Tom Stumpp, Michael Mierzejewski, Domenic Pascual, Angelika Stumpf and Peter D. Jones*

Scalable mesh microelectrode arrays for neural spheroids and organoids

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Abstract:

Introduction: Neural organoids promise to help understand the human brain and develop treatments for neurological diseases. Electrophysiological recordings are essential in neural models to evaluate the activity of neural circuits. Mesh microelectrode arrays (MEAs) have been demonstrated to be suitable for organoids and spheroids, and there is demand for easy-to-use devices that can be manufactured at scale.

Methods: We present a new mesh MEA device with an easy-to-use design. We produce mesh MEA chips on 100 mm carrier wafers and connect individual chips to PCBs by wire-bonding. The devices are completed by assembly of a two-piece well and a glass cover slip.

Results: Each device contains a suspended hammock-like mesh with 64 microelectrodes. The square grid's pitch of 200 µm makes the mesh suitable for typical organoid sizes while spreading the electrodes across a 1.4 mm region. The well is designed for fluid handling by pipetting or pump systems. Impedance measurements indicate a high yield of functional microelectrodes, although further effort is needed to produce consistent low impedances. The devices are compatible with commercial amplifiers, while adaptation of the PCB to other formats will be straightforward.

Conclusions: Using scalable production methods, we have developed a mesh MEA device design that offers improved ease-of-use. Next steps will include biological validation in collaboration with partners.

Keywords: electrophysiology, brain organoid.

Tom Stumpp, Michael Mierzejewski, Domenic Pascual, Angelika Stumpf and Peter D. Jones: NMI Natural and Medical Sciences Institute at the University of Tübingen, Markwiesenstr. 55, 72770 Reutlingen, Germany, peter.jones@nmi.de

1 Introduction

Organoids are new neural models between conventional cell culture and a living brain, which are expected to enable new possibilities to understand and treat neurological diseases [1]. Electrophysiological recordings are critical in neural models to evaluate functionally important changes correlating with the clinical endpoint [2]. Therefore, new technologies have been designed for electrophysiology in neural organoids [3].

A common concept is to produce polymer-based microelectrode arrays with flexible or mesh structures. Mesh microelectrode arrays (MEAs) support microelectrodes on thin polymer filaments with thickness on the order of 20 µm. Cells and organoids grow readily on such meshes and enable recordings from the surface and depth of organoids [4–7]. Good integration between the organoids and devices enables long-term electrophysiology over months.

For adoption by the broader community of brain organoid researchers, both usability and cost are important issues. These issues are closely connected to the manufacturability and reliability of the devices. As a result, there is a need for devices which can be reliably manufactured at an increased scale in a design which provides ease-of-use and stable performance. While the microfabrication of polymer-based microelectrode arrays is mature, packaging and assembly are two areas where progress can be made.

We present a new design developed for ease-of-use and improved packaging and assembly methods to improve manufacturability. We focus here on technical aspects and will later present biological results of spheroid/organoid culture and electrophysiology.

2 Methods

The meshes were produced using cleanroom microfabrication as previously reported [7]. In contrast to our previous work, we produced mesh chips on 100 mm silicon wafers. Briefly, a 6 µm polyimide layer (DuPont PI-2611) was spin-coated,

treated by oxygen plasma, and coated with adhesion promoter (VM-652, HDMicrosystems). A 400 nm layer of Ti/Au/Ti was sputter deposited, patterned, and etched to form traces (5 µm critical dimension). A second 6 µm polyimide layer was applied, followed by a silicon nitride hard mask by plasma-enhanced chemical vapor deposition, patterning (AZ ECI 3027), and reactive-ion etching. Polyimide was etched and the hard mask was removed. Microelectrodes were patterned with TiN using a lift-off process (AZ 10XT).

Meshes were wire-bonded to printed circuit boards (PCBs) designed for compatibility with commercial amplifiers (e.g. 60- or 256-channel amplifiers from Multi Channel Systems MCS GmbH). Using a two-component epoxy (EPOTEK 301-2FL), a two-piece polycarbonate well was glued above and below the mesh, and a glass cover slip (pretreated with aminopropyltriethoxysilane) was glued to close the bottom.

3 Results & Discussion

Our devices each contain a single well with a suspended hammock-like mesh to support one or more spheroids or organoids (Figure 1). Specifications of the devices are given in Table 1. The devices are designed to support spheroids or organoids and allow their growth in all directions, as the mesh is suspended 2.5 mm above the glass floor. Filaments with a width of 20 µm are arranged in a square 200 µm grid. Sixty-four TiN microelectrodes (30 µm diameter) are at the nodes of the mesh in an 8×8 layout spanning a 1.4 mm square region (Figure 2a,b). Therefore, the hammock-like mesh is most suitable for common organoid sizes, i.e. organoids larger than the 180 µm square hole size, and not too much larger than the 2 mm diagonal of the electrode field. In contrast to our previous work with a spider-web-like mesh with circularly arranged microelectrodes [7], the square grid simplifies data analysis and visualization. The electrode pitch of 200 µm ensures that activity from a large cross-section of an organoids will be recorded. Future versions could increase the electrode density to support spike sorting or current source density analysis.

One design objective was to maximise the well volume to minimise media changes. To accommodate typical microscope working distances, we limited the height below the mesh to 2.5 mm. This gives a volume of 116 µl below the mesh (for example, when culturing at the air–liquid interface). The height of the well above the mesh was 4 mm.

Our previous work avoided electrical packaging (which can be a source of failures) by using a large 49×49 mm² polyimide component which integrated microelectrodes, traces and contact pads but allowed only one “chip” per carrier substrate. Here, we produced mesh chips of 9×14 mm² which allows up to 38 chips per 100 mm carrier wafer. We then used rivet bonding [8], which we have recently used for flexible implants [9], to connect the polyimide components to PCBs. Rivet bond connections have a resistance below 5 Ω [10].

The devices shown in Figures 1 and 2 have a PCB designed for compatibility with 60-channel amplifiers from MCS, and therefore connect only 59 of the 64 microelectrodes and one reference electrode. Alternative PCB designs would allow connection to all 64 microelectrodes. The PCB-based format will also allow enable miniaturized devices, multiwell devices or extra functionality such as sensors, pumps or lights for optical stimulation.

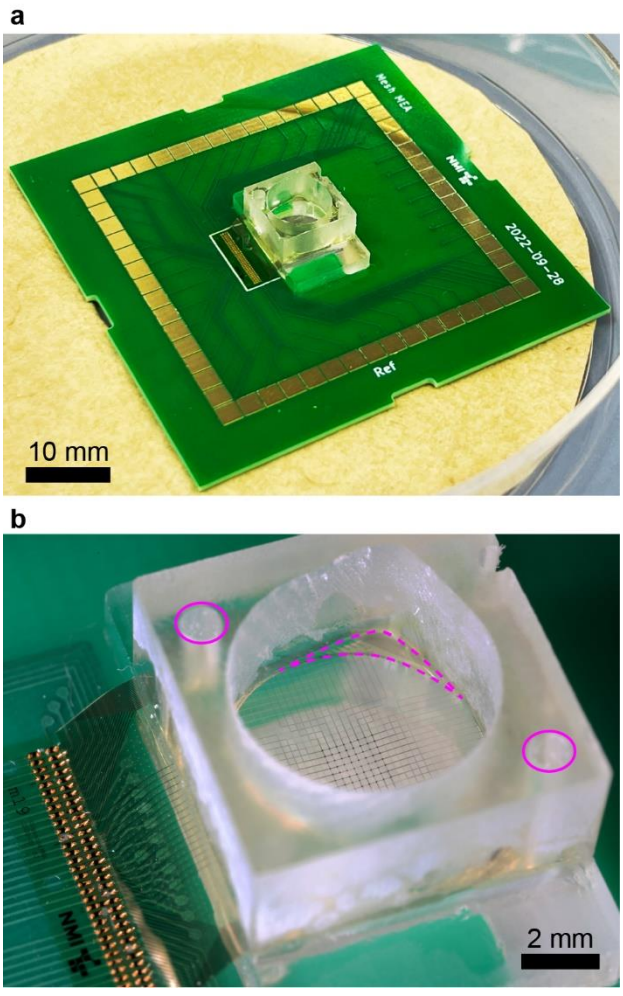

Figure 1: Photographs of the mesh MEA in a design compatible with 60-channel amplifiers. **a:** The PCB has 60 gold pads which are contacted by gold pins of the amplifier headstage. **b:** Magnification of the well, with 1 mm access ports (magenta circles), pipetting ledge (dashed magenta outline) and wire bonds (left).

Table 1: Specifications of the mesh MEA devices.

Component	Parameter	Value
Electrodes	Diameter	30 µm
	Layout	8×8, 200 µm pitch
	Quantity	59 or 64
Mesh	Grid size	200 µm
	Thickness	12 µm
	Filament width	21 µm
Well	Diameter	7 mm
	Height below mesh	2.5 mm
	Height above mesh	4 mm
	Volume below mesh	116 µl
	Max. volume above mesh	160 µl
	Diameter of access ports	1 mm

The well is designed for flexible fluidic handling by pipetting or using pump systems (Figure 2). Two access ports can be used to fill the volume below the mesh (Figure 3). Their 1 mm diameter is suitable for pipette tips or 1 mm stainless steel or polymer tubes for a pump system. Additionally, a ledge is formed by a cut-out in the side of the well above the mesh, giving a physical support to pipette at the level of the mesh without damaging the delicate filaments (Figure 2a,c).

For perfusion, we anticipate using both access ports as inlets (e.g. for normal medium and a drug solution) while a suction cannula placed over the ledge defines the level of the air-liquid interface (at the mesh or a given height above it. Such a system should enable perfusion and compound testing without introducing bubbles, while also regulating the volume in the well.

Impedance measurements (Figure 4) show that the microelectrodes are suitable for low noise recordings [11]. We suspect that the gluing process may cause the variation of impedance between devices. A small number of microelectrodes on some MEAs had high impedances, possibly due to open circuits in the gold traces or issues related to wire bonding. We are working to resolve these issues to improve the yield of fully functional devices and achieve consistent impedances.

We will report results of the culture and electrical recordings of neural spheroids on mesh MEAs. Future work may include electrical stimulation and recording of evoked activity.

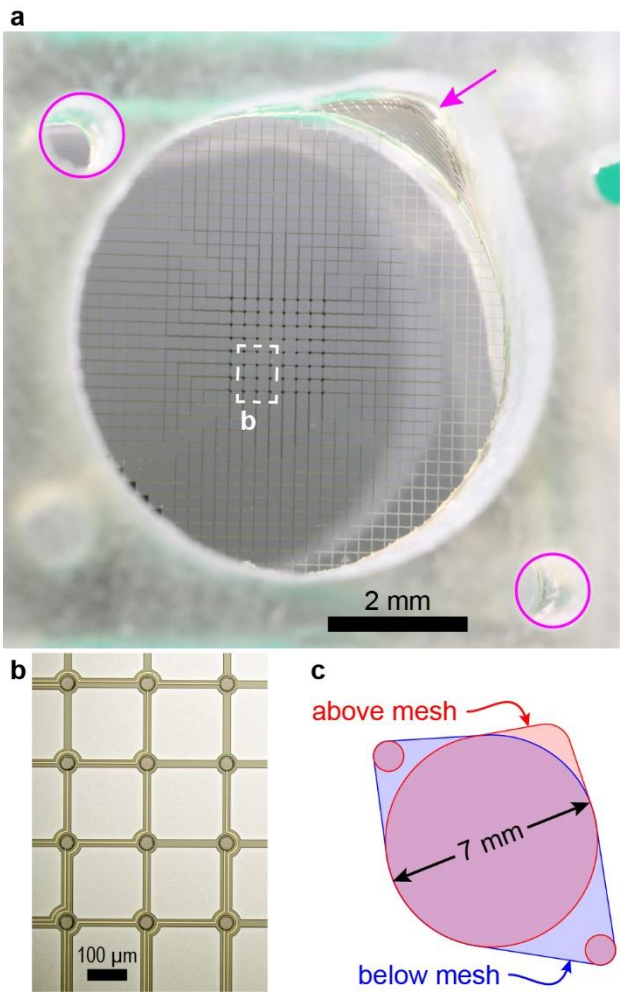

Figure 2: Detail of the well and mesh. **a:** Macro photograph of the well, showing 1 mm access ports (magenta circles) and a pipetting ledge (magenta arrow). **b:** Micrograph of a section of the microelectrode field, showing the polyimide grid containing gold traces and microelectrodes at nodes. The image was taken before removal from the carrier wafer and corresponds to the region highlighted in (a). **c:** Planar view of the well regions above (red) and below (blue) the mesh.

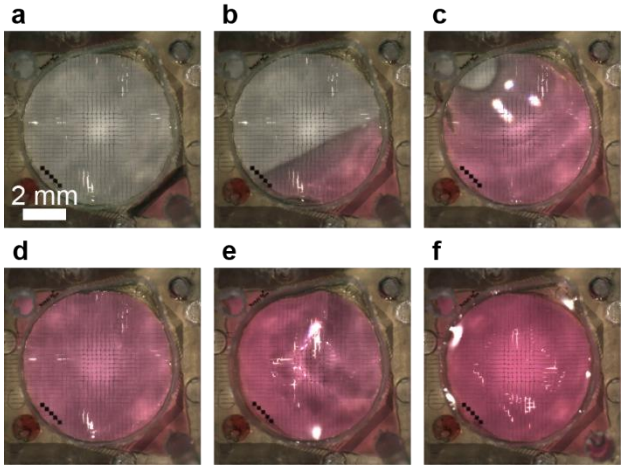

Figure 3: Filling of the well with culture medium. **a:** Medium is introduced from a pipette tip placed in the lower right access port. **b, c:** Medium flows under the mesh towards the top left access port. **d:** The volume below the mesh is filled. **e:** Medium begins to flow upwards through the mesh. **f:** The well above the mesh is uniformly filled.

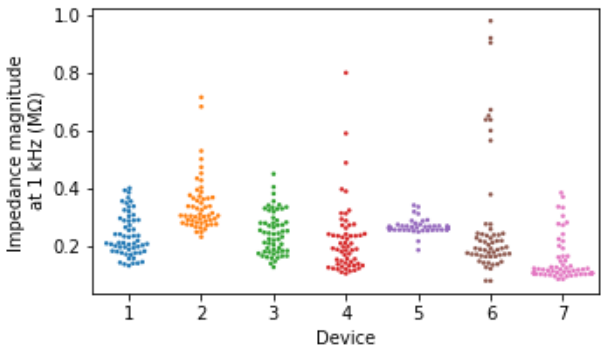

Figure 4: Impedance measurements of seven mesh MEA devices. Each dot indicates one of the 59 microelectrodes per device. Reference electrodes and defective electrodes (>1 MΩ, *n*=12) are omitted.

4 Conclusion

We have developed a new mesh MEA device for electrophysiology of neural spheroids and organoids, with a well design that simplifies both assembly and fluidic handling. The wafer-scale fabrication of polyimide mesh chips and wire bonding to PCBs reduces production effort. The improved design and efficient manufacturing approach should facilitate wider distribution to the brain organoid research community. Next steps will include biological validation in collaboration with partners.

Author Statement

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