Advanced MEA Technology for Functional Characterization of Neural Organoids

Katja Malfertheiner [1,2], Sara Pizzi [1], Francesca Garilli [1], Irma Della Corte [1], Arianna Stefan [1], Sacha Mommo [3], Andrea Lovera [3,4], Paolo Cesare [1]
[1] Eurac Research, Institute of Biomedicine, Bolzano, Italy, [2] University of Trento, Department of Cellular, Computational and Integrative Biology, Trento, Italy, [3] FEMTOprint, Agno, Switzerland, [4] AxCellerate, Agno, Switzerland

Interreg Italia – Svizzera | Italie – Suisse | Italien – Schweiz
Cofinanziato dall'Unione Europea
COHESION ITALY 21-27 ALTO ADIGE SÜDTIROL
Co-funded by the European Union
INNo-CHIP

Introduction

Human iPSC-derived brain organoids provide powerful models of human neurodevelopment and disease, but their functional characterization remains limited. Conventional 2D microelectrode arrays (MEAs) primarily record activity from the organoid surface, while many 3D systems are invasive and can disrupt tissue integrity or restrict growth. Here, we present a non-invasive organ-on-chip platform that enables high-fidelity, longitudinal electrophysiological recordings from region-specific organoids (Figure 1). By guiding axons into neurite-trapping microelectrodes, this approach preserves cytoarchitecture while providing improved spatial coverage and reproducibility, supporting accurate analysis of network maturation and region-specific activity patterns.

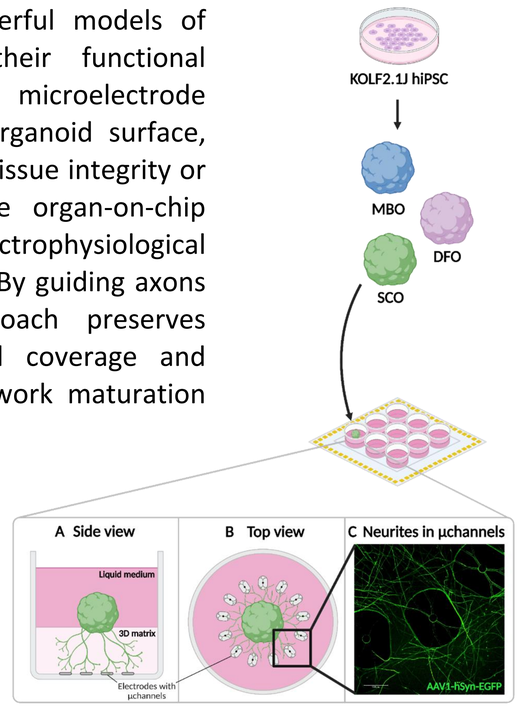

Figure 1. Concept of the MEA-NeuroCHIP for organoid recordings. Dorsal forebrain (DFO), midbrain (MBO), and spinal cord organoids (SCO) were generated from the hiPSC line KOLF2.1J using STEMdiff™ differentiation kits (STEMCELL Technologies) and transferred to the MEA-NeuroCHIP platform. (A) Side view showing the organoid positioned on a hydrogel matrix that supports neurite outgrowth toward the bottom electrodes for extracellular recording. (B) Top view of the chip illustrating the electrode layout within each well. (C) eGFP-transduced organoid neurites extending through the microtunnels toward the electrodes. (Created with Biorender.com)

Validation of Region-Specific Identity in Organoids

Upon differentiation, region-specific organoids exhibit markedly reduced expression of the pluripotency marker OCT4 compared with iPSCs. The regionalized organoids further display distinct fate-specific gene expression profiles and corresponding neurotransmitter identities (Figure 2).

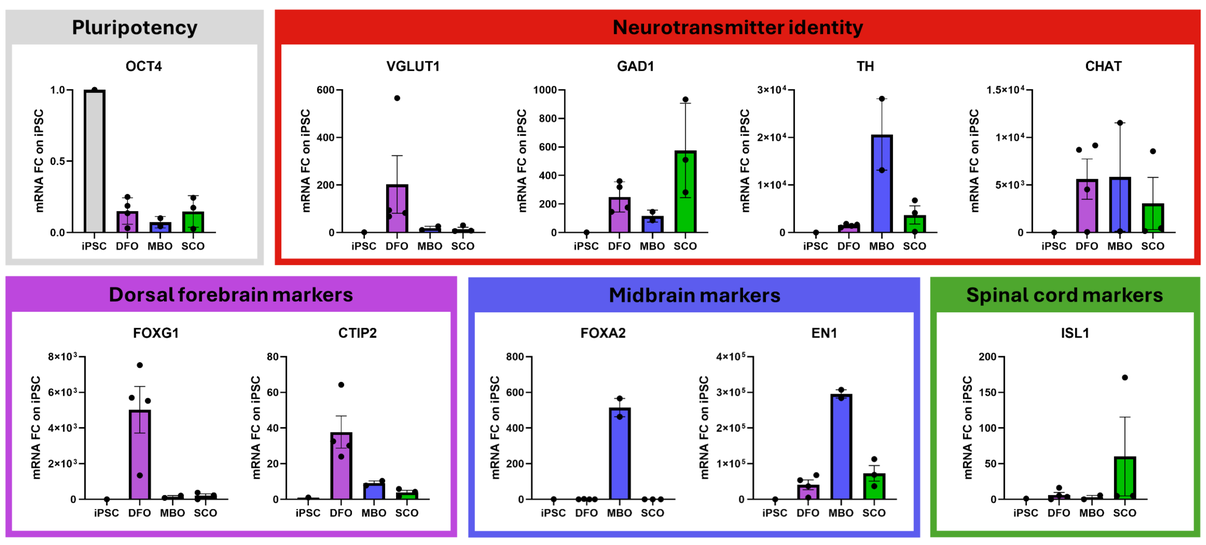

Figure 2. Region-Specific Gene Expression Analysis During Organoid Differentiation. RT-qPCR, fold change on iPSC and normalized to *GAPDH* of *OCT4, FOXG1, FOXA2, HB9* at day 50±5 of differentiation and *VGLUT1, GAD1, TH, CHAT, CTIP2, EN1, ISL1* at day 80±5 of differentiation in dorsal forebrain organoids (DFO), midbrain organoids (MBO), and spinal cord organoids (SCO). Data are shown as mean ± SEM. Each point represents one differentiation experiment with pooled RNA from around 10 organoids.

Immunohistochemical analysis confirmed tissue-specific marker expression in regionalized brain organoids: TBR1 in dorsal forebrain organoids, TH in midbrain organoids, and Islet1 in spinal cord organoids.

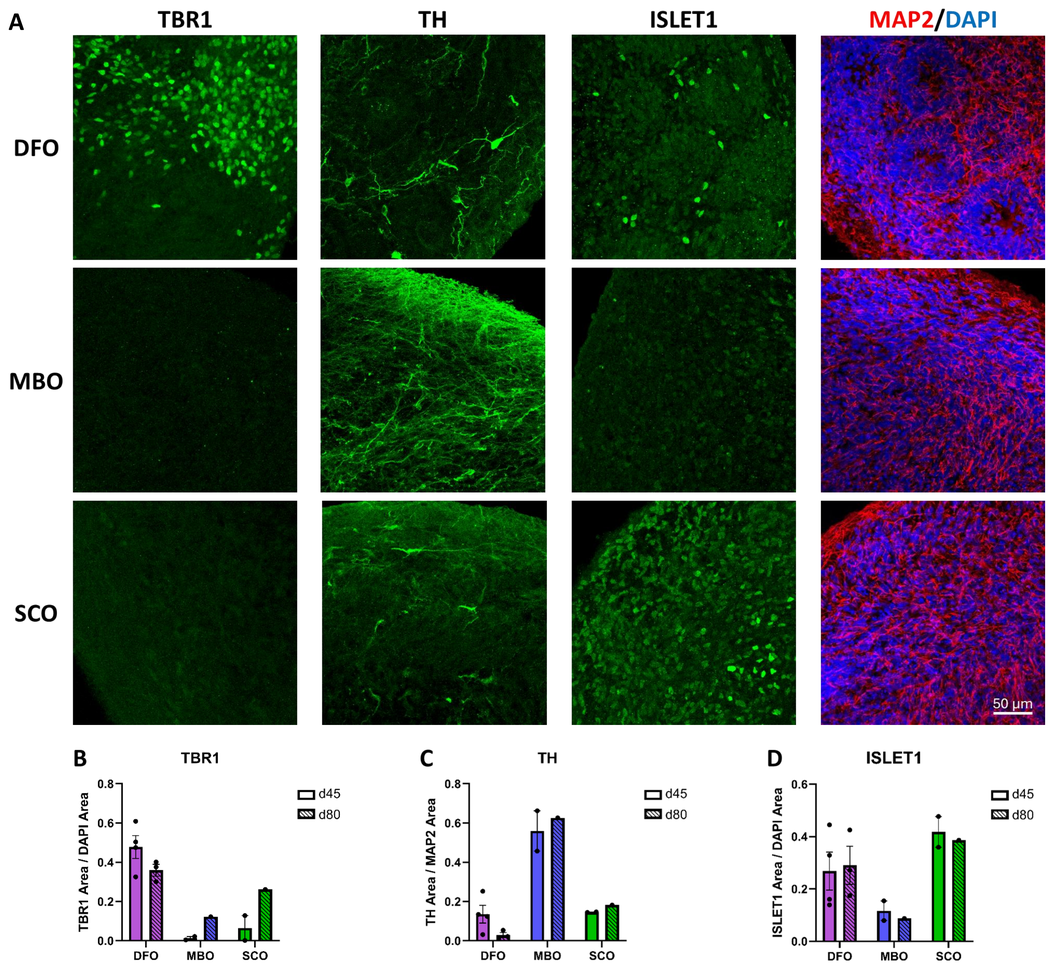

Figure 3. Immunofluorescent Analysis of Regional Identity Markers. (A) Representative images of immunohistochemical analysis of TBR1 (T-box brain transcription factor 1), TH (tyrosine hydroxylase), ISLET1 (ISL LIM homeobox 1) in dorsal forebrain organoids (DFO), midbrain organoids (MBO), and spinal cord organoids (SCO). (B) Quantification of TBR1 fluorescence area normalized to DAPI area in region-specific brain organoids at days 45 and 80 of differentiation. Bars represent mean ± SEM. (C) Quantification of TH fluorescence area normalized to MAP2 (microtubule associated protein 2) area in region-specific brain organoids at days 45 and 80 of differentiation. Bars represent mean ± SEM. (D) Quantification of ISLET1 fluorescence area normalized to DAPI area in region-specific brain organoids at days 45 and 80 of differentiation. Bars represent mean ± SEM. Each data point represents one independent organoid differentiation, with three images analysed per differentiation.

Functional Characterization of Neural Organoids

MEA recordings from region-specific organoids cultured in our MEA-NeuroCHIP were analysed to extract spike, burst, and network-burst parameters, enabling functional characterization of dorsal forebrain, midbrain, and spinal cord organoids (representative recordings shown in Figure 4, and the corresponding quantitative analyses are presented in Figure 5).

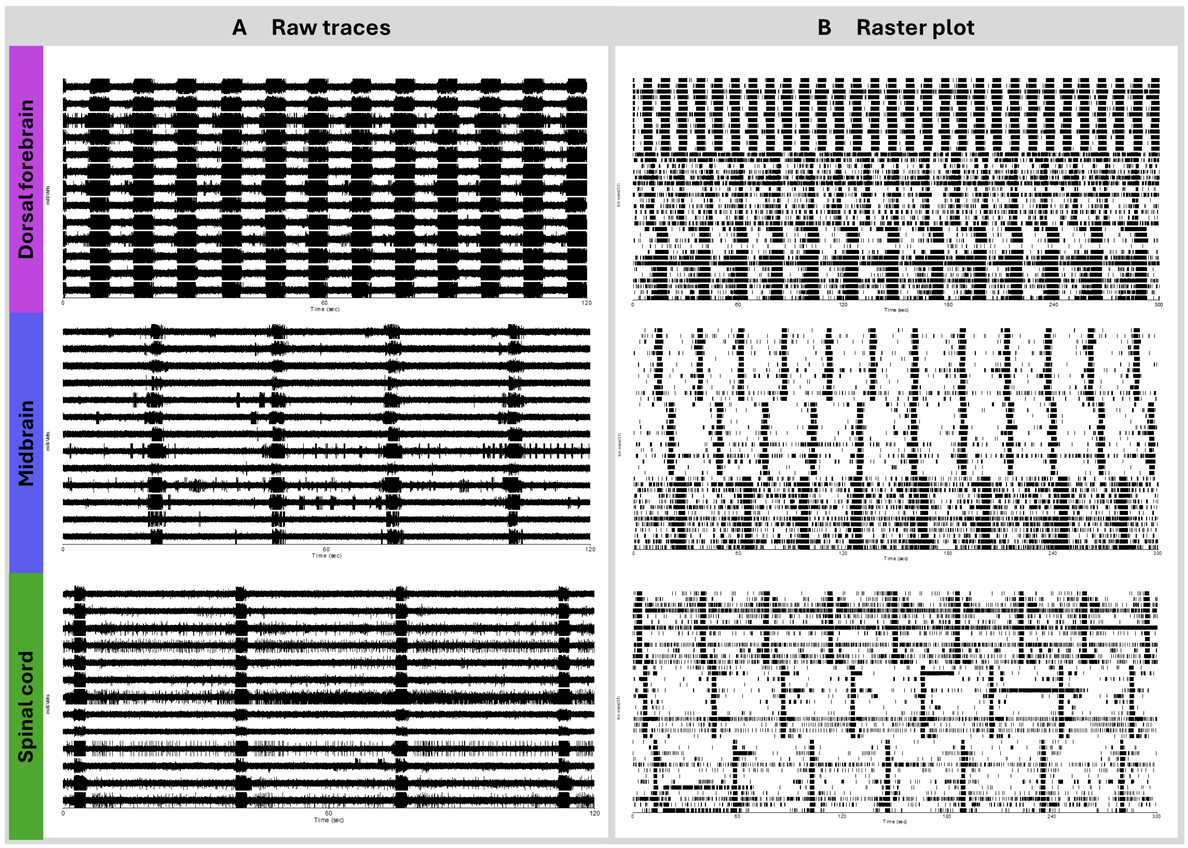

Figure 4. Representative electrophysiological recordings from region-specific human neural organoids. (A) Representative extracellular activity traces recorded from a single well containing one organoid derived from dorsal forebrain, midbrain, or spinal cord patterning. Each panel shows the simultaneous signals from 13 microelectrodes integrated within the well (one organoid per well). Recordings illustrate region-dependent differences in baseline spiking and bursting dynamics under identical acquisition conditions. (B) Representative spike raster plots across multiple wells on a single chip. Raster plots show spike timing from three representative wells per organoid type (dorsal forebrain, midbrain, spinal cord) selected from a 9-well chip. Each well contains one organoid recorded by 13 electrodes. Rasters highlight well-to-well variability and region-specific network activity patterns, including differences in burst frequency, synchrony, and occurrence of network-wide events.

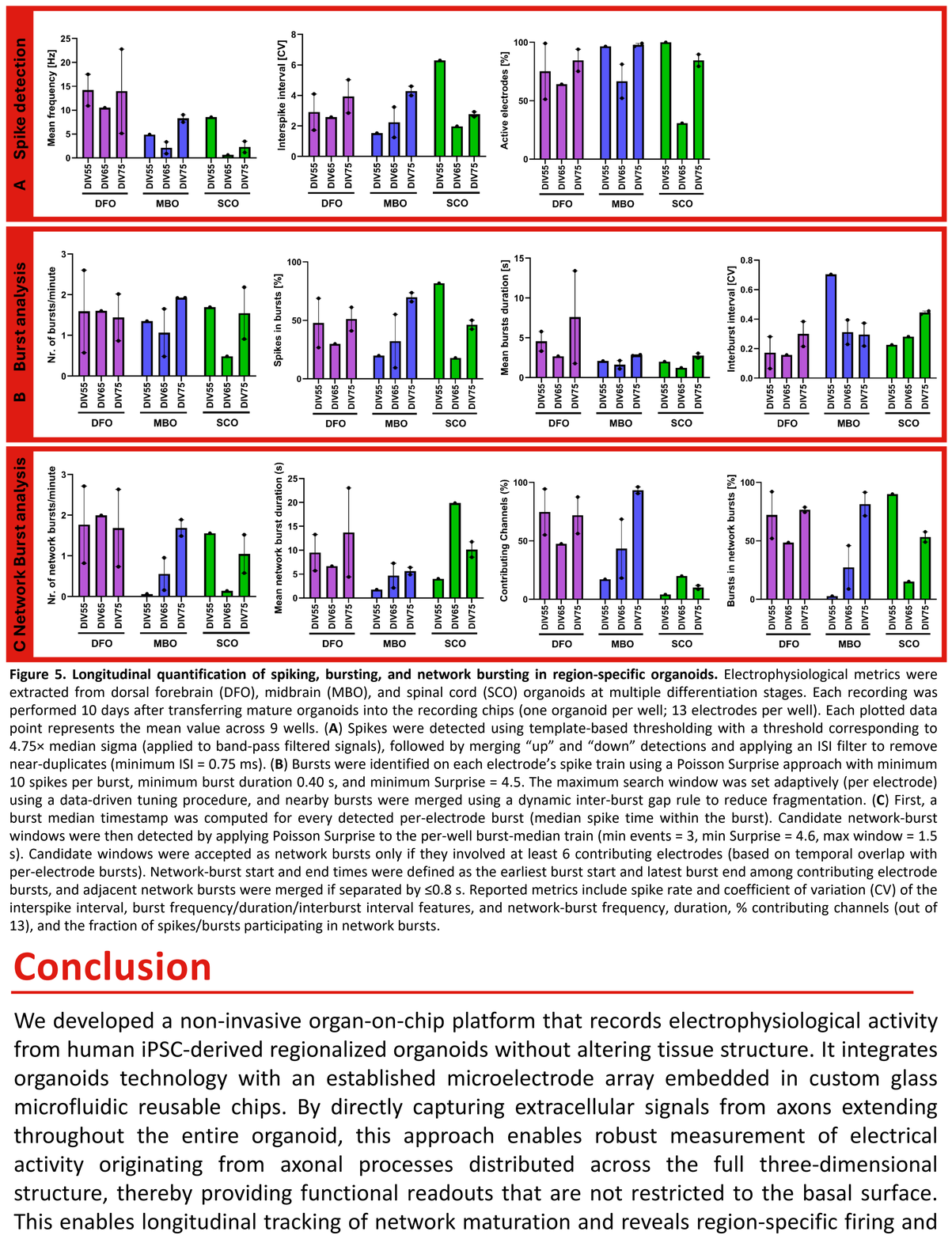

Figure 5. Longitudinal quantification of spiking, bursting, and network bursting in region-specific organoids. Electrophysiological metrics were extracted from dorsal forebrain (DFO), midbrain (MBO), and spinal cord (SCO) organoids at multiple differentiation stages. Each recording was performed 10 days after transferring mature organoids into the recording chips (one organoid per well; 13 electrodes per well). Each plotted data point represents the mean value across 9 wells. (**A**) Spikes were detected using template-based thresholding with a threshold corresponding to 4.75× median sigma (applied to band-pass filtered signals), followed by merging "up" and "down" detections and applying an ISI filter to remove near-duplicates (minimum ISI = 0.75 ms). (**B**) Bursts were identified on each electrode's spike train using a Poisson Surprise approach with minimum 10 spikes per burst, minimum burst duration 0.40 s, and minimum Surprise = 4.5. The maximum search window was set adaptively (per electrode) using a data-driven tuning procedure, and nearby bursts were merged using a dynamic inter-burst gap rule to reduce fragmentation. (**C**) First, a burst median timestamp was computed for every detected per-electrode burst (median spike time within the burst). Candidate network-burst windows were then detected by applying Poisson Surprise to the per-well burst-median train (min events = 3, min Surprise = 4.6, max window = 1.5 s). Candidate windows were accepted as network bursts only if they involved at least 6 contributing electrodes (based on temporal overlap with per-electrode bursts). Network-burst start and end times were defined as the earliest burst start and latest burst end among contributing electrode bursts, and adjacent network bursts were merged if separated by ≤0.8 s. Reported metrics include spike rate and coefficient of variation (CV) of the interspike interval, burst frequency/duration/interburst interval features, and network-burst frequency, duration, % contributing channels (out of 13), and the fraction of spikes/bursts participating in network bursts.

Conclusion

We developed a non-invasive organ-on-chip platform that records electrophysiological activity from human iPSC-derived regionalized organoids without altering tissue structure. It integrates organoids technology with an established microelectrode array embedded in custom glass microfluidic reusable chips. By directly capturing extracellular signals from axons extending throughout the entire organoid, this approach enables robust measurement of electrical activity originating from axonal processes distributed across the full three-dimensional structure, thereby providing functional readouts that are not restricted to the basal surface. This enables longitudinal tracking of network maturation and reveals region-specific firing and bursting patterns. The platform improves sensitivity, spatial coverage, and reproducibility, supporting neurodevelopmental research, disease modelling, and drug discovery.