

Supplementary material

Low impedance and highly transparent microelectrode arrays (MEAs) for *in vitro* neuron electrical activity probing.

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MEA fabrication process

Gold pads and track fabrication

In the initial fabrication step, the titanium/gold contact pads and tracks are patterned by electron beam (Ebeam) lithography step (Raith EBPG 5000Plus, exposure dose 400 $\mu\text{C}/\text{cm}^2$), followed by thermal evaporation of 10 nm of titanium (used as adhesive layer) and 100 nm of gold (Fig. 1a). Then, a lift-off process was performed to remove the unwanted Ti/Au parts and leave the pads and tracks on top of the glass substrates.

PEDOT:PSS electrode fabrication

A diluted commercial aqueous cleaning solution (2% Micro90 v/v in deionized water) is spin-coated (at 2000 rpm for 10 sec) onto the surface of the wafers. This layer acts as an anti-adhesive coating helping one to peel-off the sacrificial parylene layer. Then, a 2 μm parylene C layer is deposited *via* chemical vapor deposition process (in a C20S deposition chamber from Comelec) (Fig.1b). This first parylene layer will be used as a sacrificial layer. Openings in the parylene layer, corresponding to where the microelectrodes are positioned on the wafer, are made by Ebeam lithography step (exposure dose 70 $\mu\text{C}/\text{cm}^2$) followed by an oxygen plasma etching (O_2 150W 100mT 30sccm for 10 min) (Plasmalab 80Plus, Oxford Instruments). Next, according to the work of Mantione *et al.*, a PEDOT:PSS solution (20 mL) (CleviosTM PH1000, Heraeus) was mixed with ethylene glycol (5 mL), dodecyl benzene sulfonic acid (DBSA) (50 μL) and 1wt.% of (3-Glycidioxypropyl)trimethoxysilane (GOPS) [1] and spin-coated after activation of the

surface using UV ozone treatment for 15 min (UVO-Cleaner® Model 42-220, *Jelight* Company, Inc. 2 Mason. Irvine, CA 92618 U.S.A.) and subsequently baked at 140°C for 1 h (Fig. 1c). In order to obtain different thicknesses, the spin-coating of PEDOT:PSS was performed in a single layer at the different rotation rates, namely at 1000 rpm for 45 sec and of 800 rpm for 30 sec. It has to be noticed that the same procedure was achieved for the deposition of PEDOT:PSS layer on plain glass slides for transmission measurements. Following this, the parylene C layer is peeled-off (Fig. 1d).

In the final fabrication steps, devices are again coated with a second parylene C layer (2 μm thick), which will act as the device insulator layer. Finally, openings corresponding to the microelectrodes and the contact pads are opened by a 3rd Ebeam lithography (exposure dose 70 $\mu\text{C}/\text{cm}^2$) step followed by an oxygen plasma etching (Fig. 1e).

The cell culture chamber (made using PDMS moulding) was glued on top of the parylene C of the MEA devices using two components of the adhesive (SYLGARD 184) mixed in ratio 10:1 and hardened in the oven for 1h at 60°C. Then, the adhesive was set around the chamber in order to avoid any leakage of the fluid and was hardened again in the oven for 1h at 60°C. The impedance of the electrodes was measured after the gluing process of the chamber. No evolution of the impedance values was observed, thus proving that the gluing step has no detrimental effect on device performances (Fig. S3).

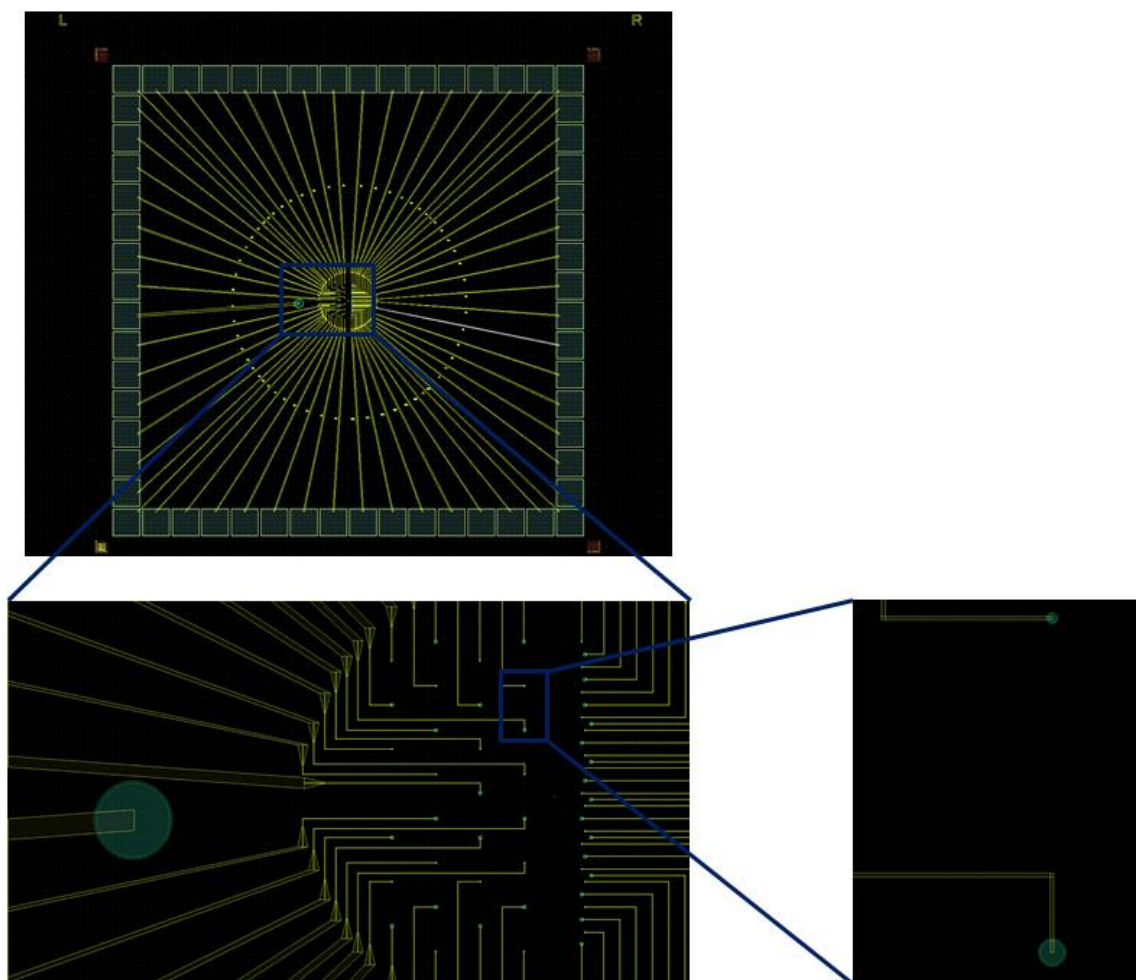


Fig. S1 Layout of the MEA device with PEDOT:PSS electrodes with size of 10 and 30 μm in diameter on the same device.

Morphological characterization

After patterning of PEDOT:PSS electrodes their surface morphology (uniformity) was characterized by atomic force microscopy (AFM). The AFM measurements were carried out in tapping mode with a scanned area of $70 \times 70 \mu\text{m}^2$. The thickness of the PEDOT:PSS electrodes was evaluated by profilometer (Dektak XT, Bruker) which is resulted to be about 100 nm for the rotation rate of 1000 rpm for 45 sec, while the thickness of the PEDOT:PSS electrodes is 200 nm for the rotation rate of 800 rpm for 30 sec. The values of root mean square of surface roughness and the average surface roughness for electrodes with diameter of 10 or 30 μm and PEDOT:PSS thicknesses of 100 or 200 nm are presented in Table S1.

Thickness of PEDOT:PSS [nm]	Electrode size [μm]	Rq [nm]	Ra [nm]
100	10	14	9

100	30	14	11
200	10	8.3	6.7
200	30	10	7.1

Table S1 The values of root mean square of the surface roughness (Rq) and the average surface roughness (Ra) obtained from AFM measurements for a selected area of $5.5 \times 5.5 \mu\text{m}^2$ for electrodes with a diameter of 10 and 30 μm and with PEDOT:PSS thickness of 100 and 200 nm, respectively.

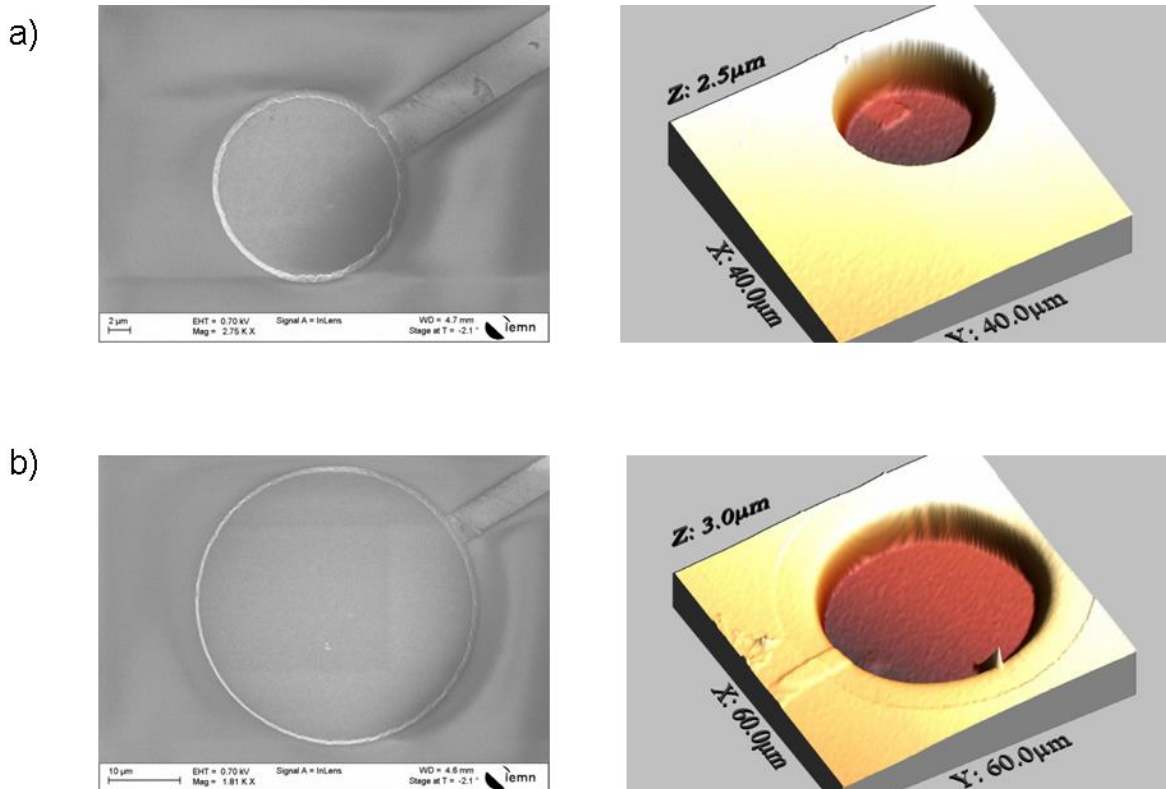


Fig. S2 Scanning electron microscope images and atomic force microscopy images of a) a fabricated electrode from PEDOT:PSS with a diameter of 10 μm and b) of 30 μm , respectively.

Cell culture

Primary culture

Rat primary cortical cells were prepared from 18-days-old Wistar rat embryos as previously ethical committee (agreement APAFIS#2264-2015101320441671 from CEEA75, Lille, France) and follows European guidelines for the use of animals. Gestating females (Janvier Labs) were housed in a temperature-controlled (20 - 22° C) room maintained on a 12 h described [2]. The present experimental research has been performed with the approval of an day/night cycle with food and water provided *ad libitum*. Culture medium was made of Neurobasal (Gibco) supplemented with B-27 (Gibco), Antibiotic-Antimycotic (Gibco) and L-

glutamine (Gibco). Cultures were kept in a cell incubator (ThermoScientific) at 37°C in a 5% CO₂ atmosphere. Culture medium was never changed but small amounts of fresh medium were added once a week to compensate medium evaporation (never more than 10% of the initial volume).

Cell culture on surfaces for biocompatibility evaluation

The samples to test were inserted into 12-well cell culture plates (Corning, USA). Coating was performed with a mix of Poly-D-lysine (0.5 mg ml⁻¹, Sigma) and laminin (10 µg ml⁻¹, Sigma). The coating solution was applied for 2 hours at room temperature then washed using sterile water (Corning) and air-dried. In each well, 200000 viable cells were seeded in 1.5 ml culture medium (57000 cells/cm²). For the biocompatibility evaluation, two different cell culture dissociations were performed from two different litters.

Cell culture on MEAs

In order to create a well for cell culture, a PDMS ring was sealed around the recording area on a MEA device with electrodes from PEDOT:PSS (Fig. 1g). Before utilization, MEAs were cleaned in ethanol then air-dried in a class II biosafety cabinet sterile for 72 hours for commercial MEAs (60MEA200/30iR-Ti with glass rings, Multi Channel Systems) for 3 hours for the MEA device with electrodes from PEDOT:PSS. The MEA recording area was coated with a mix of Poly-D-lysine (0.5 mg/ml, Sigma) and laminin (10 µg/ml, Sigma). The coating solution was applied for 30 minutes at room temperature then washed using sterile water (Corning) and air-dried.

For each MEA, one drop of a cellular suspension at 1000 cells/µl in cell culture medium was seeded on the MEA recording area (20000 cells). Ten minutes after plating, 1 ml of fresh medium was added and the MEA culture chamber was sealed with a removable hydrophobic semipermeable membrane cover (fluorinated ethylene-propylene, 12.5 microns thick, Multi Channel Systems). After cell culture, MEA were washed in water then in Tergazyme 1% (Alconox) for two hours at room temperature then keep in water at 4°C until the next utilization.

Cell viability/metabolic activity assay and cytotoxicity/cell death assay

To assay metabolic activity/number of viable cells, cellular reduction of MTS tetrazolium into formazan was quantified using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega) following the manufacturer's instructions. Cells grown into plate wells (i.e. on tissue culture treated-polystyrene) were used as a reference meaning that their metabolic activity was set as the reference value (100%). The metabolic activity of the cells grown on the other surfaces was normalized against it. To assay cell death, the release of lactate dehydrogenase (LDH) into

the cell medium was quantified using the CytoTox 96 Non-radioactive Cytotoxicity Assay (Promega) following the manufacturer's instructions. Maximum LDH release was determined by adding lysis solution (9% Triton X-100) to cells grown on tissue culture treated-polystyrene. Significant differences between results obtained for cells grown on glass and results obtained for other substrates were examined using the nonparametric Mann-Whitney and Wilcoxon tests, and p -values <0.05 were considered significant.

Confocal microscopy

0,1 μM of NeuroFluorTM NeuO (Stemcell technologies) was added in the cell cultures after 1 month *in vitro*. Then, neurons were live-imaging using an inverted confocal microscope (LSM 710, AxioObserver Zeiss) with a 20x lens and a 10x lens for wider fields at 488 nm and laser power of 25 mW (5% power). Images were processed with ZEN software (Zeiss). 10 μm orthogonal projections were displayed.

MEA recordings

The experimental setup was based on the MEA2100-system (Multi Channel Systems) and consisted in a 2 x 60 channels headstage (Multi Channel Systems) that amplifies and digitizes the signals, an interface board (MCS-IFB, Multi Channel Systems) and a personal computer. A temperature controller (TC02, Multi Channel Systems) was used to keep the temperature of the cultures at 37°C during the experiment. Real-time monitoring, signal filtering and spike detection and recording were achieved through the Multi Channel Experimenter software (Multi Channel Systems). The spontaneous activity was recorded after 14, 21 and 28 days *in vitro* (DIV) for 5 minutes after a period of rest into the experimental setup of 15 minutes. Amplitude thresholding is used for spike detection. A threshold was set for each recording electrode as 10 times the standard deviation of the baseline noise level.

Data analysis and statistics

Spike time stamps and spike cutouts recorded through the Multi Channel Experimenter software were exported to the NEX format using the Multi Channel DataManager software (Multi Channel Systems). All the analyses were done through the Neuroexplorer software (Nex Technologies). An active electrode is defined as an electrode that has recorded at least 5 spikes per minute. Spike and burst frequencies, burst duration, number of spikes per burst and peak spike intensity were extracted. Correlation quantification was performed through the cross-correlograms analysis. All the active electrodes were selected both for the analysis and as

reference electrodes and compared with each other. The analysis was performed on total recording time (5 minutes). Basically, from timestamps examination, the cross-correlograms analysis shows the conditional probability of a spike to occur in a given electrode at time t_0+t on the condition that there is a reference event (spike in the reference electrode) at time t_0 . To this end, the time axis is divided into bins we arbitrary set at 0.005 second around each reference event $[-0.2, +0.2]$. For each reference event in the referent electrode, the distance from it to all the spikes of the spike train in the analyzed electrode is calculated. When this distance falls into a bin, it allows incrementing the counter of the corresponding bin. Then a normalization is performed according to the formula: $bin_value = (bin_count - Confidence_mean) / \sqrt{Confidence_mean}$, where $Confidence_mean$ is the expected mean bin count. Then we extracted all the maximum bin_value or Z-score values obtained for all the active electrodes of a given cortical cell culture at a given time point of the culture and averaged them to obtain a global score of correlation reported in the results section. Graphical representations of the extracellular neuronal activity (timestamps) were also obtained via the Neuroexplorer software.

Influence on impedance after gluing of a cell culture chamber on top of a MEA device

The impedance of the PEDOT:PSS electrodes was measured before and after the gluing process of the cell culture chamber. No changes in the impedance were observed.

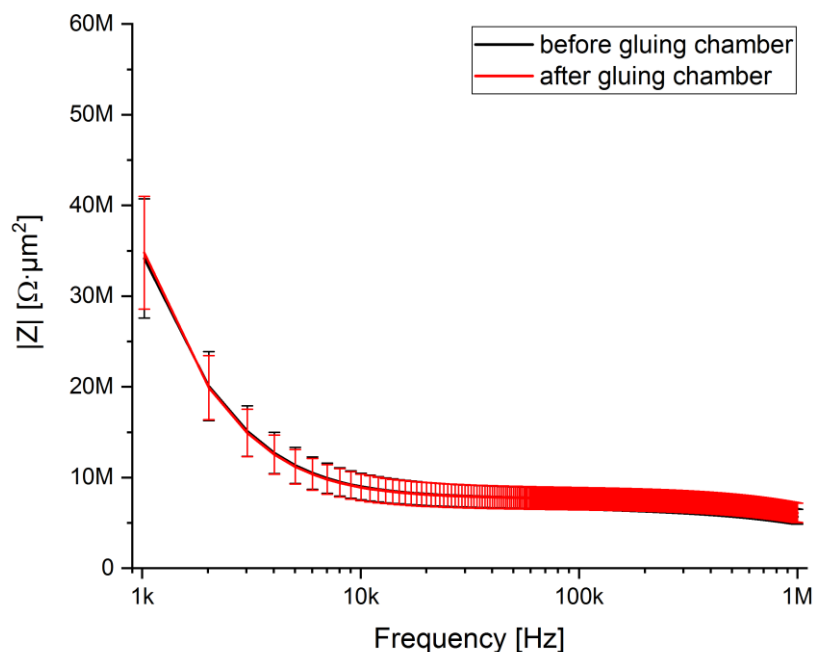


Fig. S3 Influence on impedance of gluing chamber on top of a MEA device. The averaged impedance spectra measured for all PEDOT:PSS electrodes (with a

diameter of 30 μm and with PEDOT:PSS thickness of 200 nm) of a MEA device before and after gluing the chamber on top.

Stability of electrode

Experiments were performed to demonstrate that PEDOT:PSS microelectrodes can keep identical electrical performance even after 10 measurements. To do so, the same electrode with either diameter of 10 or 30 μm (200 nm of PEDOT:PSS) have been characterized. All measurements were performed in the presence of phosphate buffer saline (PBS) solution and by applying a 100 mV sine wave with frequencies ranging from 20 Hz to 1 MHz. As we can see in Fig.S4, no changes of impedance values were observed.

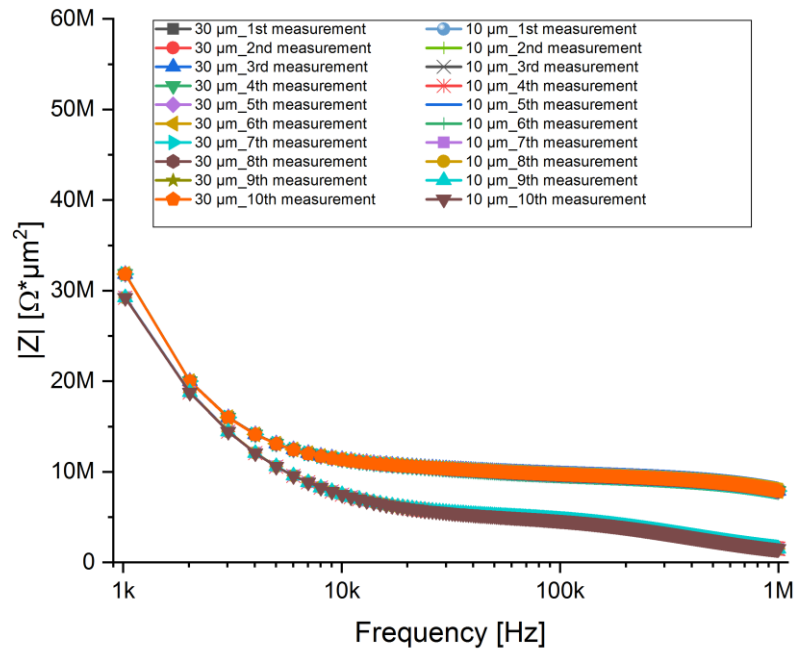


Fig. S4 Impedance variation after 10 measurements performed in PBS solution on an electrode with a diameter of 10 μm and on an electrode with a diameter of 30 μm on the same MEA device with PEDOT:PSS thickness of 200 nm.

Impedance measurements over time

Mean and standard deviation values at 1 kHz over 3 weeks measurements for electrodes with a diameter of 10 and 30 μm and with the thickness of PEDOT:PSS of 200 nm, are presented in tables S2 and S3, respectively.

day	$ Z _{\text{mean}}$ [$\text{M}\Omega \mu\text{m}^2$]	$ Z _{\text{standard deviation}}$ [$\text{M}\Omega \mu\text{m}^2$]
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1	32.90	2.61
2	36.03	2.47
3	36.70	2.59
4	36.16	2.64
7	37.15	2.72
9	37.67	2.77
11	38.49	2.85
14	40.74	3.24
16	38.12	2.99
18	36.40	2.84
21	37.68	2.82

Table S2 The averaged surface impedance values (mean \pm standard deviation) at 1 kHz of 20 electrodes with a diameter of 30 μm and with the thickness of PEDOT:PSS of 200 nm measured over 3 weeks.

day	$ Z _{\text{mean}}$ [$\text{M}\Omega \mu\text{m}^2$]	$ Z _{\text{standard deviation}}$ [$\text{M}\Omega \mu\text{m}^2$]
1	33.91	4.72
2	36.25	4.50
3	36.05	4.59
4	35.47	4.75
7	37.30	5.03
9	37.79	5.06
11	38.66	5.43
14	44.95	8.67
16	39.35	4.31
18	35.21	5.15
21	36.09	5.08

Table S3 The averaged surface impedance values (mean \pm standard deviation) at 1 kHz of 20 electrodes with a diameter of 10 μm and with the thickness of PEDOT:PSS of 200 nm measured over 3 weeks.

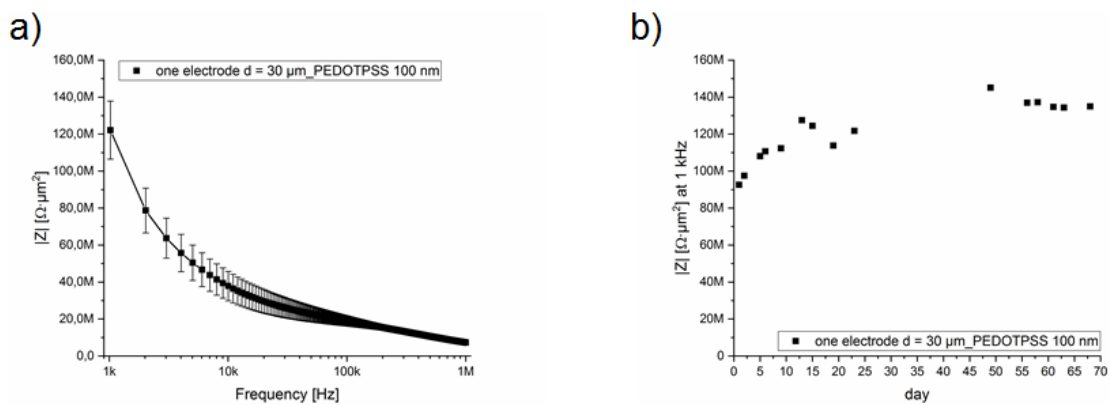


Fig. S5 (a) Averaged impedance spectra measured during 10 weeks of one electrode with a diameter of 30 μm and with PEDOT:PSS thickness of 100 nm. (b) Surface impedance values at 1 kHz of one electrode with a diameter of 30 μm during 10 weeks.

Transmission measurement

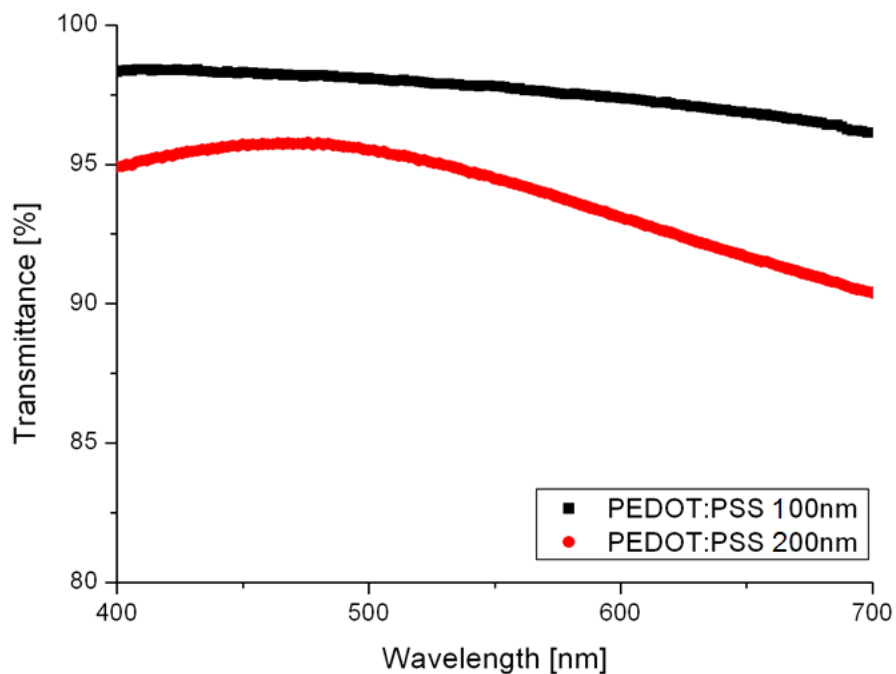


Fig. S6 Transmission for 2 single layers of PEDOT:PSS with thickness of 200 nm (red curve) and 100 nm (black curve).

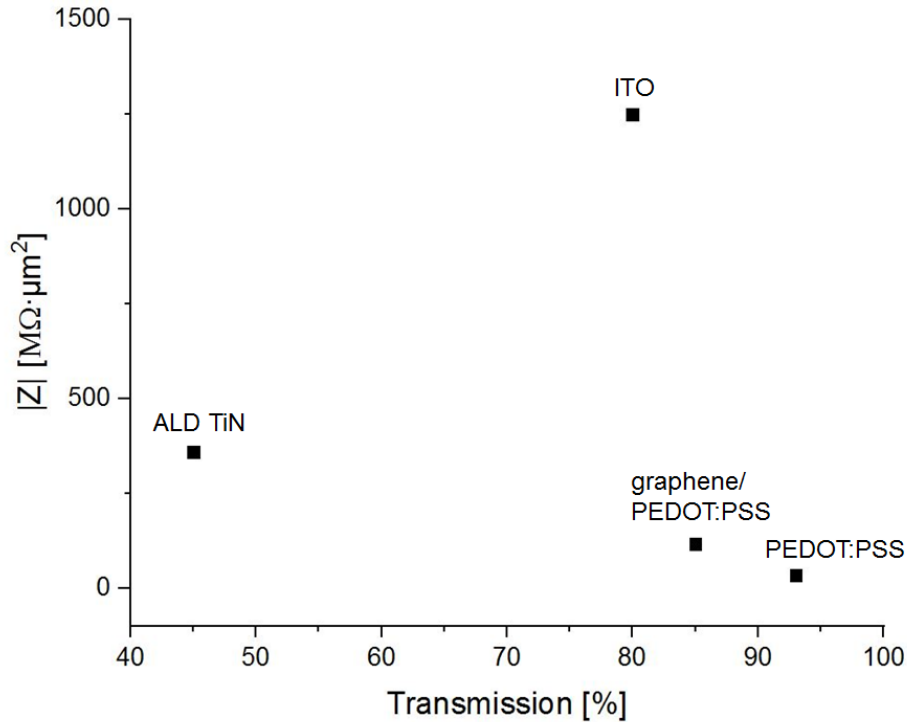


Fig. S7 Comparison of the surface impedance values at 1 kHz and transmission values for the microelectrodes from different transparent materials.

Biocompatibility assessment

Sample	Mean metabolic activity at 7 DIV	Mean metabolic activity at 14 DIV	Mean metabolic activity at 22 DIV	Mean metabolic activity at 28 DIV
Glass (reference)	102 ± 27	113 ± 5	121 ± 19	98 ± 11
PDMS	48 ± 21	61 ± 17 *	64 ± 19 *	47 ± 6 *
PEDOT:PSS	39 ± 8 *	60 ± 13 *	83 ± 20	67 ± 21
Parylene on top of PEDOT:PSS	80 ± 22	94 ± 14 *	90 ± 23	83 ± 25

Table S4 Mean metabolic activity (reflecting living cell number) of primary cortical cells cultured on top of different substrates after 7, 14, 22 and 28 DIV. $n=4$ for each condition. Significant differences between results obtained for cells grown on glass and results obtained for other substrates were examined using the nonparametric Mann-Whitney and Wilcoxon tests (*: $p < 0.05$).

Sample	Mean metabolic activity at 7 DIV	Mean metabolic activity at 14 DIV	Mean metabolic activity at 22 DIV	Mean metabolic activity at 28 DIV
Glass	0.41 ± 0.15	0.25 ± 0.05	0.22 ± 0.03	0.29 ± 0.03

(reference)				
PDMS	0.91 ± 0.37	0.44 ± 0.09	0.60 ± 0.23 *	0.81 ± 0.42 *
PEDOT:PSS	0.94 ± 0.22 *	0.36 ± 0.08	0.27 ± 0.01	0.31 ± 0.07
Parylene on top of PEDOT:PSS	0.58 ± 0.17	0.34 ± 0.09	0.30 ± 0.10	0.34 ± 0.02

Table S5 Mean mortality (ratio of cell death on metabolic activity) of primary cortical cells cultured on top of different substrates after 7, 14, 22 and 28 DIV. $n=4$ for each condition. Significant differences between results obtained for cells grown on glass and results obtained for other substrates were examined using the nonparametric Mann-Whitney and Wilcoxon tests (*: $p < 0.05$).

Cell culture on the PEDOT:PSS electrodes

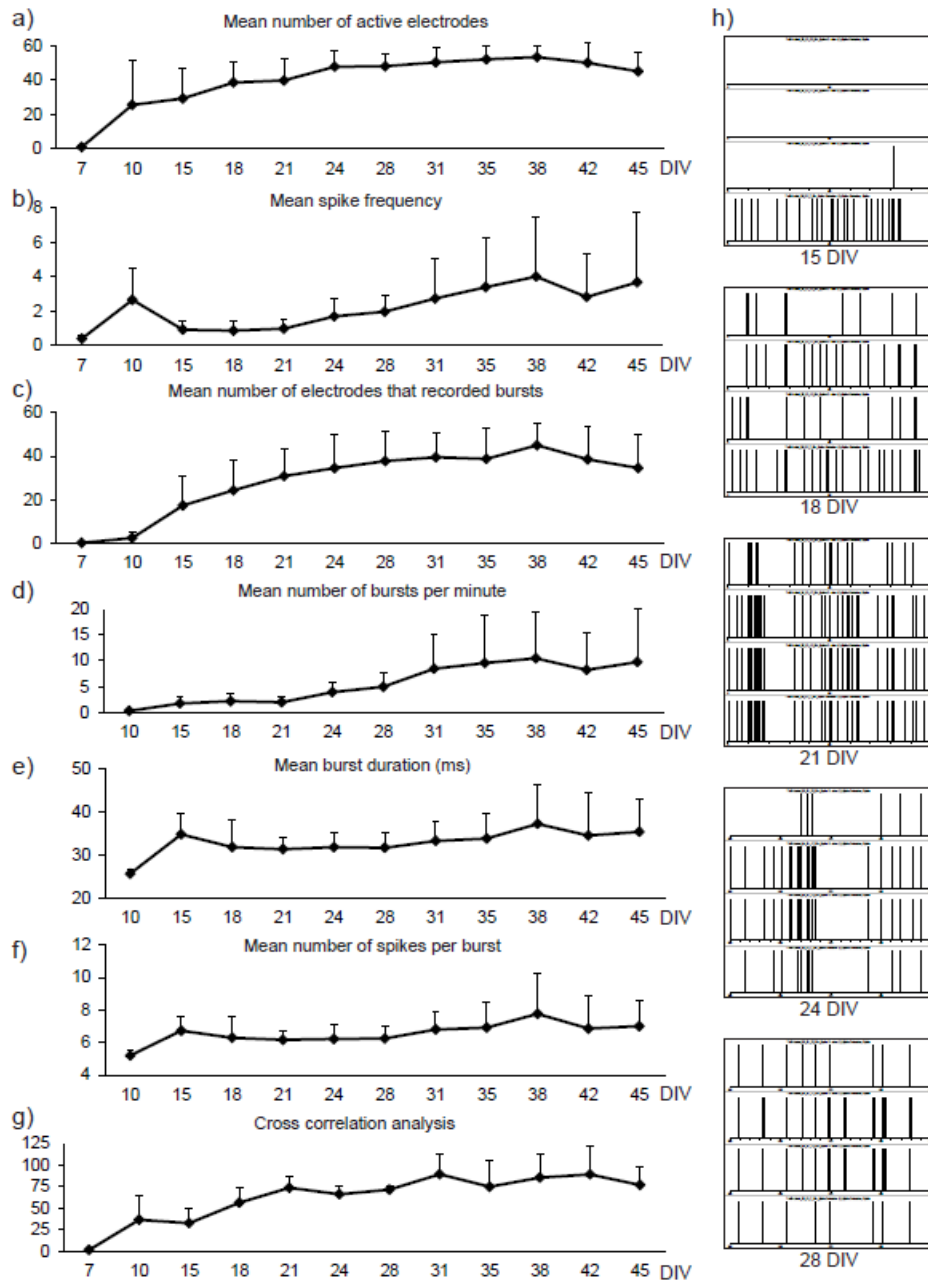


Fig. S8 Spontaneous activity of rat cortical cells cultured on commercial MEAs ($n = 4$) during 45 days of culture. The mean number of active electrodes (a), the mean spike frequency (b), the mean number of electrodes that recorded bursts over time (c), the mean number of bursts per minute (d), the mean burst duration in ms (e), the mean number of spikes per burst (f) and the results of cross-correlation analysis (f) are reported every 3 days from 10 DIV. Are also shown representative examples of extracellular traces of cortical networks at 15, 18, 22, 24 and 28 DIV. The recording duration is 40 seconds and the recordings obtained from four nearby electrodes are displayed (h).

MEA	PEDOT:PSS MEA culture 1	PEDOT:PSS MEA culture 2	Commercial MEA 1	Commercial MEA 2	Commercial MEA 3
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Mean spike peak intensity	-0.027 mV	-0.028 mV	-0.028 mV	-0.029 mV	-0.041 mV
Max. spike peak intensity	-0.176 mV	-0.186 mV	-0.162 mV	-0.110 mV	-0.120 mV
Min. spike peak intensity	-0.015 mV	-0.016 mV	-0.020 mV	-0.020 mV	-0.034 mV

Table S6 Comparison of the mean peak value, the maximal spike peak and the minimal spike peak detected with each MEA after 14 DIV

MEA	PEDOT:PSS MEA culture 1	PEDOT:PSS MEA culture 2	Commercial MEA 1	Commercial MEA 2	Commercial MEA 3
Mean spike peak intensity	-0.024 mV	-0.030 mV	-0.026 mV	-0.045 mV	-0.044 mV
Max. spike peak intensity	-0.092 mV	-0.209 mV	-0.098 mV	-0.152 mV	-0.093 mV
Min. spike peak intensity	-0.015 mV	-0.015 mV	-0.019 mV	-0.032 mV	-0.031 mV

Table S7 Comparison of the mean peak value, the maximal spike peak and the minimal spike peak detected with each MEA after 21 DIV

MEA	PEDOT:PSS MEA culture 1	PEDOT:PSS MEA culture 2	Commercial MEA 1	Commercial MEA 2	Commercial MEA 3
Mean spike peak intensity	-0.030 mV	-0.024 mV	-0.026 mV	-0.050 mV	-0.044 mV
Max. spike peak intensity	-0.162 mV	-0.142 mV	-0.100 mV	-0.214 mV	-0.165 mV
Min. spike peak intensity	-0.016 mV	-0.016 mV	-0.018 mV	-0.033 mV	-0.029 mV

Table S8 Comparison of the mean peak value, the maximal spike peak and the minimal spike peak detected with each MEA after 28 DIV

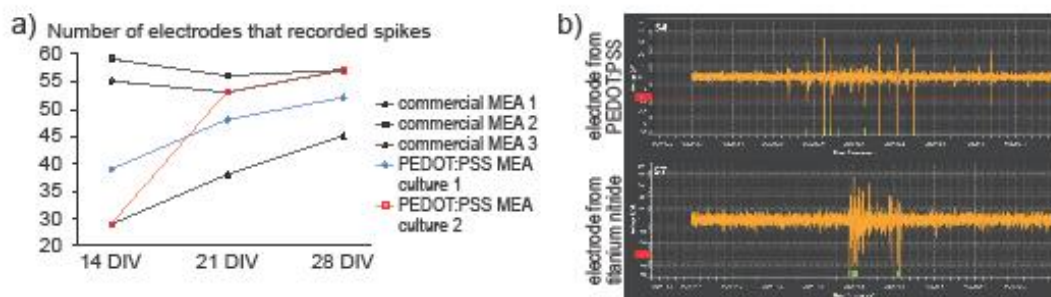


Fig. S9 Number of active electrodes over time for each culture on MEA included in the comparative study (a) and representative filtered signals (in yellow) monitored

from cortical cells cultured on an electrode from PEDOT:PSS (b, top) and cortical cells cultured on an electrode from titanium nitride (b, bottom) after 21 DIV. The threshold for spike detection (in red) is set as 10 times the standard deviation of the baseline noise level. Spike timestamps are visualised in green. **Are also shown representative examples of extracellular traces (timestamps) of cortical cells cultured on PEDOT:PSS MEA at 14 DIV (c, left). The duration is 1 minute and the recordings obtained from four nearby electrodes are displayed. Results of cross-correlation analysis performed in pairs on these four electrodes are also displayed (c, right).**

Reference

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- [2] M. Galas, P. Dourlen, D. Blum, M. Hamdane, L. Bue, The Peptidylprolyl cis / trans - Isomerase Pin1 Modulates Stress- induced Dephosphorylation of Tau in Neurons IMPLICATION IN A PATHOLOGICAL MECHANISM RELATED TO ALZHEIMER DISEASE *, 281 (2006) 19296–19304. <https://doi.org/10.1074/jbc.M601849200>.