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Air-liquid interface micro-electrode arrays - well-adapted tools for long-term *in vitro* monitoring of 3D neural tissues

https://doi.org/10.1515/cdbme-2025-0168

Abstract: Despite the large variety of traditional, flexible and stretchable Micro-Electrode Array (MEA) devices available for use with organoids, most of them present drawbacks when applied for long-term 3D neural tissue monitoring applications. In many cases, 3D neural tissues can only be monitored for acute and/or short-term durations due to tissue necrosis that occurs due to the lack of tissue oxygenation under submerged culture conditions. This paper presents alternative MEA devices, called ALI-MEA. These ALI-MEA allow the maintenance of 3D neural tissues at air-liquid interface, which provides excellent tissue oxygenation and long-term survival conditions, resulting in the capability to stimulate and record electrophysiological activity from the tissues for long-lasting experiments, up to several months. This paper illustrates these findings with results from several typical 3D neural tissue preparation experiments showing that long-term experiments can be easily achieved using the ALI-MEA devices.

Keywords: Micro-electrode array, Air-liquid interface, Long-term monitoring, 3D Neural tissues, Organoids.

1 Introduction

Micro-Electrode Array (MEA) devices were developed a couple decades ago for stimulation of and recording from dissociated cell and sliced tissue cultures in vitro. These "traditional" MEA devices are based on glass substrates with 2D MEA and 3D MEA electrode configurations. Many ex-vivo models based on brain slices have been developed and studied with success in acute experiments using 2D MEA and 3D MEA devices [1].

Recently, animal derived cell models are more and more replaced by human stem-cell based models issued from reprogrammed iPSC cells. 3D reconstructed human neural tissues including neurospheres, organoids and assembloids have become the new standard to study effects of compounds for drug discovery/validation and toxicology applications. Traditional 2D and 3D MEA are well suited for mainly acute to short-term experiments when using entire 3D tissues.

For long-term experimentation on 3D neural tissues, these MEA devices are not well suited due to lack of oxygenation induced by the culture conditions, i.e. the tissue is submerged by culture medium that is limited in tissue available oxygen presence over time. Because diffusion allows oxygen and nutrients to penetrate less than 1 mm into the tissue, cell viability in deep parts of 3D tissues is thus a problem and 3D neural tissues/organoids tend to develop a necrotic core.

Several flexible and stretchable MEA devices have been designed for use with 3D neural tissues [2]. They mainly surround the 3D neural tissues and provide electrophysiological information from around the tissues. Also mesh MEA configurations [3] have been developed to be embedded into the 3D neural tissue with time, allowing monitoring of electrical activity from the inside of the tissues.

However, most of these devices require constant perfusion or regular/daily exchange of medium to avoid necrosis in the centre of the tissues when used for long-term cultures, which makes the work more tedious to maintain the preparations in good conditions for survival.

Besides the solution of direct perfusion of medium through the tissue to overcome the oxygenation problem, this work presents another solution allowing facilitated long-term culture of 3D neural tissue: the Air-Liquid Interface (ALI) culture approach [4]. This technique, where the 3D neural tissues are not completely immersed in the nutrient medium, but remain at an ALI, provides sufficient oxygenation for long-term tissue survival, with the tissues being supplied from below through a porous membrane by capillary action of the medium [5]. Under such conditions, the tissues can, after

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maturation is reached, be placed onto an ALI adapted MEA device for electrical monitoring and experimentation.

This paper presents examples of ALI adapted MEA devices and illustrates the power of the ALI culture method for long-term experimentation by experimental results from 3D neural tissues and brain organoids.

2 Methods

2.1 ALI-MEA and Data Acquisition Platform

The MEA biochip named ALI-MEA developed in this work is designed to be fully compatible with a commercial 60-channel data acquisition system of Multi-Channel Systems MCS GmbH (MCS), Reutlingen, Germany. It is composed of a printed circuit board substrate for connection to external electronics, a fluidic channel covered with a PTFE membrane (0.45 µm pore size) where the inlet and outlet are based on Luer-lock format and enable gentle and efficient media perfusion of the 3D neural tissue from the bottom, and a thin-film polyimide (PI) membrane integrating 59 platinum black coated recording electrodes and 1 large reference electrode that is placed on top of the PTFE membrane. Since the polyimide membrane is porous, the perforated design of the ALI-MEA allows for continuous exchange of media through the tissue sample and enables conformal contact with the neural tissues without inducing any damage (see Fig. 1A).

The fabrication process of the ALI MEA is following the protocol previously described in Stoppini et al. [6]. Briefly, the polyimide membranes are manufactured under cleanroom conditions and are released from the substrate, mounted onto a printed circuit board, and sealed with epoxy. The obtained platinum electrodes are coated electrochemically with a platinum black layer to reduce the electrode impedances, providing low noise electrode characteristics. Finally, laser-cut PMMA parts defining the fluidic channel are assembled on both sides of the ALI-MEA (see Fig. 1B).

The resulting ALI-MEA devices are composed of a flexible 8 µm thick, porous PI membrane. Electrodes are arranged on an 8x8 matrix without corner electrodes, have a diameter of 30 µm and are located on a 200 µm grid. A 10% area membrane porosity is achieved by etching ø7.5 µm holes on a 20 µm grid through the membrane (see Fig. 1C).

Data acquisition was performed using MEA2100-Mini System hardware and the "Multi Channel Experimenter" and "Multi Channel Analyzer" software from MCS. Briefly, the hardware allowing the detection of single unit action potentials and the cut-out of the activity related time-window data is time-multiplexed among 60 channels. Each channel is sampled at a frequency of 10 kHz in the presented experiment. The amplifier bandpass filter was set from 0.1 Hz to 5.0 kHz (1st order high-pass, 3rd order Butterworth low-pass). The acquisition hardware allows the real-time detection of spikes by applying a simple threshold-crossing criterion. A voltage variation larger than six times the standard deviation is considered as a spike. Each time that the threshold is reached a 3 ms window is recorded (starting 1 ms before the event).

2.2 3D Neural Tissues and Organoids Generation

Neural stem cells derived from induced pluripotent stem cells were used for the generation of 3D neural tissue according to Govindan et al. [7]. Cultures were maintained in orbital agitation. For the presented experiments, 1-year-old 3D neural tissues with a diameter between 300 to 500 µm were used.

Commercially available healthy female patient-derived hiPSC purchased from Phenocell were used for the generation of dorsal cortical brain organoids following a previously published protocol [8]. Briefly, 2M hiPSC we aggregated on day 0 and sequentially exposed to cocktails of patterning

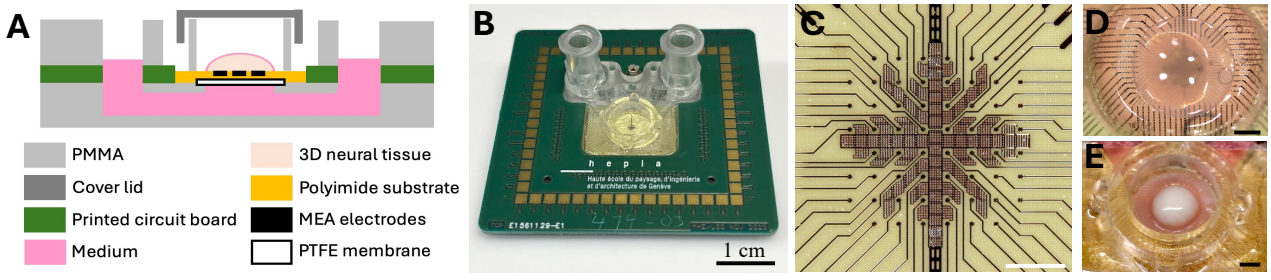

Figure 1: A) Schematic view of an ALI-MEA60 biochip allowing stimulation and recording of electrophysiological activities from 3D neural tissues at air-liquid interface. A thin layer of culture medium is covering the neural tissues. B) Picture of an ALI-MEA60 with Luer-lock connectors for injection or perfusion of culture medium. C) Closer view of the working area at center of the ALI-MEA60. Pictures of an assembloid built from 12-months-old 3D neural tissues on a pre-cut porous membrane patch (D) and a 1.3-months-old dorsal brain organoid (E) covering the ALI-MEA60 working area. Scale bars: C = 500 µm; D & E = 1mm.

molecules over the course of 43 days before culture on agitation in maturation medium (Neurobasal with SM1-A).

2.3 Air-Liquid Interface Principle

ALI cultures were originally developed for organotypic cultures of brain slices, and it has been shown to retain many essential organizational features of the host tissue [9]. An important feature of the ALI culture method is the improved exchange between air and tissue, allowing the development of a relatively thick 3D tissue culture without hypoxic cell death. In fact, by addition of the culture medium only underneath a porous membrane, the neural tissue lying on top of this membrane is covered by capillarity by a very thin film of medium, allowing important air diffusion within the entire thickness of the tissues. Mature (12-month-old) 3D neural tissues have been transferred onto ø6 mm patches of circular hydrophilic membrane supported by a six well insert at air-liquid interface. 3D neural tissues can be left in these conditions for several days up to several months if needed. The use of membrane patches facilitates the tissue manipulation for further imaging or electrophysiology experiments. It results that the previously described 3D neural tissues culturing technique allows the generation of many mature 3D neural tissues ready to be transferred onto ALI-MEA for electrophysiological experiments. This approach represents an interesting advantage since it is not necessary to culture the tissues directly on the ALI-MEA for a long period of time prior to recordings. Electrical activity can therefore be recorded shortly after placing the tissue in contact with the ALI-MEA.

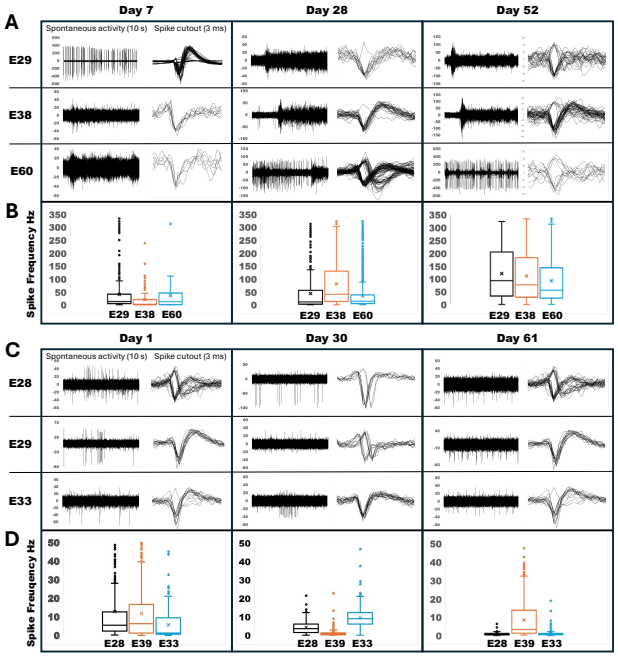

Figure 2: Examples of raw data recorded (A) from a 3D neural tissues assembloid at 7, 28, and 52 days and (C) from an organoid at 1, 30 and 61 days on the ALI-MEA showing 10 sec traces of spontaneous action potential activities from three different electrodes (left) and overlay spike plots of 3 ms duration (right). The amplitude scales are indicated in µV. Boxplots showing the spike frequency recorded (B) from the 3D neural tissues assembloid and (D) from the organoid. All the values used in the boxplots correspond to the spike frequency measured per individual electrodes during a time frame of 10 min. The cross in each boxplot quartile indicates the mean frequency and the bar the median value.

2.4 Experimental Protocols

Mature 3D neural tissues and organoids were positioned onto two types of patches of membrane: 1) with a central hole of diameter 2.2 mm and 2) without a hole. In condition 1), the patch of membrane is then transferred with forceps on an ALI-MEA working area. In condition 2), the patch of membrane is placed upside down to allow a direct contact of neural tissues to the electrodes. Spontaneous activities were then recorded for 10 min after tissue deposition and subsequent days up 52 days for 3D neural tissues and up to 61 days for organoids.

3 Results

3.1 Using 3D Neural Tissues

To cover the entire surface of the electrode area, 3 to 4 3D neural tissues were pre-aggregated onto pre-cut patches of membrane to form an assembloid by fusion (see Fig. 1D). Spontaneous electrophysiological activity can be recorded starting 10 to 60 min after their positioning onto the ALI-MEA. For this first series of experiments, we performed a 10 min recording of electrical activities every day or two days for 52 days. Electrodes exhibiting more than 10 spikes per min were considered active and were included in further analysis. 17, 36 and 49 electrodes were considered active at days 7, 28 and 52 respectively. The noise level at electrodes was ±20 µV as shown in Fig. 2A where examples of traces of raw data from three different electrodes (E29; E38 and E60) are displayed at three time points (days 7, 28 and 52). At the end of each raw data traces, representative examples of superposed action potential cut-outs are shown. Depending on the proximity of electrodes to neurons, amplitudes of spikes can vary from about 40 µV (E38 at day 7) to 400 µV (E29 at day 7 and E60 at day 52). The action potential frequency over 10 min for the three electrodes were analysed and shown in Fig. 2B. An increase of spike frequency over time can be seen for the three

electrodes. The raster plots recorded during the whole experiment indicate a general increase of electrophysiological activities from the overall 3D neural tissues over time (data not shown). Concomitantly, an increase of burst activity per min was also recorded with an average of 24 burst/min at day 7, 141 burst/min at day 28, and up to 487 burst/min at day 52.

3.2 Using Organoids

A 1.3-month-old organoid was placed onto an ALI-MEA to follow-up the electrophysiological activities of the tissue over up to two months (Fig. 2C and 2D). We could record spontaneous activity after 1h. We started a 10-min recording the day after the placement of the organoid within the ALI-MEA and every subsequent day or two days. After one day, 22 electrodes were considered as active, 10 at day 30 and 11 at day 61. Examples of raw data of the spontaneous activity from three electrodes (E28, E29, E33) at days 1, 30, 61 and superposed action potential cut-outs are shown. A large variation of the shapes, amplitudes and frequencies of the action potentials recorded from the three different electrodes can be observed. Amplitudes of action potentials were comprised between 40 µV to 100 µV with frequency comprised between 5 Hz and 12 Hz at day 1, between 1 Hz and 9 Hz at day 30 and between 0.8 Hz and 8 Hz at day 61. We could also record some burst activities although their presence was random from 58 burst recorded at day 1, 35 at day 2, and up to 129 at day 9 and 50 at day 30. Then onward, only few bursts could be recorded until the end of the experiment with only one burst detected at day 61.

4 Conclusion

We have introduced a novel air-liquid interface ALI-MEA to analyse 3D neural network activity. In this configuration, gas can diffuse within the entire thickness of the tissue allowing a good survival in static condition. There is no need for any MEA surface treatment to ensure good coupling of the MEA electrodes with the 3D neural tissues. Since we can detect action potentials within a very short time after tissue placement, we can therefore select tissues which are responding and rapidly discard non-active 3D neural tissues before starting long-term experiments. During the validation process of this new approach, we analysed different parameters to describe the general neuronal activity. Spontaneous spike activity, as well as burst structures of multiple spike trains to confirm that the functionality of neural tissues was like previous work using different MEA [10].

Author Statement

Research funding: We thank HES-SO and Multi Channel Systems MCS GmbH for their funding. Conflict of interest: The authors declare that they have no conflict of interest. The authors thank O. Lavielle and L. Aubert from FCBG for providing organoids, and E. Garretas and L. Nikles from HEPIA for assistance on manufacturing and tissue cultures.

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