FABRICATION OF SOFT AND TRANSPARENT 3D MICROELECTRODE ARRAYS FOR *IN VITRO* ELECTROPHYSIOLOGICAL RECORDING

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ABSTRACT

The mechanisms governing the onset and eventual progression of several neurodegenerative disorders remain poorly understood or even undiscovered. This lack of pathophysiological insight can be partly attributed to reliance on inaccurate *in vitro* models. Notwithstanding research efforts towards recapitulating brain functions on flat devices, mimicking the brain's three-dimensional (3D) architecture *in vitro* remains a prime target, as 3D models more closely resemble the functional behavior and dynamic responses of *in vivo* organs. In this work, we present a novel, wafer-scale approach for microfabrication of soft and transparent 3D microelectrode arrays (MEAs) for *in vitro* electrical recording and optical inspection of electrogenic cell cultures. The proposed 3D MEAs entail 90 µm-high polydimethylsiloxane-based micro-pyramids featuring multiple, electrically-distinct and vertically-stacked titanium nitride electrodes on their slanted facets. Our innovative 3D MEAs will facilitate the development of physiologically-accurate brain-on-a-chip models capable of monitoring 3D electrical communication in neuronal networks while allowing their simultaneous optical characterization.

KEYWORDS

Brain-on-chip, electrophysiology, 3D microelectrode arrays, MEA, microfabrication, organ-on-chip

INTRODUCTION

The mechanisms driving the onset and progression of neurodegenerative disorders remain poorly understood, partly due to the limitations of conventional *in vitro* models and unreliable animal studies. Efforts to replicate brain function on planar (2D) devices [1], [2] have yet to overcome these challenges. Hence recapitulating the brain's inherently three-dimensional (3D) architecture *in vitro* remains essential, as 3D models better emulate the functional behavior and dynamic responses of *in vivo* organs [3].

The key to unravelling neurodegenerative diseases lies in understanding neuronal communication mechanisms and their alterations in response to diseases. Among traditional electrophysiological methods of analysis, such as patch clamping [4] and calcium and voltage imaging [5], microelectrode arrays (MEAs) stand out for recording neuronal activity with sub-millisecond temporal and micrometric spatial resolution. However, 2D MEAs cannot capture the full extent of 3D neuronal interactions. The latter requires brain-on-chip (BoC) devices with microelectrode arrays distributed in 3D space. This gap recently motivated significant research efforts towards developing 3D MEAs, wherein independent electrodes are arrayed across all spatial directions [3], [6], [7]. Along with geometry, critical requirements for effective 3D MEAs are notably optical transparency of the substrate, to ensure compatibility with analytic microscopy, and matching between tissue and substrate stiffnesses to mimic *in vivo* physiological conditions. Going beyond the state of the art, here we propose a combination of Si micromachining and polymer molding to fabricate transparent polymeric 3D MEAs compatible with electrophysiological readout systems (Fig. 1).

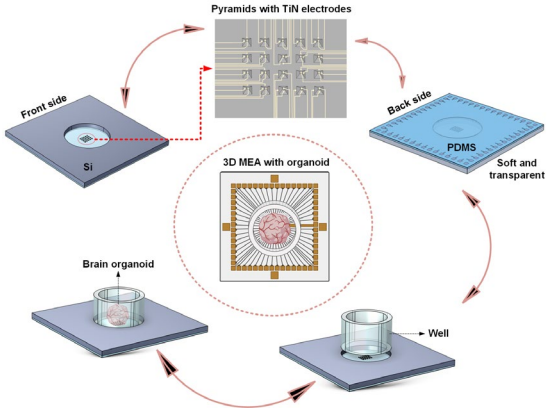

Figure 1: Schematic representation of the proposed soft and transparent 3D MEAs for brain-on-chip application.

The 3D MEAs entail an array of 5×4, 90 µm-high truncated pyramids with 20 µm-wide top plateaus. Each pyramid features three distinct titanium nitride (TiN) microelectrodes – respectively on the top, sidewall, and base plane – for a total of 60 distinct electrodes per chip. The biocompatibility of the 3D MEAs was demonstrated by culturing SH-SY5Y neuroblastoma cells onto the fabricated devices for 72 hours before being fixed. The 3D MEAs were further evaluated electrically and optically, respectively through impedance spectroscopy and optical transmittance. This work paves the way for a new class of wafer-scale microfabricated soft and transparent 3D MEAs tailored for measuring 3D electrogenic cell cultures.

DESIGN AND FABRICATION

The proposed 3D MEA devices entail a 5×4 array of truncated, polydimethylsiloxane (PDMS)-based pyramids, each measuring 90 µm in height with a 20 µm-wide top plateau. Every pyramid incorporates three distinct TiN microelectrodes—positioned on the top, sidewall, and base

plane, respectively—resulting in a total of 60 unique electrodes per die. A 4-inch, 525 µm-thick, double-side polished P-type <100> Si wafer was used as the substrate for batch wafer-level fabrication of the 3D MEA devices.

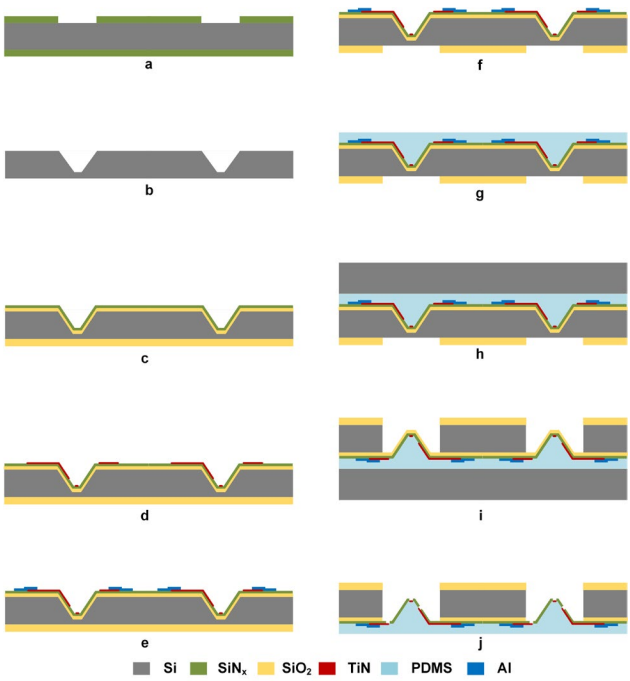

Figure 2: Fabrication process flow. (a) Patterning of a double-side polished Si wafer with SiN hard mask. (b) Timed anisotropic etching of Si in TMAH and subsequent stripping of the SiN hard mask. (c) Deposition of PECVD SiO_2 passivation layer on both sides of substrate followed by the deposition of PECVD SiN on the top (cavity) side. (d) Sputter deposition and subsequent patterning of TiN to define electrodes and interconnects. (e) Sputter deposition and subsequent patterning of AlSi to define the bond pads for the electrodes. (f) Patterning and wet etch of the back SiO_2 hard mask for the Bosch process. (g) PDMS spin-coating on the patterned cavities. (h) Wafer mounting on a carrier wafer. (i) Bosch process for Si anisotropic back etch and release of the pyramids. (j) Plasma etching to remove the SiO_2 layer on the pyramids, and subsequent patterning and plasma etching of the SiN layer to create openings at the tip of electrodes for direct interface with cells.

The fabrication process flow is represented in Fig. 2. A 400 nm-thick SiN masking layer was deposited by low-pressure chemical vapor deposition (LPCVD) on both sides of the Si substrate, later photolithographically patterned and plasma-etched on the top (cavity) side to create openings for timed anisotropic Si bulk etching (Fig. 2a). The masked Si wafers were etched in 25% TMAH at 80 ºC to achieve 90 µm-deep inverted pyramidal cavities. The residual SiN was stripped using hot phosphoric acid at 145 ºC (Fig. 2b). The substrate was then passivated with a 4 µm-thick layer of SiO_2 deposited by plasma-enhanced CVD (PECVD); subsequently a 400 nm-thick PECVD SiN layer was additionally deposited on the inverted pyramids side (Fig. 2c). On the wafer backside, PECVD SiO_2 was additionally deposited for a total thickness of 7 µm. To define the microelectrodes and their interconnects, a 20/300 nm-thick Ti/TiN layer was sputtered, patterned and plasma-etched (Fig. 2d), and a 1 µm-thick Al/Si layer was sputtered, patterned and dry-etched to define the bond pads (Fig. 2e). The oxide layer on the wafer backside was patterned and subsequently etched in buffered hydrofluoric acid (BHF) to define the hard mask for the ensuing backside Si etch (Fig. 2f). A 10 µm-thick PDMS layer (10:1 monomer-to-crosslinker ratio, Sylgard) was spin-coated on the cavity side of the wafer, degassed, and oven-cured at 90 ºC for 1 hour (Fig. 2g). The wafer was mounted on an Al-coated carrier wafer (Fig. 2h), and Bosch-type deep reactive ion etching (DRIE) was carried out on the masked wafer backside for 45 min to land on the oxide layer and release the PDMS membranes (Fig. 2i). The oxide coating was stripped with plasma etching, and patterning and plasma-etching of the SiN-protected PDMS pyramids was conducted to selectively expose only the tips of the TiN electrodes (Fig. 2j). Finally, the fully-processed wafer (Fig. 3e) was diced into 12, 20×20 mm^2 chips (Fig. 3f), and each chip was glued and wire-bonded to a custom-designed, 2-layer printed circuit board (PCB) compatible with a commercial readout system (not shown).

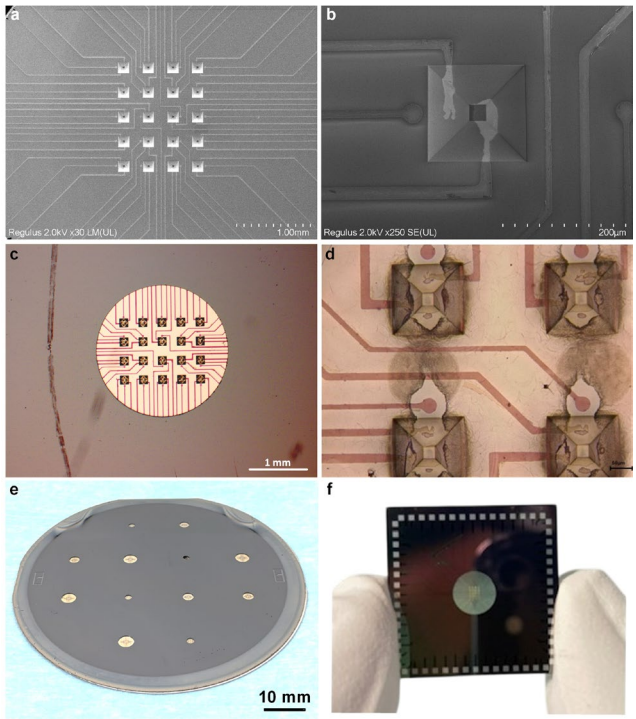

Figure 3: (a) Scanning electron micrograph of cavities in Si with patterned electrodes, and (b) of an individual inverted pyramid with electrodes patterning on the inner sidewalls. (c) Optical micrograph of a released PDMS pyramid array with TiN microelectrodes. (d) Optical micrograph highlighting the distinct TiN electrodes on the PDMS pyramids with the selective opening of the SiN layer over the tips of the electrodes. (e) Photograph of the top (pyramid) side a fully processed wafer with the released PDMS-based membranes supporting the 3D MEAs over optical windows of different diameters. (f) Optical photograph of a single, 2x2 cm^2 die, with the soft and transparent 3D MEAs at the center of the optical window of the Si die.

The integrity and continuity of the electrodes were assessed visually by means of both optical and scanning electron microscopy (SEM). Fig. 3a shows an SEM micrograph of TiN electrodes patterned on the inverted pyramidal cavities on the Si wafer. Three distinct electrodes patterned at distinct depths of a single Si cavity are evidenced in Fig. 3b. Fig. 3c shows a full array of released PDMS-based pyramids with patterned TiN electrodes. Fig. 3d highlights the continuity of the distinct TiN electrodes on the pyramids, along with the spatially-selective openings in the protecting SiN layer for interfacing only the electrode tips with the cells. Further confirmation of the electrical continuity of the microelectrodes was gathered by the electrical impedance measurements described in the next section.

EXPERIMENTAL RESULTS

Custom-designed, double-sided, 2-layer PCBs were used to support and interconnect electrically each Si chip containing the 3D MEAs. This packaging solution makes the devices readily suitable for subsequent data acquisition experiments, to be conducted using a commercial electrophysiological recording setup (MEA2100 from Multichannel Systems, not shown). Fig. 4a shows a single packaged 3D MEA chip bonded to the custom PCB and completed by a customized PDMS well for cell culture.

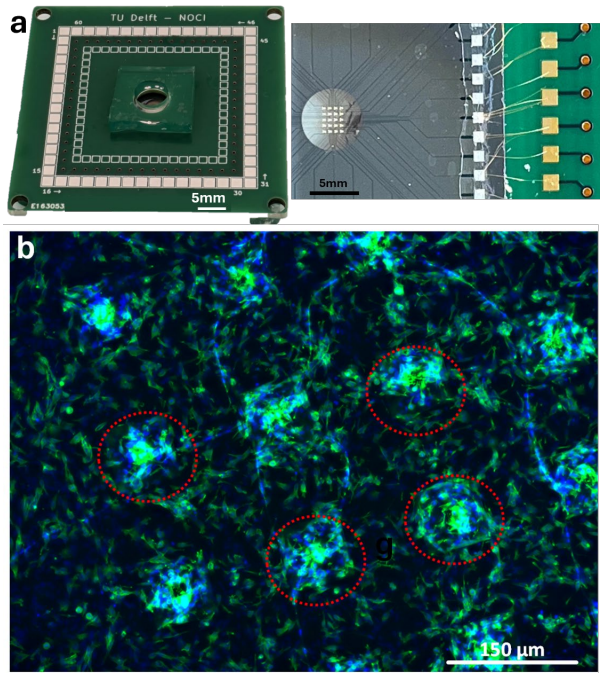

Figure 4: (a) Packaging of the 3D MEA chip with custom-designed, 2-layer PCB compatible with commercial electrophysiological recording setup. Left: frontside, showing the PDMS-based central well mounted over the chip for cell culture. Right: backside, showing the wire bonds connecting the chip's bond pads to the PCB. (b) Fluorescent image of SH-S5Y5 neuroblastoma cells grown on-chip and stained for actin (green) and DAPI (nucleus, blue).

To assess the biocompatibility of the PDMS pyramids, neuroblastomas were cultured on the device. The pyramids were oxygen plasma-activated and subsequently seeded with SH-S5Y5 neuroblastoma cells. The cells were fixed after 5 days in vitro and stained with actin (cell body, green) and DAPI (nucleus, blue). The fluorescent micrograph in Fig. 4b shows the neuronal cells growing on the top plateaus (red circles) and sidewalls of the pyramids.

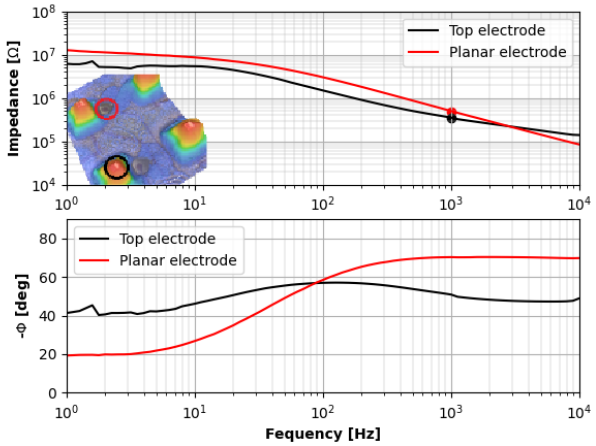

Figure 5: Electrode characterization in 0.1 M phosphate-buffered saline by 2-probe electrical impedance spectroscopy. The inset shows a 3D laser scan of the pyramids evidencing the electrodes used for the measurements, respectively on the top plateau (top) and base plane (planar).

To assess the electrochemical performance of the electrodes, the MEAs were characterized in 0.1M phosphate-buffered saline (PBS) using three-probe electrical impedance spectroscopy (EIS). The impedance magnitude (Z) and phase (ϕ) were recorded over a frequency range of 1 Hz to 10 kHz. As can be observed in the spectrum shown in Fig. 5, the impedance decreases with increasing frequency, with both planar and top electrodes having an impedance of around 400 kΩ at 1 kHz. The decrease in impedance can be attributed to the capacitive electrode-electrolyte interface (electric double-layer capacitance). The corresponding change in phase value from -20 to -70 deg also supports the capacitive behaviour at the interface of the 3D MEAs, crucial for bio-interfacing with cell cultures. We attribute the high impedance values we measured to imperfect patterning of the TiN electrodes and incomplete etch of the protective SiN layer over the tips of the electrodes, which are conducted over the considerable substrate topography respectively of the cavities and pyramids. We also note that, in separate 2-point electrical probe measurements, we observed values of resistance as low as 10 kΩ, which are in line with typical values reported for TiN microelectrodes in (2D) MEAs [6].

Finally, to confirm the transparency of the 3D MEAs, the fabricated devices were characterized for optical transmittance. As observed from the plot in Fig. 6 showing optical transmittance versus wavelength, the PDMS substrate exhibits negligible transmission in the ultraviolet region. Within the visible spectrum, it significantly attenuates blue light, with transmission rising sharply around 500 nm. Beyond 550 nm, the membrane maintains a transmittance of at least 60% across the visible spectrum. The highest transmittance (73%) is observed in the green region, agreeing with the sample's green appearance to the naked eye. In the infrared region (beyond 780 nm), the membrane transmits over 70% of the incident light. Furthermore, the transmis-

sion spectra of PDMS exhibit interference fringes, indicating that the MEA is a poor scatterer of light and does not introduce noticeable haze to the sample. The test confirmed that the PDMS substrate supporting the 3D MEAs is highly transparent across a significant portion of the visible spectrum, which is critical for optical microscopy applications.

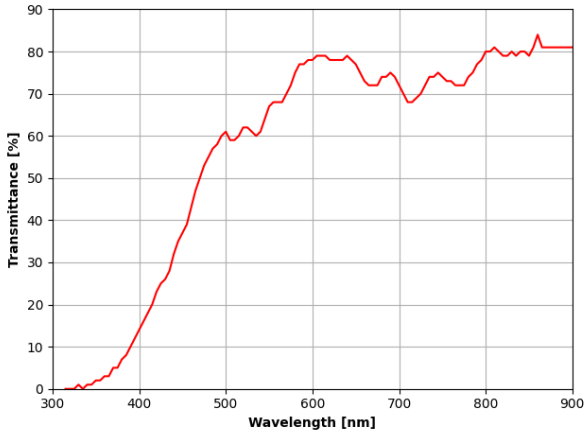

Figure 6: Measured optical transmittance of the PDMS-based 3D MEAs in the visible band of wavelengths.

CONCLUSION

In this work, going beyond the state of the art, we have demonstrated a new class of soft and transparent 3D microelectrode arrays tailored for applications involving 3D electrogenic cell cultures. The proposed wafer-scale microfabrication method combines established batch MEMS processing with PDMS-based soft-lithography to achieve a segmented array of TiN microelectrodes across three dimensions. A cytotoxicity assay performed on the 3D MEAs demonstrated biocompatibility with electrogenic cell cultures. The 3D MEAs were wire bonded to a custom-designed PCB for seamless electrical interfacing with commercial data acquisition setups. The electrodes were also characterized through impedance spectroscopy, demonstrating a partially-capacitive response of the 3D MEAs in simulated physiological conditions and inherently the electrical continuity of the microelectrodes. Finally, the PDMS substrates were characterized for optical transmittance, demonstrating more than 60% transmittance in the visible spectrum (400 to 700 nm wavelength). The 3D MEAs proposed here will inspire a future generation of soft and transparent microelectrode arrays compatible with organoids and true 3D cell cultures, better mimicking physiological conditions and reducing the need for animal testing.

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