# A mesh microelectrode array for non-invasive electrophysiology within neural organoids

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## Abstract

Organoids are emerging *in vitro* models of human physiology. Neural models require the evaluation of functional activity of single cells and networks, which is best measured by microelectrode arrays. The characteristics of organoids clash with existing *in vitro* or *in vivo* microelectrode arrays. With inspiration from implantable mesh electronics and growth of organoids on polymer scaffolds, we fabricated suspended hammock-like mesh microelectrode arrays for neural organoids. We have demonstrated the growth of organoids enveloping these meshes, their cultivation for at least nine months, and could measure spontaneous electrical activity within organoids. Our concept should enable a new class of microelectrode arrays for *in vitro* models of three-dimensional electrically active tissue.

## 1 Introduction

Neural organoids show promise as physiologically relevant *in vitro* models of the human brain.[1] Electrophysiology is critical to demonstrate functionality of translational models of the central nervous system.[2] Electrical activity has been observed in organoids by adapting existing tools (Figure 1B–F), however, there remains an unmet need for more appropriate tools and methods specific for electrophysiology of organoids.[3]

What is needed for organoid electrophysiology? Scientists may have different demands: Can we extract relevant information using only a handful of electrodes?[4] Can we measure the activity of all neurons in an organoid,[5] and would this be useful? How does the activity of organoids relate to human physiology?[1] Should we consider new ethical concerns?[6] In fact, current knowledge of the functional electrical activity within organoids does not allow these questions to be addressed and thus we suggest a new technical approach to tackle them.

Neural organoids are on a spectrum between conventional cell culture and a living brain. Research tools are available for both ends of this spectrum, yet are not ideal for organoids. In conventional cell cultures, cells adhere onto planar microelectrode arrays (MEAs). Typical *in vitro* models relate action potentials and bursting activity to cellular or synaptic effects of applied compounds. In contrast, animal models attempt to resolve the activity of many single neurons over an extended period of time, and to relate it to parameters such as behavior.

In either case, microelectrodes may be sparsely distributed to efficiently capture independent activity or may be densely packed to capture all activity at a high resolution. In the simplest *in vitro* models, network activity can be captured by only a few electrodes, yet many independent samples are needed to measure small effects.[4] An ultimate vision in neuroscience is to record all cells in the brain at millisecond resolution *in vivo*.[5,7]

Capturing the activity of single cells requires close proximity to electrodes – and tracking cells over time requires a stable structure. Therefore, an alternative to conventional methods of growing freely floating organoids is needed. Organoids can be grown on 2D-MEAs for several months,[8] but they flatten as neurons migrate and spread on the MEA surface (Figure 1C). CMOS MEAs can capture activity in tissue at a high resolution and detect activity to a depth of 100 µm.[9] The ability to resolve

internal activity similar to electroencephalography (EEG) cannot be assumed in organoids, as EEG measures coordinated activity in structured cell layers.[10] Deeper activity can be recorded by MEAs with protruding microelectrodes (Figure 1D) and slicing of organoids[11] or implantable neural probes (Figure 1E). In addition to the technical challenge of implantation, tissue damage limits recordings to a single endpoint.[12] Beyond such electrical methods, optical methods can record superficial activity (Figure 1F) but with lower sensitivity.[13]

To move beyond these limited approaches, we were inspired by the growth of organoids on artificial structures[14] and demonstrations of implantable mesh electronics with cellular or subcellular structure sizes.[15,16] We believe that the combination of these approaches should enable guided growth of neural organoids with non-invasive electrophysiology (Figure 1A).

A similar device has been demonstrated for acute experiments and suggested for organoids.[17] Superficial electrophysiology of cardiac spheroids has been demonstrated with a flexible self-rolling (but not mesh) device.[18]

In this work, we present a mesh microelectrode array specifically designed for physically suspended growth and long-term electrophysiology of neural organoids (Figure 1A). With a hammock-like artificial structure, the device suspends organoids far from solid surfaces to enable their unimpeded growth. Our successful recording of spontaneous extracellular action potentials supports the use of such devices for non-invasive electrophysiology of activity within and throughout neural organoids.

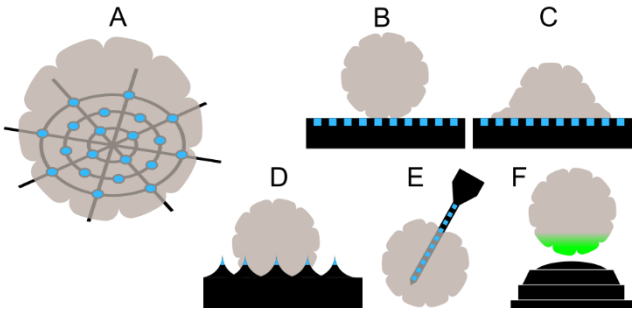


*Figure 1: Technical possibilities for recording electrical activity of neural organoids. A: Mesh MEA (this work). B: Acute use of a planar MEA. C: Cultivation on a planar MEA. D: Planar MEA with protruding electrodes. E: Probe implantation. F: Calcium or voltage imaging.*

# 2 Methods

## 2.1 Design

In designing the device, we prioritized compatibility with organoid culture methods, minimization of technical complexity and material choices for long-term device stability. We adopted methods previously used for fabrication of perforated MEAs.[19] Microelectrodes (30 µm diameter) were distributed across a suspended region with a diameter of 2 mm. Minimum dimensions of 4 µm (lines and spaces) ensured a high fabrication yield. We focused on a design compatible with 256-channel amplifiers from Multi Channel Systems MCS GmbH (Reutlingen) with minimal complexity of electrical packaging.

## 2.2 Fabrication

Our devices consist of flexible microfabricated meshes assembled into glass-bottomed wells (Figure 2). The meshes were produced on carrier wafers using cleanroom microfabrication.[19] Electrical paths (Ti/Au/Ti, 400 nm) were insulated on both sides by 6 µm of polyimide (DuPont PI-2611). Metals were deposited by sputter coating and structured by plasma etching. Polyimide (PI) was structured by plasma etching against a silicon nitride hard mask. Microelectrodes (30 µm diameter) of titanium nitride (TiN, 500 nm) were structured by sputter coating and lift-off using a photoresist mask. After release from carrier substrates, we assembled the meshes into wells comprising multiple machined polymer and glass components using EPOTEK 301-2SL (Figure 2E). Glass components were pretreated with aminopropyltriethoxysilane before gluing.

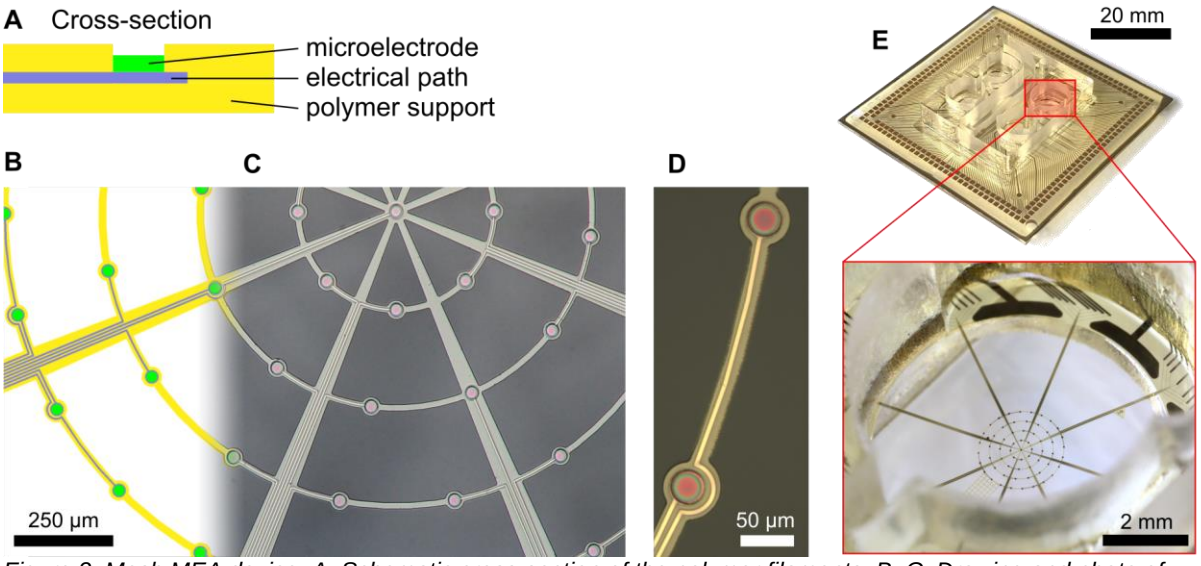


*Figure 2: Mesh MEA device. A: Schematic cross-section of the polymer filaments. B, C: Drawing and photo of the suspended mesh. Regions between polymer filaments are open. D: Microelectrodes on a single filament. E: Photos of the whole device and a single well with a suspended hammock-like mesh.*

## 2.3 Cell and organoid culture

Here, we present results of two types of neural organoids generated from human induced pluripotent stem (iPS) cells. Cells were verified pluripotent and contamination-free, confirmed negative for mycoplasma, and pluripotent stem cells were maintained feeder-free. Detailed characterization of these organoids is ongoing and will be reported elsewhere.

Cerebral organoids (Figure 3) were generated following the protocol published by Lancaster et al.[20] Human iPS cells were obtained in house via OSKM reprogramming.[21] Briefly, at day 0, 7000 cells from single cell suspensions were plated in 96-well U-bottom low attachment plates to generate embryoid bodies. After 6 days in vitro, media composition was changed to induce the formation of anterior neuroectoderm. At day 10 the neuroectodermal aggregates were embedded in Matrigel to provide them with a scaffold that would allow for further differentiation, maturation and growth.

For a second type of neural organoid (Figure 4), human iPS cells were obtained from Thermo Fisher Scientific (A18945). On day 0 of organoid culture, iPS cells (passage < 50, approximately two thirds confluence) were dissociated from plates by EDTA (Sigma) treatment and plated in ultra-low-binding plates (Corning) containing mTeSR™1 medium (STEMCELL Technologies) with blebbistatin (Calbiochem) added. Neural induction was induced by gradually exchanging mTeSR™1 medium with neural induction medium [DMEM/F12 (Gibco), N-2 supplement (Gibco), Glutamax (Gibco), MEM-NEAA (Gibco) and heparin (Sigma)] from day 1 on. At day 9, tissues were embedded into Matrigel (Corning) and thereafter grown in differentiation media [DMEM/F12 and Neurobasal (Gibco) containing N-2 supplement, B27 supplement (Gibco), 2-mercaptoethanol (Sigma), insulin (Sigma), Glutamax, penicillin-streptomycin (Gibco) and MEM-NEAA].

We transferred individual organoids onto mesh MEAs by pipetting. For cerebral organoids, low media volumes for the first 5 days on the mesh kept the organoids at the air–liquid interface to facilitate enveloping of the mesh. Later, full media changes covering the entire organoid were performed every other day. Before placing our second type of organoids, MEAs were coated with poly-D-lysine to promote cell adhesion: MEAs were treated with air plasma, incubated with 1 mg/ml poly-D-lysine for 1 h, then rinsed three times with saline. Media was changed every other day and maintained to keep organoids at the air–liquid interface.

## 2.4 Electrophysiology

Spontaneous electrical activity was recorded by a multichannel amplifier (USB-MEA256 from Multi Channel Systems MCS GmbH) at a sampling rate of 40 kHz. Recordings were high-pass filtered (200 Hz second-order Butterworth) and action potentials were identified by threshold detection.

# 3 Results and discussion

## 3.1 Device

The devices (Figure 2) contain four wells with diameters of 7 mm. Each well can support a single organoid on a central spider-web-like mesh with a diameter of 2 mm and 61 microelectrodes. The mesh has four concentric ring filaments (radii of 0.25, 0.5, 0.75 and 1 mm) containing 8, 12, 16 and 24 electrodes, and one electrode at the center of the mesh. The rings are suspended 2 mm above the glass bottom. Eight radial filaments provide structural support and electrical connections to the electrodes. The filaments have a thickness of 12 µm and width of 20 µm. The width of the radial filaments increases to 80 µm to accommodate all electrical connections. Outside of the wells, the electrical paths extend to gold pads at the device perimeter, which are contacted directly by the amplifier headstage.

We designed this device to allow unrestricted growth in all directions. In the suspended plane of the mesh, 85 % of the central 2 mm circle is open space. With its thickness of 12 µm, the artificial structure would occupy only 0.1 % of the total volume of a spherical 2-mm-diameter organoid. The impact on the development of such an organoid should be greatly reduced in comparison to organoids grown on planar MEAs. Suspending the organoid prevents its adhesion on solid surfaces, thereby reducing the need for mechanical shaking. Nutrients and oxygen can be delivered from all sides, although perfusion could improve delivery. No flow was used in the presented results.

We designed our mesh to support microelectrodes in a fixed arrangement for simple placement of organoids and electrode localization even when embedded in tissue. However, the materials used to produce the mesh would allow flexibility or stretching if modified to include serpentine structures.[22] Our microelectrodes are connected by unbroken thin film metal paths rather than complicated electrical packaging.[23,17,22] The microfabricated structures of our device are similar to the flexible devices reported by Soscia et al., although their device is intended for 3D cultures of cells suspended in hydrogel.[24] In comparison to the use of rigid devices for recording in 3D cell cultures,[11,12] devices such as ours or those of Soscia et al. which integrate electrode on flexible filaments should allow for a better long-term cultivation of organoids.

We have observed no problems of leakage or technical instability, even after cultivation of organoids over a period of 9 months.

## 3.2 Organoids

After their placement on a mesh MEA, organoids enveloped the filaments and continued to grow. Figure 3 shows a cerebral organoid which had grown on the mesh for ten days. The decentered placement of this example – although unintentional – revealed how an organoid can grow on filaments of a mesh MEA. Thin regions of tissue reveal the embedded microelectrodes, while thicker regions hide the filaments and microelectrodes. We have cultivated organoids on these devices for longer than nine months — and counting!

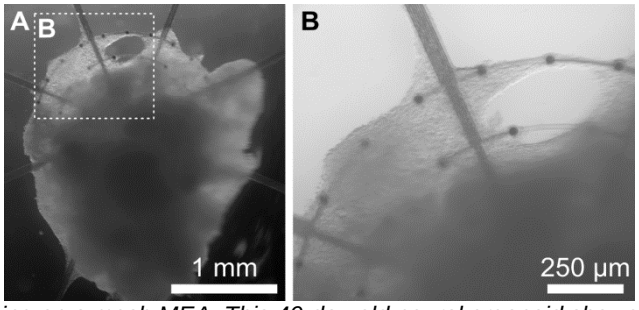


*Figure 3: Organoids growing on a mesh MEA. This 40-day-old neural organoid showed outgrowth of cells along the polymer filaments after ten days on the mesh. Thin tissue reveals embedded microelectrodes (black circles in B). Images were taken from above.*

A limitation of our design is the large open spaces peripheral to the central electrode field. Adhesion of organoids after placing them on meshes is not immediate, but requires the organoids to be returned to an incubator. Even careful handling may cause some organoids to fall off of their meshes. Although readily solved by closing these regions with structural filaments, a user-friendly solution such as well inserts may simplify handling.[25]

We have demonstrated the continued growth of organoids after their placement on mesh MEAs. We envision that growth from earlier states (embryoid bodies or stem cells) may be possible. However, this will require additional adaptation of biological protocols.

### 3.3 Electrophysiology

A neural organoid was placed on the mesh at an age of 197 days. Recordings of spontaneous activity were performed 35 days later (day 232). We observed spontaneous activity on most microelectrodes across a distance of 2 mm in the organoid (Figure 4). We present this result as a demonstration of the device's functionality. Signal amplitudes decrease with increasing distance between electrodes and active cells,[9] and our measured amplitudes suggest that active cells are not directly adjacent to the microelectrodes. Time-dependent studies to compare cell types and morphologies with electrophysiology will be possible by histology of organoids containing embedded mesh microelectrode arrays.[16]

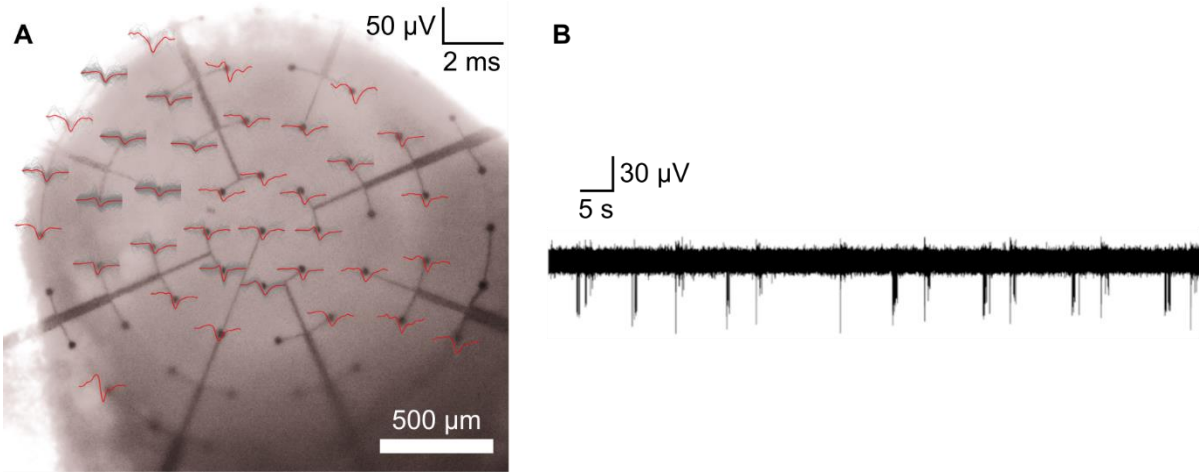


*Figure 4: Electrical activity in a neural organoid, shown with detected waveforms overlaid at electrode positions (A) and a single electrode trace (B).*

## 4 Conclusions

We have demonstrated biohybrid neural organoids and the first microelectrode array device designed for long-term, non-invasive electrophysiology within neural organoids. With this device, we hope to contribute to a better understanding of functional activity in organoids.

Full benefits of organoid electrophysiology may be obtained by considering both the device design and organoid culture techniques. Methods which place organoid precursors such as embryoid bodies or stem cells may require modified devices. Assembly of organoids of various brain regions or other tissues may be enabled by the development of specific designs. Future implementations may balance the numbers of wells and microelectrodes for targeted applications. Further technical advancements could include stretchable meshes, mechanical or chemical sensors, or three-dimensional meshes.

## 5 Conflicts of interest

There are no conflicts of interest to declare.

## 6 Acknowledgements

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# 7 References

1. Benito-Kwiecinski, S. & Lancaster, M. A. Brain Organoids: Human Neurodevelopment in a Dish. *Cold Spring Harb Perspect Biol* a035709 (2019) doi:10.1101/cshperspect.a035709.
2. Engle, S. J., Blaha, L. & Kleiman, R. J. Best Practices for Translational Disease Modeling Using Human iPSC-Derived Neurons. *Neuron* **100**, 783–797 (2018).
3. Keller, J. M. & Frega, M. Past, Present, and Future of Neuronal Models In Vitro. in *In Vitro Neuronal Networks* (eds. Chiappalone, M., Pasquale, V. & Frega, M.) vol. 22 3–17 (Springer International Publishing, 2019).
4. Negri, J., Menon, V. & Young-Pearse, T. L. Assessment of Spontaneous Neuronal Activity In Vitro Using Multi-Well Multi-Electrode Arrays: Implications for Assay Development. *eNeuro* **7**, ENEURO.0080-19.2019 (2020).
5. Marblestone, A. H. *et al.* Physical principles for scalable neural recording. *Frontiers in Computational Neuroscience* **7**, 137 (2013).
6. Koplin, J. J. & Savulescu, J. Moral Limits of Brain Organoid Research. *J Law Med Ethics* **47**, 760–767 (2019).
7. Hong, G. & Lieber, C. M. Novel electrode technologies for neural recordings. *Nat Rev Neurosci* **20**, 330–345 (2019).
8. Trujillo, C. A. *et al.* Complex Oscillatory Waves Emerging from Cortical Organoids Model Early Human Brain Network Development. *Cell Stem Cell* **25**, 558-569.e7 (2019).
9. Obien, M. E. J., Hierlemann, A. & Frey, U. Accurate signal-source localization in brain slices by means of high-density microelectrode arrays. *Sci Rep* **9**, 788 (2019).
10. Jackson, A. F. & Bolger, D. J. The neurophysiological bases of EEG and EEG measurement: A review for the rest of us: Neurophysiological bases of EEG. *Psychophysiol* **51**, 1061–1071 (2014).
11. Giandomenico, S. L. *et al.* Cerebral organoids at the air–liquid interface generate diverse nerve tracts with functional output. *Nat Neurosci* **22**, 669–679 (2019).
12. Quadrato, G. *et al.* Cell diversity and network dynamics in photosensitive human brain organoids. *Nature* **545**, 48–53 (2017).
13. Kirihara, T. *et al.* A Human Induced Pluripotent Stem Cell-Derived Tissue Model of a Cerebral Tract Connecting Two Cortical Regions. *iScience* **14**, 301–311 (2019).
14. Lancaster, M. A. *et al.* Guided self-organization and cortical plate formation in human brain organoids. *Nat Biotechnol* **35**, 659–666 (2017).
15. Liu, J. *et al.* Syringe-injectable electronics. *Nature Nanotechnology* **10**, 629–636 (2015).
16. Yang, X. *et al.* Bioinspired neuron-like electronics. *Nature Materials* (2019) doi:10.1038/s41563-019-0292-9.
17. Kireev, D., Rincón Montes, V., Stevanovic, J., Srikantharajah, K. & Offenhäusser, A. N3-MEA Probes: Scooping Neuronal Networks. *Front. Neurosci.* **13**, 320 (2019).
18. Kalmykov, A. *et al.* Organ-on-e-chip: Three-dimensional self-rolled biosensor array for electrical interrogations of human electrogenic spheroids. *Sci. Adv.* **5**, eaax0729 (2019).
19. Stett, A. *et al.* Improving of signal-to-noise ratio of electrophysiological recordings from explanted retinas by using perforated microelectrode arrays. in *Mikrosystemtechnik Kongress* (VDE Verlag GmbH, 2007).
20. Lancaster, M. A. *et al.* Cerebral organoids model human brain development and microcephaly. *Nature* **501**, 373–379 (2013).
21. Max Planck Institute for Molecular Biomedicine (MPI). MPIi001-A. *Human pluripotent stem cell registry* https://hpscreg.eu/cell-line/MPIi001-A (2019).
22. Li, Q. *et al.* Cyborg Organoids: Implantation of Nanoelectronics via Organogenesis for Tissue-Wide Electrophysiology. *Nano Lett.* **19**, 5781–5789 (2019).
23. Musick, K., Khatami, D. & Wheeler, B. C. Three-dimensional micro-electrode array for recording dissociated neuronal cultures. *Lab on a Chip* **9**, 2036 (2009).
24. Soscia, D. A. *et al.* A flexible 3-dimensional microelectrode array for in vitro brain models. *Lab Chip* 10.1039.C9LC01148J (2020) doi:10.1039/C9LC01148J.
25. Day, J. H. *et al.* Injection molded open microfluidic well plate inserts for user-friendly coculture and microscopy. *Lab Chip* **20**, 107–119 (2020).