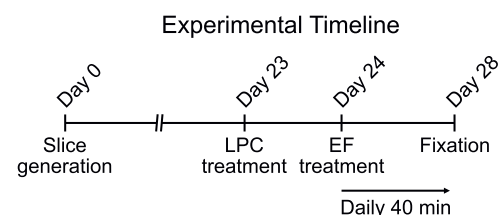


Fig. 2. Experimental timeline. OSCs were cultured for 23 days. Demyelination was then induced using LPC. After the 17 h LPC treatment, EF stimulation was applied for 40 min per day over five consecutive days. After the EF stimulation, OSCs were fixed.

controlled exposure conditions (Hawker et al., 2024; Birgbauer et al., 2004). Following LPC exposure, EF stimulation was applied for 40 min per day over five consecutive days. The stimulation duration and treatment period were selected based on two considerations: (i) low EF strengths are expected to act through slow cellular mechanisms, requiring exposure on the order of at least several minutes (Lu et al., 2023; Shaner et al., 2023), and (ii) multi-day treatment was demonstrated to be successful in animal models, making it a highly relevant condition to reproduce (Jing et al., 2015; Bacova et al., 2022; Fang et al., 2021; Zhang et al., 2014; Harland et al., 2025). Devices were reused for up to ten stimulation sessions. Impedance measurements were available only for the LP-Low EF group (see Methods). Across repeated use, impedance generally increased but VTs stayed comparable (Fig. S2B,C). After five days of stimulation, OSCs were analyzed for viability and fixed for assessment of myelination via MBP immunostaining. MBP is a component of myelin, which can appear prior to full maturation of oligodendrocytes (Hawker et al., 2024; Birgbauer et al., 2004).

2.2. OSCs remain viable following repeated submersion and EF stimulation

CNS slices require adequate oxygen and nutrient supply, which is typically achieved by culturing at the air-liquid interface. In this culture method, the slice sits on a membrane with nutrient-rich medium below and is exposed to the atmosphere above for oxygenation. However, to ensure a uniform EF, OSCs must be fully submerged during stimulation (Lu et al., 2023). As the stimulation period was limited to 40 min per day, we employed passive submersion without external medium flow that could otherwise supply oxygenated medium and potentially extend viable stimulation durations (Huang et al., 2012). This raised the question of whether OSCs would tolerate repeated 40-minute submersion. In addition, although the stimulation platform was designed to ensure biocompatible electrical stimulation (*i.e.*, PEDOT:PSS coating and agar salt bridges), it was important to experimentally verify that these measures were successful. Furthermore, factors beyond electrochemical effects may still compromise cell viability, for instance, the exposure to sufficiently strong EF can induce membrane permeabilization and apoptosis, although this typically occurs at higher EF strengths (Al-Sakere et al., 2007). In addition, repeated EF stimulation can contribute to cumulative effects even when individual stimulation remains within safe limits (Bikson et al., 2016). We therefore assessed cell viability under the tested stimulation parameters to validate the

platform (Fig. 3A).

Live cells stained with calcein-AM were observed across all groups (Fig. 3B). A smaller number of EthD-1 + dead cells were present, predominantly at the edges of the OSCs, consistent with increased cell damage in regions affected by the sectioning. Quantification of cell viability, normalized to native slices, showed no significant differences between groups, indicating that LPC exposure, repeated submersion, and EF stimulation did not adversely affect cell viability (Fig. 3C, one-way ANOVA, $F(5,20) = 0.602$, $p = 0.699$).

2.3. Application of platform to LPC-induced demyelination reveals differences in MBP signal

The spinal cord is organized with central gray matter (GM) surrounded by white matter (WM), where GM primarily contains neuronal cell bodies and WM consists mainly of myelinated axons supported by glial cells, including oligodendrocytes (Alizadeh et al., 2015; Diaz and Morales, 2016). Longitudinal sectioning preserves the boundary between WM and GM, allowing distinction of both regions in the OSCs. As myelin is predominantly localized in the WM, our analysis of MBP signal was focused on this region (Fig. 4A). To demonstrate the biological utility of the stimulation platform, OSCs were exposed to LPC, a glial toxin known to induce demyelination (Hawker et al., 2024; Birgbauer et al., 2004), and EF stimulation was applied. The precise mechanism of myelin loss is unclear as LPC may directly disrupt myelin or injure oligodendrocytes (Birgbauer et al., 2004). MBP is a well-established marker for myelination, as it is part of the myelin sheath and is typically expressed during the early phases of remyelination (Hawker et al., 2024; Birgbauer et al., 2004). In the rat OSC model used here, endogenous remyelination following LPC exposure has been reported to reach approximately 60% of MBP levels after one week in culture, which remains at this level over the 5-week observation period compared to native controls (Hawker et al., 2024). Therefore, we used MBP as a marker to assess whether EF stimulation can restore MBP signal after LPC treatment.

OSCs were stained for MBP following the 40-minute daily EF stimulation over five consecutive days. MBP staining was observed in all groups (Fig. 4B). Quantification of the MBP intensity revealed a significant difference between groups (one-way ANOVA, $F(5,18) = 4.116$, $p = 0.011$) (Fig. 4C). *Post hoc* analysis (Tukey's HSD) showed that the SP-Low EF ($p = 0.969$), SP-High EF ($p = 0.709$), and LP-Low EF ($p = 0.280$) groups did not differ significantly from the LPC-treated

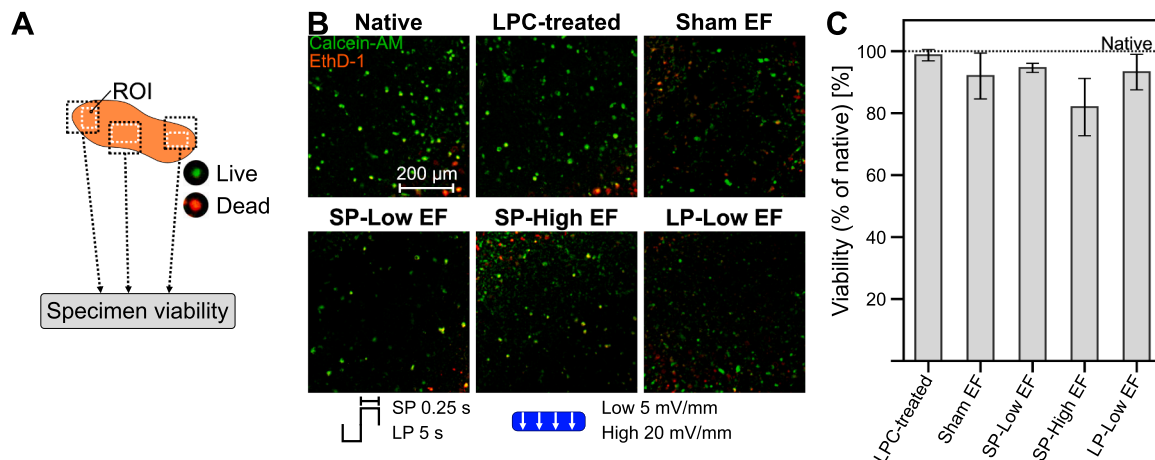


Fig. 3. OSCs remain viable when submerged and stimulated for 40 min daily over five consecutive days. **A)** OSCs were stained with Calcein-AM (live) and EthD-1 (dead). Three images were acquired at random locations per slice. The region of interest (ROI) was defined within the central area of the slice to exclude border regions, where increased cell death is expected due to the sectioning. Viability was then calculated by counting live and dead cells to obtain a specimen viability value. **B)** Representative images of stained OSCs. **C)** Quantification of the proportion of live cells, normalized to native slices ($n = 4, 5, 4, 5, 2, 6$ for Native, LPC-treated, Sham EF, SP-Low EF, SP-High EF, and LP-Low EF, respectively). No statistically significant differences were observed between groups using one-way ANOVA. Data are presented as mean $\pm$ SEM.

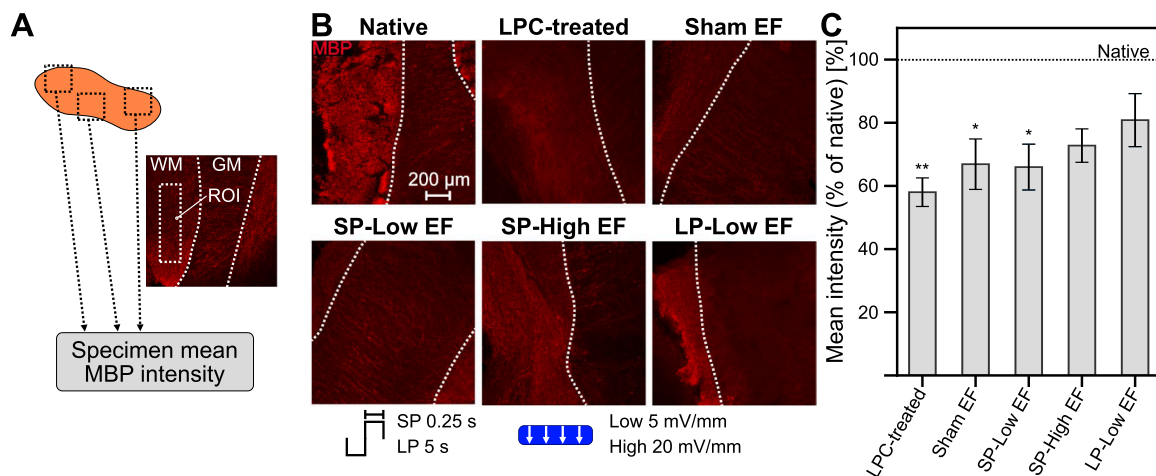


Fig. 4. MBP signal following LPC-induced demyelination under the tested EF stimulation conditions. **A)** OSCs were immunostained for MBP. Three images per slice were acquired at locations where the boundary between white matter (WM) and gray matter (GM) was clearly identified. Mean MBP fluorescence intensity was quantified in the WM for each image and averaged to obtain a specimen intensity value. **B)** Representative images of MBP staining in OSCs following the 5-day treatment period. MBP signal was detected in all groups. WM is oriented to the left of each image with the white line indicating the boundary to GM. **C)** Quantification of MBP intensity, normalized to native controls, showed a significant reduction in signal for LPC-treated, sham EF, and SP-Low EF groups compared to native slices. None of the EF-treated groups differed significantly from the LPC-treated group. Each bar represents mean $\pm$ SEM ($n = 4$). Statistical significance was determined using one-way ANOVA followed by Tukey's HSD post hoc test with * $p < 0.05$, and ** $p < 0.01$.

controls. However, MBP intensity remained significantly below levels in native controls in the LPC-treated ($p = 0.008$), sham EF ($p = 0.047$), and SP-Low EF ($p = 0.039$) groups, whereas the SP-High EF ($p = 0.138$) and LP-Low EF ($p = 0.456$) groups did not differ significantly from native controls.

3. Discussion and conclusions

A limited number of previous studies (Meng et al., 2011; Shaner et al., 2023) have demonstrated the feasibility of applying EFs to organotypic CNS slice cultures. However, these studies have not fully exploited the potential of OSCs in long-term culturing to allow repeated stimulation and assessment. This is a critical limitation, as both animal and human studies commonly apply EFs across multiple days or sessions (Jing et al., 2015; Bacova et al., 2022; Fang et al., 2021; Zhang et al., 2014; Shapiro, 2014; Shapiro et al., 2005); therefore, adopting similar repeated stimulation paradigms in OSCs is essential to enable meaningful translation between *ex vivo*, *in vivo*, and clinical findings. The aim of this study was to develop and validate an accessible experimental platform and protocol for repeated, spatially uniform, and biocompatible EF stimulation of OSCs. Using this platform, we first demonstrated that EF stimulation can be applied repeatedly without adversely affecting slice viability, thereby validating the platform and protocol for multi-day OSC stimulation. We then applied the platform to LPC-induced demyelinated OSCs to demonstrate its biological utility for studying EF-mediated CNS repair. Our results show differences in MBP signal among the tested stimulation conditions, supporting further investigation of how EF parameters influence remyelination.

OSCs remained viable during repeated submersion and EF stimulation (Fig. 3). Prior studies have limited submersion and stimulation to short, single sessions, such as 20 min in organotypic brain slice cultures (Shaner et al., 2023) or up to five hours in OSCs (Meng et al., 2011). This is not surprising, as maintaining tissue viability under submerged conditions is challenging. Given the high oxygen consumption of neural tissue, repeated submersion might be expected to impact viability. However, the absence of such an effect following daily 40-minute submersion over five days in our study indicates that these conditions are well tolerated. Although microfluidic devices can support continuous fluid flow and potentially prolong submersion periods by maintaining the delivery of oxygen-saturated media, we show that a simple,

accessible, and scalable platform is sufficient for the repeated EF stimulation protocol tested here. Because the platform is straightforward to fabricate and implement, it may facilitate broader adoption and adaptation to other research questions. Importantly, no differences in viability were observed between experimental groups, indicating that the applied EF stimulation itself was biocompatible. Several design features likely contributed to this. Electrodes were coupled via agar salt bridges to delay the diffusion of stimulation by-products into the media (Schopf et al., 2016), and coated with PEDOT:PSS to enhance their capacity to deliver long pulses in a biocompatible manner (Fig. 1A). The applied charge densities (0.12 – 2.33 mC/cm²) fall within the range of predominantly capacitive charge transfer in LIG, minimizing the generation of harmful faradaic reaction products (Matter et al., 2023). Moreover, the stimulation was performed within the electrochemical water window measured in PBS (Fig. 1E,F), thereby avoiding water electrolysis and the rapid generation of toxic species such as hydrogen, which can cause pH shifts (Matter et al., 2024). PBS was used to facilitate comparison with previous studies; however, the experimentally observed water window depends on both electrolyte composition and electrode material and may therefore differ in cell culture medium (Matter et al., 2024; Ehlich et al., 2022, 2024). Other faradaic reactions can occur within the water window, and definitive assessment would require direct identification and quantification of reaction products.

EF stimulation influenced MBP signal in OSCs and although our study did not allow exhaustive investigations of the parameter space, some conclusions can still be drawn. LPC exposure induced robust demyelination in WM, with MBP levels comparable to those reported previously (Fig. 4) (Hawker et al., 2024). While the MBP signal was significantly reduced in the sham EF and SP-Low EF groups (250 ms biphasic pulses, 5 mV/mm), no significant reduction was observed in the SP-High EF (250 ms biphasic pulses, 20 mV/mm) and LP-Low EF groups (5 s biphasic pulses, 5 mV/mm) compared to native controls. However, MBP signal in none of the EF-treated groups differed significantly from the LPC-treated slices. The observed pattern is therefore consistent with possible partial recovery of MBP signal under the SP-High EF and LP-Low EF conditions but does not demonstrate a statistically significant rescue effect. Nevertheless, the different MBP intensities after EF stimulation suggest that EF strength and pulse duration warrant further systematic investigation. In this context, previous work has proposed that cellular responses to EF stimulation may depend on

cumulative exposure. The effective stimulation dose was suggested to be the threshold time-integrated EF intensity (McCaig and Rajniecek, 1991; Tang-Schomer, 2018; Patel and Poo, 1984). Indeed, in our study, the time-integrated EF intensity was 4- and 20-fold higher in the SP-High EF and LP-Low EF groups, respectively, compared to the SP-Low EF group. The stimulation parameters used in this study were motivated by previous work in a rat model of thoracic spinal cord injury, in which stimulation parameters similar to the SP-Low EF group were applied, although with lower EF strength (Harland et al., 2025). In that study, improved locomotor function was observed without clear evidence of changes in neuronal structural integrity or axonal regeneration, suggesting that alternative mechanisms may have contributed to recovery highlighting the need for complementary studies such as the one presented here. We did not observe a significant effect in the SP-Low EF group on MBP signal in the present study, which would most closely mimic the parameters used in animals. However, the relatively short stimulation period in OSCs (five days), compared to the prolonged treatment in animals (1 h daily over 3 months), resulted in a substantially lower time-integrated EF intensity, which may explain this discrepancy. Further studies are needed to determine whether extended EF stimulation significantly increases MBP signal and promotes remyelination.

Although the present study did not directly investigate the underlying cellular mechanisms, the hypothesis that EF stimulation may influence processes relevant to remyelination is in line with current understanding and literature. It is known that early stages of myelination can be influenced by the electrical activity of axons. These effects involve the regulation of neuronal cell adhesion molecules required for myelination (Stevens et al., 1998; Itoh et al., 1995), as well as the release of signalling molecules from electrically active axons which can promote OPC proliferation and differentiation (Fields and Stevens, 2000; Stevens et al., 2002; Lee et al., 2017), and further through astrocyte-mediated cytokine release modulating myelination by mature oligodendrocytes (Ishibashi et al., 2006). An important limitation of this study is that MBP intensity alone cannot confirm remyelination. MBP can be expressed by differentiated oligodendrocytes before mature myelin formation (Birgbauer et al., 2004), and the present analysis did not determine whether MBP-positive processes were aligned with axons. Future studies should therefore combine additional oligodendrocyte lineage markers (e.g., OLIG2, SOX10, NKX6.2, O4, CC1, O1, and MOG) with markers for axonal integrity and approaches that assess myelin-axon association or ultrastructural wrapping. Shaner et al., 2023 demonstrated that a weak EF strength of 4.7 mV/mm does not induce measurable increases in neuronal activity, as assessed by calcium imaging and c-Fos expression, whereas substantially higher field strengths (~140 mV/mm) are required to elicit neuronal activation in organotypic brain slice cultures. This suggests that the EF strengths applied in the present study (5–20 mV/mm) are likely below the threshold required to directly trigger electrical activity of axons and thereby promote remyelination. At the same time, EF-induced increases in OPC proliferation and differentiation have been reported at lower EF strengths (0.4 – 0.5 mV/mm) than applied in this work, with long-duration (min) pulses (Jing et al., 2015; Fang et al., 2021; Zhang et al., 2014). Although the underlying mechanisms remain unclear, these effects may arise because such subthreshold stimulation enhances neuronal excitability, thereby indirectly influencing neural activity (Barra et al., 2022; Balaguer et al., 2025), or from direct effects such as the release of signalling molecules.

Future studies could extend this approach to additional OSC injury models, such as transection (Bonnici and Kapfhammer, 2008), to examine the role of EF stimulation in axonal guidance and regeneration. In the present work, the biological application was intentionally limited to an established LPC-induced demyelination model and MBP intensity as a proof-of-concept marker. We hypothesized that remyelination would be largely independent of EF directionality, as myelination primarily depends on local cell-to-cell interactions. In contrast, processes such as axonal guidance are inherently direction-dependent and would

therefore require a modified experimental design to ensure directionality of the applied EF. For example, this could be achieved by incorporating microchannels connected to the culture wells to generate a longitudinal EF (Lin et al., 2025). Furthermore, expanding the platform to enable live-cell imaging for direct evaluation of stimulation effects (e.g., calcium-imaging) would allow systematic optimization and investigation of EF parameters to promote not only remyelination but also broader CNS repair processes. Together, these findings establish a practical and well-controlled experimental framework for systematically investigating EF-mediated effects in CNS tissue, enabling more reproducible comparisons across studies and accelerating the development of EF-based treatments.

4. Methods

4.1. Electrode preparation

LIG electrodes (bottom electrode: 32 mm diameter; top electrode: 22 mm diameter; 2 mm wide connection lines) were fabricated by laser-induced carbonization of 75 μm -thick Kapton sheets (Kapton HN, DuPont) using a laser cutter (VLS3.60DT, Universal Laser Systems). The laser was operated at a power of 6.3 W and a scanning speed of 100 mm/s. The LIG electrodes were subsequently coated with PEDOT:PSS via electropolymerization at 0.9 V from an aqueous solution containing 5 mg/mL sodium polystyrene sulfonate (poly(sodium 4-styrenesulfonate), Sigma-Aldrich) and 0.01 M 3,4-ethylenedioxythiophene monomer (3,4-ethylenedioxythiophene, 97%, Sigma-Aldrich). The deposited charge was monitored, and the process was terminated upon reaching 300 mC/cm². Coated electrodes were soaked in 1xPBS overnight. The bottom electrodes were connected to wires using copper tape, and the connection lines were insulated by manual coating with silicone (DowSil 734, Dow Corning). The silicone was dried for 48 h.

4.2. Device preparation and characterization

Devices consisted of a 3D-printed well and a lid. The well (35 mm diameter, 2 mm wall thickness) was printed from PETG (PET-G, Prusa i3 MK3S). The lid was printed from a resin (Clear Resin, Form 3B+, Formlabs Inc.). The bottom electrode was glued to the base of the well using cyanoacrylate adhesive (UHU Super Glue). The well was then filled with 3% (w/v) agar (Select Agar, Invitrogen) prepared in 1xPBS (2 mL) to form an approximately 2 mm-thick salt bridge. The top electrode was heated to 60°C on a hot plate and prewetted with 1xPBS. Agar solution (450 μL) was then pipetted onto the electrode surface, forming an approximately 1 mm-thick salt bridge. Subsequently, the top electrode was glued to the lid and connected to wires using copper tape. Finally, the connection lines and junctions were insulated with silicone. Voltage transients at 250ms and 5 s pulse width of fully assembled devices consisting of top and bottom electrode were recorded in 1xPBS using a PGSTAT204 potentiostat (Metrohm Autolab B.V.). Impedance (4 points per decade, 100 mV peak to peak amplitude, 1 Hz – 10 kHz) was measured in 1xPBS before use.

4.3. Computational modeling

The EF distribution was simulated in COMSOL Multiphysics® software (version 5.3) using the Electric Currents module. The stimulation well was modeled as a cylinder with an inner diameter of 35 mm and a total height of 9 mm, bound by 1 mm thick polystyrene walls. Two culture media domains were defined, the lower domain with 35 mm diameter and 2.5 mm height, and the upper domain representing the media filled insert with 25 mm diameter and 4.5 mm height. The electrodes were modeled as conductive disks with a height of 0.5 mm, positioned at the bottom and top of the well (bottom electrode 32 mm diameter, top electrode 22 mm diameter). The OSC was positioned inside the upper medium at the interface to the lower medium domain.

The OSC was modeled as cylinder with a height of 0.3 mm and diameter of 4 mm. The conductivity and relative permittivity values used in the model are shown in Table 1. The bottom electrode was defined as electrical ground, while the top electrode was assigned a terminal condition with a swept current amplitude. The walls of the well were modeled as ideal electrical insulators. The model was validated by simplifying the geometry to a 22 mm diameter well filled with media and comparing numerical with analytical results (see Supplementary Note: FEA simulation).

4.4. Animals

Postnatal 9- to 11-day old Sprague-Dawley male and female rat pups were used in this study. Animals were housed in a 12 h light-dark cycle with access to food and water *ad libitum*. Animal euthanasia was in accordance with the New Zealand Animal Welfare Act 1999 and was approved by the University of Auckland Animal Ethics Committee (R27796).

4.5. Slice generation

The protocol for slice generation is adapted from previous work (Hawker et al., 2024). The spinal cord was isolated and cut in two equally long pieces along the caudal-cranial axis. The cord was mounted onto a vibratome chuck (Leica Biosystems) using cyanoacrylate adhesive (UHU Super Glue). The vibratome chamber was filled with ice-cold medium (Advanced DMEM/F-12 with 1% penicillin-streptomycin, Thermo Fisher Scientific #12634010 and #15140148). The cord was sectioned in the longitudinal plane at 300 μ m thickness. Generated slices were then mounted onto the membrane of inserts in 6-well plates (Corning, #COR3450) and cultured at 35°C with 5% CO₂. 1 mL medium was added below the inserts. For the first three days, slices were cultured in serum-containing media (MEM with Hanks balanced salts, 1% penicillin-streptomycin, 25% horse serum, Thermo Fisher Scientific #11575032 and #16050130) spiked with mitotic inhibitors (4.4 mM of Sigma #U3003, #F0503 and #C1768). The slices were then transitioned to serum-free culture medium (Advanced DMEM/F-12, 2% B-27, 1% N-2, 1% penicillin-streptomycin, Thermo Fisher Scientific, #17504044 and #17502048) and media was exchanged every Monday, Wednesday, and Friday.

4.6. LPC treatment

After 23 days in culture, slices were divided into 6 groups: LPC-treated, LPC-treated with sham EF, LPC-treated with long-pulse EF, LPC-treated with short-pulse EF, LPC-treated with high-intensity short-pulse EF, and untreated native controls. To induce demyelination, slices were incubated in 0.5 mg/mL LPC (Sigma, #62962) for 17 h.

Table 1
Conductivities and relative permittivity.

Component/ Tissue	Conductivity [S/m]	Relative Permittivity	Reference
PEDOT:PSS- coated electrode	2000	1	(Shaner et al., 2023)
Media	1.9	80	(Puttaswamy et al., 2010), (Shaner et al., 2023)
OSC	0.6	2×10^7	(Baumgartner et al., 2025)
Polystyrene	1×10^{-14}	2.6	(Qi et al., 2011), (Rumble, 2017)

4.7. EF stimulation

OSCs were electrically stimulated, beginning on Day 24, immediately following LPC-induced demyelination. Stimulation was applied for five consecutive days for 40 min daily. During each session, four slices, one from each experimental group (LPC with sham EF, LP-EF, SP-EF, SP-High EF) were stimulated simultaneously. OSCs were stimulated in an incubator at 35°C with 5% CO₂. Each stimulation device was used a maximum of ten times limited to twice per day, to allow electrodes to refresh, with the exception of the sham device, which was reused across sessions. Slices were stimulated in fresh serum-free culture media. Prior to the first stimulation and after each daily use, devices were disinfected by soaking in 70% (v/v) ethanol for 30 s. Devices were then filled with culture medium to allow hydration of the salt bridges and subsequently UV-sterilized. OSCs were stimulated at 5 and 20 mV/mm EF magnitude. Based on computational modeling, these EF magnitudes corresponded to an applied current of 1.77 mA, and 7.08 mA. The SP-Low EF and SP-High EF (250ms biphasic pulses, 100 μ s interphase, 100ms interpulse) were stimulated using an STG5 Stimulus Generator (Multi Channel Systems). To confirm successful stimulation, voltage transients were recorded with an oscilloscope (Teledyne T3DSO1204). The LP-Low EF group (5 s biphasic pulses, 100 μ s interphase, 100ms interpulse) was stimulated using a PGSTAT204 potentiostat (Metrohm Autolab B.V.), due to its higher compliance voltage. The impedance of the platform and the voltage transient during stimulation was recorded with the potentiostat.

4.8. Cell viability

Cell viability was assessed with the live cell marker calcein-AM (Thermo Fisher Scientific, #C1430) and the dead cell marker ethidium homodimer-1 (Thermo Fisher Scientific, #E1169). Slices were incubated in 2 μ M calcein-AM and 4 μ M EthD-1 for 40 min at room temperature. Subsequently, slices were washed in PBS three times and immediately imaged with a Nikon TE2000E inverted fluorescent microscope. Three fluorescence images were acquired per slice. Images were processed using Fiji. Regions of interest were selected from the middle of the slices, excluding the edges where increased numbers of dead cells were observed due to the longitudinal cut. Images were cropped and converted to 8-bit grayscale. Background was subtracted using a rolling-ball algorithm (radius 20 pixels), followed by Gaussian smoothing. Automated thresholding (default method) was applied to generate binary masks. Morphological opening and watershed segmentation were used to separate adjacent cells. Particles were quantified using the Analyze Particles function. Statistical analysis was performed in Python using SciPy. Viability was calculated per slice as the ratio of live to total cells and normalized to the mean viability of native slices. Statistical significance was assessed using one-way ANOVA on unnormalized specimen-level viability.

4.9. Immunohistochemistry

Slices were fixed in 4% paraformaldehyde for 24 h at 4°C. Slices were then stained using a shortened version of the optical clearing technique iDISCO as previously described (Hawker et al., 2024). In short, after 24 h of permeabilization, slices were blocked and then incubated in primary antibody for 36 h. Demyelination and remyelination were assessed by the expression of myelin basic protein (MBP) (1:250, MAB386). Alexa Fluor goat anti-rat 594 conjugated secondary antibody was used to fluorescently label the MBP. The membrane-bound slices were imaged using an inverted confocal microscope (Zeiss LSM 800) after they were dehydrated in a series of increasing concentrations of methanol and cleaned in dibenzyl ether. MBP fluorescence images were processed using Fiji. For each image a region of interest in white matter was manually selected. Images were converted to 8-bit grayscale. Background was subtracted using a rolling-ball algorithm (radius 1000

pixels). A consistent threshold was applied to all images and the mean intensity was measured. For visualization, values were normalized to the mean of native slices. For statistical testing, unnormalized values from individual images were used. Statistical significance was assessed using one-way ANOVA on unnormalized specimen-level MBP intensity followed by Tukey's post hoc test.

Ethics declaration

This study was conducted in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. This study was approved by the University of Auckland Animal Ethics Committee (Approval No. R27796).

CRediT authorship contribution statement

LM: Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **SCK:** Conceptualization, Funding Acquisition, Supervision, Validation, Writing – review & editing. **AC:** Methodology, Resources, Supervision, Validation, Writing – review & editing. **BH:** Methodology, Resources, Supervision, Validation. **SL:** Methodology, Writing – review & editing. **BruH:** Resources, Writing – review & editing. **DS, MA:** Conceptualization, Resources, Funding Acquisition, Supervision, 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.

Acknowledgments

This work was supported by funding from the CatWalk Spinal Cord Injury Trust (Cure Programme) and the Chalmers Gender Initiative for Excellence (GENIE). The research stay of LM at the University of Auckland was supported by The Solveig & Karl G. Eliassons memorial and supplementary funds. BruH was supported by a HRC Sir Charles Hercus Fellowship. We thank the Biomedical Imaging Research Unit (BIRU) at the University of Auckland for technical support with confocal image acquisition. We also thank Dr. Manju Ganesh for research technical support.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jneumeth.2026.110908](https://doi.org/10.1016/j.jneumeth.2026.110908).

Data availability

Data will be made available on request.

References

- Al-Sakere, B., et al., 2007. Tumor ablation with irreversible electroporation. *PLoS One* 2, Alizadeh, A., Dyck, S.M., Karimi-Abdolrezaee, S., 2015. Myelin damage and repair in pathologic CNS: Challenges and prospects. *Front. Mol. Neurosci.* 8.
- Andersen, J., et al., 2020. Generation of functional human 3D cortico-motor assembloids. *Cell* 183, 1913–1929.
- Ao, Z., et al., 2022. Human spinal organoid-on-a-chip to model nociceptive circuitry for pain therapeutics discovery. *Anal. Chem.* 94, 1365–1372.
- Bacova, M., Bimbova, K., Kisucka, A., Lukacova, N., Galik, J., 2022. Epidural oscillating field stimulation increases axonal regenerative capacity and myelination after spinal cord trauma. *Neural Regen. Res.* 17, 2730–2736.
- Balaguer, J.M., et al., 2025. Neural mechanisms underlying the recovery of voluntary control of motoneurons after paralysis with spinal cord stimulation. *Neuron* 113, 3846–3862.e6.
- Barra, B., et al., 2022. Epidural electrical stimulation of the cervical dorsal roots restores voluntary upper limb control in paralyzed monkeys. *Nat. Neurosci.* 25, 924–934.
- C. Baumgartner et al. ITIS database for thermal and electromagnetic parameters of biological tissues, 5, 2025. <https://itis.swiss/virtual-population/tissue-properties/overview>.
- Becker, D., Gary, D.S., Rosenzweig, E.S., Grill, W.M., McDonald, J.W., 2010. Functional electrical stimulation helps replenish progenitor cells in the injured spinal cord of adult rats. *Exp. Neurol.* 222, 211–218.
- Bikson, M., et al., 2016. Safety of transcranial direct current stimulation: evidence based update 2016. *Brain Stimul.* 9, 641–661.
- Birgbauer, E., Rao, T.S., Webb, M., 2004. Lysolcithin induces demyelination in vitro in a cerebellar slice culture system. *J. Neurosci. Res.* 78, 157–166.
- Bonnici, B., Kapfhammer, J.P., 2008. Spontaneous regeneration of intrinsic spinal cord axons in a novel spinal cord slice culture model. *Eur. J. Neurosci.* 27, 2483–2492.
- Borgens, R.B., 1999. Electrically mediated regeneration and guidance of adult mammalian spinal axons into polymeric channels. *Neuroscience* 91, 251–264.
- Borgens, R.B., Blight, A.R., Murphy, D.J., Stewart, L., 1986. Transected dorsal column axons within the guinea pig spinal cord regenerate in the presence of an applied electric field. *J. Comp. Neurol.* 250, 168–180.
- Brummer, S.B., Turner, M.J., 1977. Electrochemical considerations for safe electrical stimulation of the nervous system with platinum electrodes. *IEEE Trans. Biomed. Eng.* 24, 59–63.
- Cogan, S.F., 2008. Neural stimulation and recording electrodes. *Annu. Rev. Biomed. Eng.* 10, 275–309.
- Diaz, E., Morales, H., 2016. Spinal cord anatomy and clinical syndromes. *Semin. Ultrasound CT MRI* 37, 360–371.
- Dobson, R., Giovannoni, G., 2019. Multiple sclerosis – a review. *Eur. J. Neurol.* 26, 27–40.
- Ebert, A.D., Svendsen, C.N., 2010. Human stem cells and drug screening: Opportunities and challenges. *Nat. Rev. Drug. Discov.* 9, 367–372.
- Ehlich, J., et al., 2022. Direct measurement of oxygen reduction reactions at neurostimulation electrodes. *J. Neural Eng.* 19, 036045.
- Ehlich, J., et al., 2024. Shattering the water window: comprehensive mapping of faradaic reactions on bioelectronics electrodes. *ACS Appl. Mater. Interfaces* 16, 53567–53576.
- Fang, C., Sun, J., Wei, L., Gao, F., Qian, J., 2021. Oscillating field stimulation promotes recovery from spinal cord injury in rats by regulating the differentiation of endogenous neural stem cells. *Exp. Ther. Med.* 22, 1–8.
- Fields, R.D., Stevens, B., 2000. ATP: an extracellular signaling molecule between neurons and glia. *Trends Neurosci.* 23, 625–633.
- Franklin, R.J.M., 2002. Why does remyelination fail in multiple sclerosis? *Nat. Rev. Neurosci.* 3, 705–714.
- Harland, B., et al., 2025. Daily electric field treatment improves functional outcomes after thoracic contusion spinal cord injury in rats. *Nat. Commun.* 16.
- Hawker, B., Dhakal, M., Connor, B., McCaughey-Chapman, A., 2024. Modeling demyelination and endogenous remyelination in spinal cord ex vivo rat organotypic slice cultures. *Front. Cell. Neurosci.* 18.
- Heidemann, M., Streit, J., Tschertner, A., 2015. Investigating functional regeneration in organotypic spinal cord co-cultures grown on multi-electrode arrays. *J. Vis. Exp.* (103).
- Heo, C., et al., 2011. The control of neural cell-to-cell interactions through non-contact electrical field stimulation using graphene electrodes. *Biomaterials* 32, 19–27.
- Hernández-Labrado, G.R., Polo, J.L., López-Dolado, E., Collazos-Castro, J.E., 2011. Spinal cord direct current stimulation: finite element analysis of the electric field and current density. *Med. Biol. Eng. Comput.* 49, 417–429.
- Hinkle, L., Mccaig, C.D., Robinson, K.R., 1981. The direction of growth of differentiating neurons and myoblasts from frog embryos in an applied electric field. *J. Physiol.* 314.
- Huang, Y., Williams, J.C., Johnson, S.M., 2012. Brain slice on a chip: opportunities and challenges of applying microfluidic technology to intact tissues. *Lab. Chip* 12, 2103–2117.
- Ishibashi, T., et al., 2006. Astrocytes promote myelination in response to electrical impulses. *Neuron* 49, 823–832.
- Itoh, K., Stevens, B., Schachner, M., Fields, R.D., 1995. Regulated expression of the neural cell adhesion molecule L1 by specific patterns of neural impulses. *Science* (1979) 270, 1369–1372.
- Jack, A.S., Hurd, C., Martin, J., Fouad, K., 2020. Electrical stimulation as a tool to promote plasticity of the injured spinal cord. *J. Neurotrauma* 37, 1933–1953.
- Jaffe, L.F., Poo, M.-M., 1979. Neurites grow faster towards the cathode than the anode in a steady field. *J. Exp. Zool.* 209, 115–128.
- Jing, J.H., Qian, J., Zhu, N., Chou, W.B., Huang, X.J., 2015. Improved differentiation of oligodendrocyte precursor cells and neurological function after spinal cord injury in rats by oscillating field stimulation. *Neuroscience* 303, 346–351.
- Johnson, H.A., Buonomano, D.V., 2009. A method for chronic stimulation of cortical organotypic cultures using implanted electrodes. *J. Neurosci. Methods* 176, 136–143.
- Karimi-Abdolrezaee, S., Eftekharpour, E., Fehlings, M.G., 2004. Temporal and spatial patterns of Kv1.1 and Kv1.2 protein and gene expression in spinal cord white matter after acute and chronic spinal cord injury in rats: implications for axonal pathophysiology after neurotrauma. *Eur. J. Neurosci.* 19, 577–589.
- Leal, J., Shaner, S., Matter, L., Böhrer, C., Asplund, M., 2023. Guide to leveraging conducting polymers and hydrogels for direct current stimulation. *Adv. Mater. Interfaces* 10, 2202041.
- Lee, H.U., et al., 2017. Subcellular electrical stimulation of neurons enhances the myelination of axons by oligodendrocytes. *PLoS One* 12.

- Li, G., et al., 2020. Epidural spinal cord stimulation promotes motor functional recovery by enhancing oligodendrocyte survival and differentiation and by protecting myelin after spinal cord injury in rats. *Neurosci. Bull.* 36, 372–384.
- Li, Q., Brus-Ramer, M., Martin, J.H., McDonald, J.W., 2010. Electrical stimulation of the medullary pyramid promotes proliferation and differentiation of oligodendrocyte progenitor cells in the corticospinal tract of the adult rat. *Neurosci. Lett.* 479, 128–133.
- Lin, Y., Yodh, J.S., Rodriguez, C., Kreymborg, P., Cohen, D.J., 2026. SCHEPHERD: A modular, programmable, direct current platform to control cell behavior. *Sci. Adv.* 12 (32), ead5802. <https://doi.org/10.1126/sciadv.aed5802>.
- Liu, J., et al., 2015. Electric signals regulate directional migration of ventral midbrain derived dopaminergic neural progenitor cells via Wnt/GSK3 β signaling. *Exp. Neurol.* 263, 113–121.
- Liu, R., et al., 2022. From 2D to 3D co-culture systems: a review of co-culture models to study the neural cells interaction. *Int. J. Mol. Sci.* 23.
- Lu, H., Shaner, S., Otte, E., Asplund, M., Vlachos, A., 2023. A microfluidic perspective on conventional in vitro transcranial direct current stimulation methods. *J. Neurosci. Methods* 385.
- Matter, L., Abdullaeva, O.S., Shaner, S., Leal, J., Asplund, M., 2024. Bioelectronic Direct Current Stimulation at the Transition Between Reversible and Irreversible Charge Transfer. *Adv. Sci.* 11.
- Matter, L., Harland, B., Raos, B., Svirsksis, D., Asplund, M., 2023. Generation of direct current electrical fields as regenerative therapy for spinal cord injury: a review. *APL Bioeng.* 7.
- McCaig, C., Rajnicek, A., 1991. Electrical fields, nerve growth and nerve regeneration. *Exp. Physiol.* 76, 473–494.
- Meng, X., et al., 2011. PI3K mediated electrotaxis of embryonic and adult neural progenitor cells in the presence of growth factors. *Exp. Neurol.* 227, 210–217.
- Negi, S., Bhandari, R., Rieth, L., Van Wagenen, R., Solzbacher, F., 2010. Neural electrode degradation from continuous electrical stimulation: comparison of sputtered and activated iridium oxide. *J. Neurosci. Methods* 186, 8–17.
- Ogura, T., Sakaguchi, H., Miyamoto, S., Takahashi, J., 2018. Three-dimensional induction of dorsal, intermediate and ventral spinal cord tissues from human pluripotent stem cells. *Development (Cambridge)* 145.
- Pandamooz, S., Nabiuni, M., Miyan, J., Ahmadiani, A., Dargahi, L., 2016. Organotypic spinal cord culture: a proper platform for the functional screening. *Mol. Neurobiol.* 53, 4659–4674.
- Patel, N., Poo, M.-M., 1982. Orientation of neurite growth by extracellular electric fields. *J. Neurosci.* 2, 483–496.
- Patel, N.B., Poo, M.-M., 1984. Perturbation of the direction of neurite growth by pulsed and focal electric fields. *J. Neurosci.* 4, 2939–2947.
- Plemel, J.R., Liu, W.-Q., Wee Yong, W., 2017. Remyelination therapies: a new direction and challenge in multiple sclerosis. *Nat. Rev. Drug. Discov.* 16, 617.
- Puttaswamy, S.V., et al., 2010. Enhanced cell viability and cell adhesion using low conductivity medium for negative dielectrophoretic cell patterning. *Biotechnol. J.* 5, 1005–1015.
- Qi, X.Y., et al., 2011. Enhanced electrical conductivity in polystyrene nanocomposites at ultra-low graphene content. *ACS Appl. Mater. Interfaces* 3, 3130–3133.
- Rumble, J., 2017. *CRC Handbook of Chemistry and Physics*, 102. CRC Press, Boca Raton, FL.
- Schopf, A., Boehler, C., Asplund, M., 2016. Analytical methods to determine electrochemical factors in electrotaxis setups and their implications for experimental design. *Bioelectrochemistry* 109, 41–48.
- Shaner, S., et al., 2023. Brain stimulation-on-a-chip: a neuromodulation platform for brain slices. *Lab. Chip* 23, 4967–4985.
- Shaner, S.W., et al., 2022. Skin stimulation and recording: moving towards metal-free electrodes. *Biosens. Bioelectron.* X 11, 100143.
- Shapiro, S., et al., 2005. Oscillating field stimulation for complete spinal cord injury in humans: a phase 1 trial. *J. Neurosurg. Spine* 2, 3–10.
- Shapiro, S., 2014. A review of oscillating field stimulation to treat human spinal cord injury. *World Neurosurg.* 81, 830–835.
- Simons, M., Nave, K.A., 2016. Oligodendrocytes: myelination and axonal support. *Cold Spring Harb. Perspect. Biol.* 8.
- Stevens, B., Porta, S., Haak, L.L., Gallo, V., Fields, R.D., 2002. Adenosine: a neuron-glia transmitter promoting myelination in the CNS in response to action potentials. *Neuron* 36, 855–868.
- Stevens, B., Tanner, S., Fields, R.D., 1998. Control of myelination by specific patterns of neural impulses. *J. Neurosci.* 18, 9303–9311.
- Tang-Schomer, M.D., 2018. 3D axon growth by exogenous electrical stimulus and soluble factors. *Brain Res.* 1678, 288–296.
- Yao, L., Li, Y., 2016. The role of direct current electric field-guided stem cell migration in neural regeneration. *Stem Cell. Rev. Rep.* 12, 365–375.
- Yao, L., McCaig, C.D., Zhao, M., 2009. Electrical signals polarize neuronal organelles, direct neuron migration, and orient cell division. *Hippocampus* 19, 855–868.
- Yao, L., Shanley, L., McCaig, C., Zhao, M., 2008. Small applied electric fields guide migration of hippocampal neurons. *J. Cell. Physiol.* 216, 527–535.
- Yildirim, L., et al., 2019. Engineering three-dimensional microenvironments towards in vitro disease models of the central nervous system. *Biofabrication* 11.
- Zhang, C., et al., 2014. Oscillating field stimulation promotes spinal cord remyelination by inducing differentiation of oligodendrocyte precursor cells after spinal cord injury. *Biomed. Mater. Eng.* 24, 3629–3636.