Electronic Supplementary Material (ESI) for Materials Advances.

Supplementary Information: Additive manufacturing of multielectrode arrays for biotechnological applications

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Choice of additive manufacturing apparatus

Preliminary prototyping was carried out with PLA filament (3mm) using a Z-Morph 2SX Multi-Tool 3D Printer, an Ultimaker 2+, and a Stratasys J750 3D printer (**Figure S1**). The fidelity of the printing process was assessed by measurements of the dimensions of the printed structures and their differences from the intended dimensions in the CAD files, with resulting error levels calculated (**Table S1**). Surface roughnesses of protoypes from the AM machines were measured using a Mitoyo SJ-400 surface roughness tester as the stylus was moved over distances of 8 mm over the substrate (**Table S2**). Based upon the results presented in **Figure S1**, **Table S1** and **Table S2**, the Stratasys J750 3D printer was found to be the optimal printer available; the uptake of a physiologically relevant buffer phosphate buffered saline (PBS) was assessed by determination of mass differences in printed substrates without tracks before/after incubation in PBS for 17 h, assessed to be 2.2%.

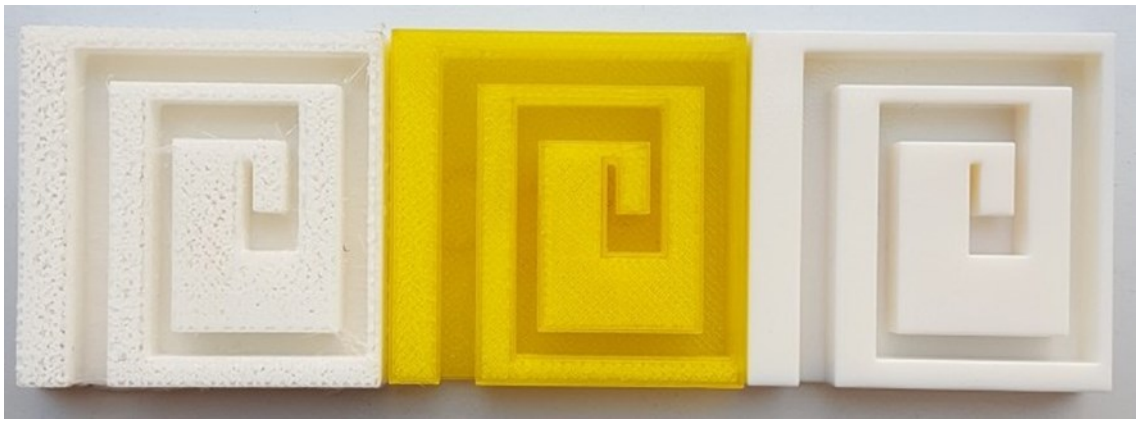

Fig. S1 Photographs of the results of preliminary prototyping with PLA filament (3mm) using various AM apparatus. Left) Z-Morph 2SX Multi-Tool 3D Printer. Centre) Ultimaker 2+. Right) and Stratasys J750.

Table S1 Standard deviations associated with the results of preliminary prototyping with PLA filament (3mm) using various AM apparatus.

Design Value (mm)	Z-morph (mm)	Ultimaker (mm)	Stratasys (mm)
5.0	0.09	0.05	0.01
4.5	0.04	0.02	0.05
4.0	0.08	0.04	0.02
3.5	0.05	0.03	0.01
3.0	0.07	0.02	0.02
2.0	0.06	0.03	0.06
1.5	0.03	0.03	0.01

Table S2 Surface profilometry of preliminary prototypes.

Design Value (mm)	Z-morph (µm)	Ultimaker (µm)	Stratasys (µm)
R_A (mean ± s.d.)	19.8 ± 5.0	2.7 ± 1.0	0.7 ± 0.2
R_Z (mean ± s.d.)	97.8 ± 25.7	15.1 ± 9.2	3.0 ± 0.8

Prototype specification

The initial prototype MEA specification used in this study is detailed in **Table S3**.

Table S3 Prototype MEA Specification

Feature	**Specification**
Substrate area	50 x 50 mm
Part thickness	3 mm
Number of parts	2
Number of electrodes	16
Electrode layout	4 x 4 square array
Inter-electrode distance	2.5 mm edge-to-edge
Electrode diameter	1.5 mm
Channel width	1.5 mm
Connector size	1.5 x 1.5 mm square
Electrode-connector distance	14.25 mm (outer electrodes)
Connector-substrate edge distance	2.5 mm
Guide slot size	3 x 3 mm square, 1 mm deep
Track material	Silver particles (commercially available paste) or CNTs/PDMS (paste composed of CNTs and uncured PDMS)
Electrode coating	Silver paint (silver electrodes only)
Substrate adhesive	Superglue (Cyanoacrylate)
Channel adhesive	Silver paint

Printing non-conductive MEA substrates with tracks to fill with injectable pastes

Non-conductive MEA substrates were printed using VeroMagenta^{TM} RGD851 Stratsys and a Stratasys J750 3D printer.

Injectable pastes

The optimal silver paste formulations were observed to be: silver paste (4.0 g), acetone (1.5 mL), or silver paste (4.0 g), α-terpineol (1.2 mL), respectively.

The optimal CNTs-PDMS paste formulation was observed to be: a degassed suspension of 2.0 g CNTs in 8.0 g silicone elastomer (composed of base and curing agent at a ratio of 10:1 base:curing agent, ca. 7270 mg base with 730 mg curing agent).

Injection of the pastes into the tracks was through a 0.5 mm diameter BD Microlance 3 needle attached to a standard syringe with a Luer-Lok tip. Significant force had to be applied to the syringe to drive the pastes out of the syringes and into the tracks.

MEA production

The MEA production process and time allocation per 10 MEAs with silver electrodes is detailed in **Table S4** and **Figure S2**. The MEA production process and time allocation per 10 MEAs with CNTs/PDMS electrodes is detailed in **Table S5** and **Figure S3**.

Table S4 Prototype production process and time allocation per 10 MEAs with silver electrodes.

Step	Action	Time
1	Print top-half and bottom-half CAD model on Stratasys J750 using VeroMagenta™ RGD851 Stratsys.	105 minutes
2	Remove any support material with high pressure water cleaner.	30 minutes
3	Mix 25g containers of silver paste with 7.5 mL of α-terpineol per container and pour into syringes.	30 minutes
4	Inject paste into channels 4 times (average number of injections).	30 minutes per injection session, waiting 90 minutes between sessions
5	Evaporate any remaining dispersing agent in oven at 80°C.	90 minutes
6	Surface finish top and bottom parts with sandpaper to remove any dried paste spilled.	30 minutes
7	Apply silver paint to end of each channel on bottom part and each corresponding connector/electrode on top part, and a small drop of superglue to each corner of bottom half.	20 minutes
8	Clamp and leave to dry.	360 minutes
9	Apply silver paint to top surface.	10 minutes
10	Cure complete MEA in vacuum oven at 50°C.	90 minutes
Total	All steps.	19.25 hours

Table S5 Prototype production process and time allocation per 10 MEAs with CNTs/PDMS electrodes.

Step	Action	Time
1	Print top-half and bottom-half CAD model on Stratasys J750 using VeroMagenta™ RGD851 Stratsys.	105 minutes
2	Remove any support material with high pressure water cleaner.	30 minutes
3	Add 2.0 g CNTs to 8.0 g silicone elastomer (composed of base and curing agent at a ratio of 10:1 base:curing agent, ca. 7270 mg base to 730 mg curing agent), mix very thoroughly, degas under high vacuum in a vacuum dessicator for 30 minutes, then transferred into syringes.	60 minutes
4	Inject paste into channels 1 time (average number of injections).	90 minutes
5	Cure in oven at 80°C.	60 minutes
6	Surface finish top and bottom parts with sandpaper to remove any dried paste spilled.	30 minutes
7	Apply silver paint to end of each channel on bottom part and each corresponding connector/electrode on top part, and a small drop of superglue to each corner of bottom half.	20 minutes
8	Clamp and leave to dry.	360 minutes
9	Cure complete MEA in vacuum oven at 120°C.	10 minutes
Total	All steps.	12.75 hours

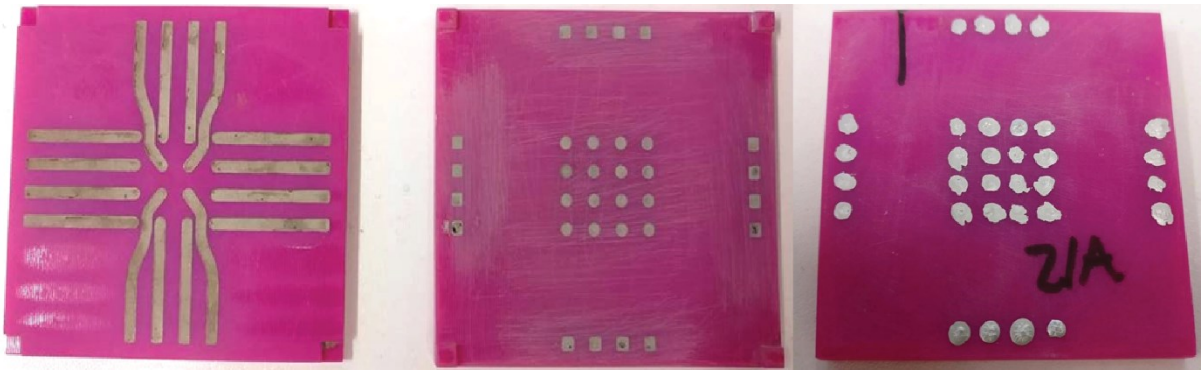

Fig. S2 Photographs of MEA substrates filled with silver. Left) Bottom half. Middle) Top half. Right) Complete MEA with silver paint finished electrodes.

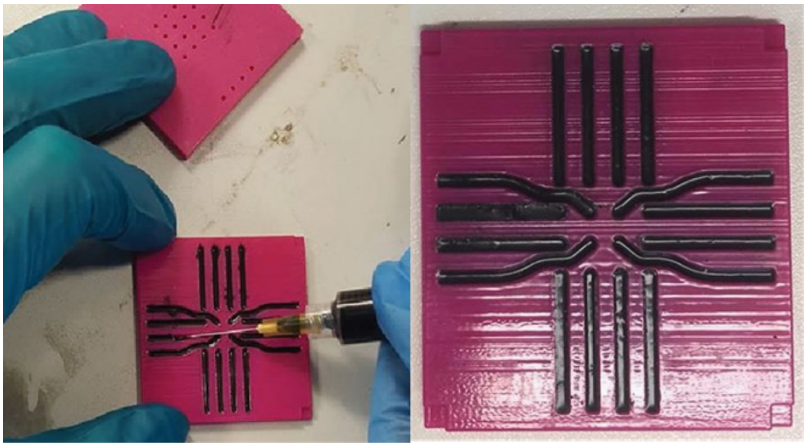

Fig. S3 MEA substrate being filled with CNTs/PDMS (left) and partially filled (right).

Electrical characterisation

Electrode surfaces were characterised by four point probe measurements using four equally spaced steel nails (2mm diameter with wires attached via crocodile clips) forming the electrodes in a strip of plastic. A 5V DC power supply (with an ammeter in series) was applied across the outer most nails and the voltage measured via a multimeter across the inner nails. Four rigs were made with 4, 6, 8, and 10 cm between extreme electrodes.

Through channel resistivity and conductivity could be found by applying equations S1 and S2:

Equation S1: $$\sigma = \frac{1}{\rho}$$

Equation S2: $$\rho = R\frac{A}{l}$$

Where σ is the conductivity in Siemens per metre (S/m), ρ is the electrical resistivity in Ohm metres (Ωm), R is the resistance in Ohms (Ω), A is the cross-sectional area in mm^2 and ℓ is the channel length. The cross-sectional areas and lengths for the long and short channels were evaluated from the corresponding SolidWorks model.

Thermogravimetric analysis (TGA)

TGA was carried out using NETZSCH STA 449 F3 Jupiter® thermal analyser. Samples of material inside aluminium crucibles were studied over the temperature range starting at 30 °C and ending at 500 °C at a heating rate of 10 °C/min. The TGA of VeroMagenta™ RGD851 Stratsys after printing is displayed in **Figure S4** and the DTG curve of VeroMagenta™ RGD851 Stratsys after printing, showing phase transitions within the material is displayed in **Figure S5**.

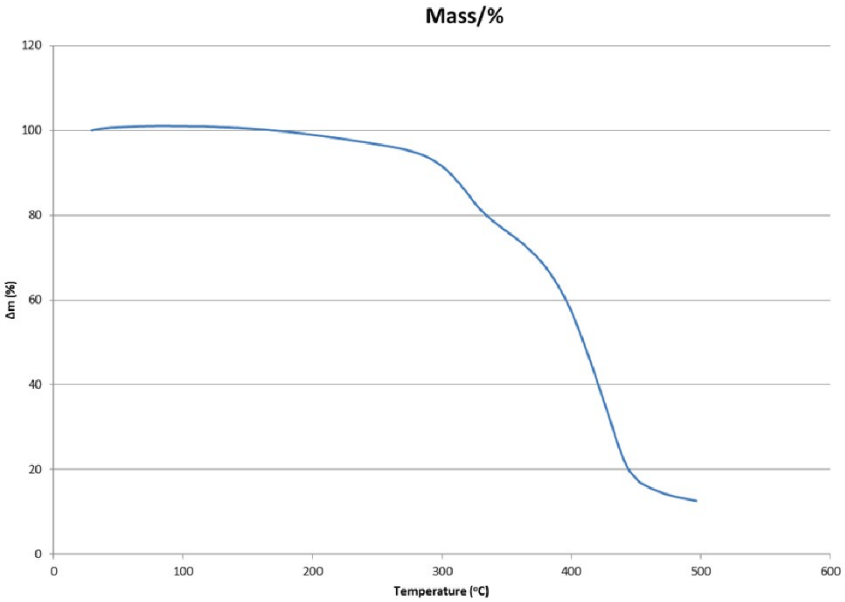

Fig. S4 TGA of VeroMagenta™ RGD851 Stratsys after printing.

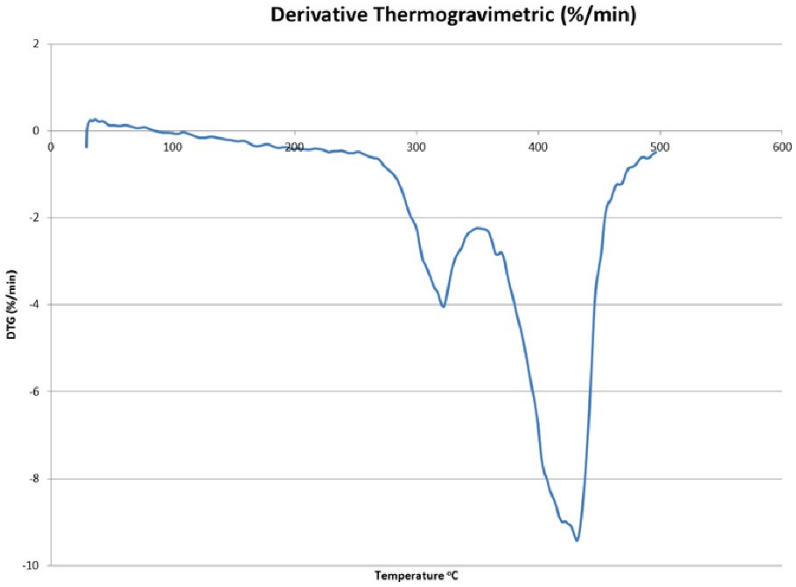

Fig. S5 The DTG curve of VeroMagenta™ RGD851 Stratsys after printing, showing phase transitions within the material. Major phase changes can be seen in a range from ca. 319 °C to 322 °C, and a range of ca. 419 °C to 429 °C, the latter of which is most likely a decomposition stage (since the exact

material composition is unknown [albeit presumed to contain PLA] a precise declaration of the exact phase change is not possible).

Sterilisation

Samples were sprayed with an aqueous solution of either ethanol (ethanol:water, 70:30 vol%) or isopropanol (isopropanol:water, 70:30 vol%) and allowed to dry under UV illumination (in a Holten Laminair 1.8 class II, type A2 biological safety cabinet) for 3.5 h.

Mechanical testing

The robustness of the MEAs towards the likely external forces acting upon them was tested by measuring the force required to break the MEAs using an experimental paradigm replicating an individual holding the MEA with their index and middle finger beneath it while applying pressure to the top surface with their thumb (based on studies finding forces of up to 179 N exerted using index fingers, and up to 290 N exerted using thumbs). Mechanical tests were undertaken using an Instron 8802 (250kN) Fatigue Testing System. Three-point flexural load testing was completed on substrates where the machine was configured to stop either when the MEA failed completely, or when the extension at a certain load became greater than 3mm. The setup was adjusted for shear testing to simulate a shear force being applied to the MEA, with steel sheets (3 mm thick) attached to the MEA sides using Araldite; additional steel blocks were attached to each end to act as a balance, and to facilitate clamping into the Instron 8802. Three-point flexural testing stopped either when the MEA failed completely or when the extension at a certain load was greater than 3mm. The MEAs withstood a maximum compressive load of 13190 N (with the compressive stress at that load being 0.53 MPa), the MEAs did not undergo complete failure, however, were not in a reusable state (**Figure S6**). Given that the MEA design was composed of two parts, shear testing was undertaken to assess the robustness of the parts without/with tabs added to the corners of the MEAs to align the two parts. MEAs without tabs on the corners could withstand a force of 0.77 kN before the two faces separated, whereas MEAs with tabs on the corners could withstand a force of 3.45 kN, thereby demonstrating the MEAs with tabs were robust enough to withstand pressure with a thumb or finger being applied in shear under most circumstances (**Figure S7**).

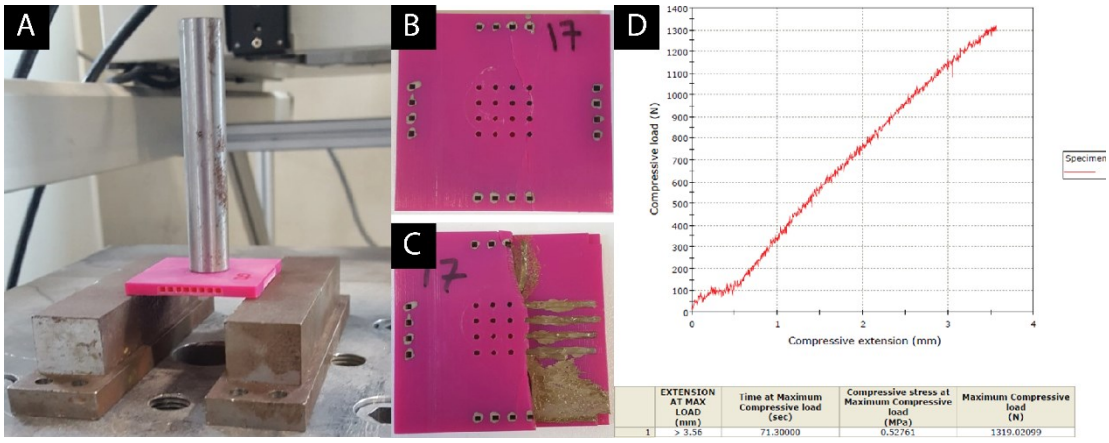

Fig. S6 Three point flexural testing. A) Example prototype MEA in the Instron 8802. B) Example prototype MEA with tabs (and unoptimised electrode filling) before testing. C) Example prototype

MEA with tabs (and unoptimised electrode filling) after testing. D) Example prototype MEA with tabs after testing.

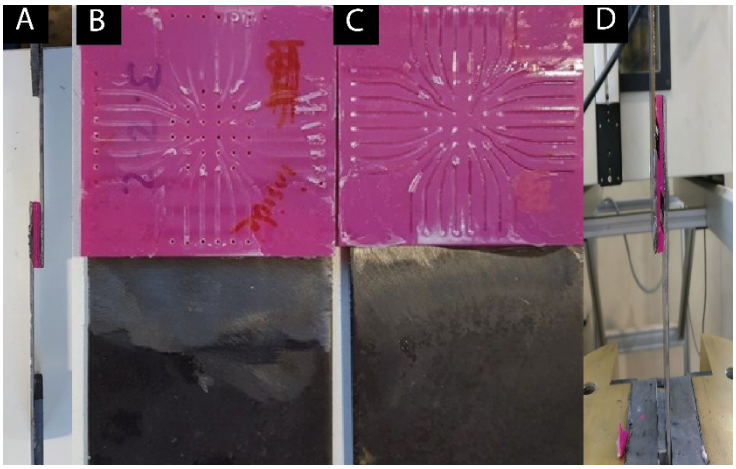

Fig. S7 Shear force test piece set up. A) Example prototype MEA in the Instron 8802. B) Example prototype MEA with tabs (and unoptimised electrode filling) before testing. C) Example prototype MEA with tabs (and unoptimised electrode filling) after testing. D) Example prototype MEA with tabs after testing.

Stability of the silver MEAs

The electrical properties of the MEAs whilst submerged in phosphate-buffered saline (PBS) were monitored over 14 days to test their stability in conditions comparable to their intended use as biomedical sensors. The electrical properties were determined using a 4-point impedance measurement conducted using Kelvin probes (Kelvin-Test Lead HZ184, Rohde & Schwarz GmbH & Co. KG) with an impedance bridge (LRC Bridge HM8118, Rohde & Schwarz GmbH & Co. KG), this was repeated with three separate electrode arrays. The potential release of silver (ppm) from the arrays was subsequently assessed by inductively coupled plasma optical emission (ICP-OES) spectroscopy (Agilent Technologies 5100 ICP-OES, Agilent Technologies Inc., Santa Clara, CA) against the blank (PBS) and 0.2 ppm silver standard (prepared by dilution of a 1000 ppm in 1M nitric acid silver standard [Thermo Fisher Scientific, Stafford, UK] with ultrapure water); this was conducted in triplicate. In short, the protocol was as follows: the impedance of four tracks of equal length (the longest corner tracks were considered here) of an assembled electrode array were measured by applying an electrode (one half of a Kelvin-Test Lead R&S®HZ184) of the impedance bridge (R&S® HM8118) to both ends of each track in turn and the impedance measured at 100 Hz, 1 kHz, 10 kHz and 10 kHz. This provides the initial 'as assembled values' of the track impedance. Submerge the electrode array fully in 250 mL 0.01 M PBS, pH 7.4, (Sigma Aldrich) and seal to prevent contamination. After 23 hours (24 hours from start), remove array, dry and repeat impedance measurement. Take a sample (10 mL aliquots) of PBS from container and store for ICP-OES analysis. Re-submerge array in same container with PBS and cover/seal. Repeat process on days 2, 3, 4, 5, 8, 11 and 14.

In silico studies

In silico toxicity and mutagenicity screening was carried out using Derek Nexus (v. 6.0.1, Nexus: 2.2.2) and Sarah Nexus (Sarah Nexus: v. 3.0.0, Sarah Model: 2.0) supplied by Lhasa Limited (Leeds, UK). Note that PLA is an FDA-approved polymer that is "Generally Recognized as Safe" (GRAS), and acetone is also GRAS listed.

Table S6 Simplified molecular-input line-entry system (SMILES) notations for the chemical species studied.

Chemical	**SMILES**
Acetone	CC(C)=O
α-terpineol	C1CC(CC=C1C)C(C)(C)O
CNTs	C1=6C5=C4C=3C2=C1C7=CC%12=C2C=%11C%10C=3C(C=C4C=C9C5=C(C=8C=6C(=C7)C=CC=8)CC=C9)C=CC%10=CCC=%11C=C%12
PDMS	O([Si@](O[Si@@](C)(C)C)(C)C)[Si@](C)(C)C
Silver	[Ag]

Brain slice preparation and electrical stimulation

Acute hippocampal brain slices were prepared from a 1-month-old male C57Bl/6j mouse as described in Cummings et al 2015. Briefly, the mouse was decapitated in accordance with the UK Animals (Scientific Procedures) Act 1986 and the brain rapidly extracted into artificial cerebrospinal fluid and slices cut using a vibrotome transverse to the long axis of the hippocampus. Slices were allowed to recover for >60 minutes following preparation.

Field excitatory postsynaptic potentials (fEPSPs) were evoked by constant voltage (20 V, 2 μs) stimulation via a contact in the MEA to CA3-CA1 axons in the hippocampus. fEPSPs were recorded using a standard glass field electrode (~2 MΩ resistance, filled with artificial cerebrospinal fluid) placed in stratum radiatum, as described in Medawar et al 2019. Electrodes were connected via a x1 preamplifier headstage to an AxoClamp 1B amplifier (Molecular Devices). Potentials were then amplified (10X) and low-pass filtered (3 kHz) prior to digitisation (20 kHz) into WinWCP (John Dempster, Strathclyde University, UK).

A 10-minute baseline was recorded by evoking pairs (50 ms inter-stimulus interval) of fEPSPs at 0.1 Hz. A tetanic conditioning train was then applied consisting of 20 stimuli at 100 Hz, repeated 3 times, once every 1.5 s. Following conditioning, fEPSPs were again evoked in pairs at 0.1 Hz. At the end of the experiment, the voltage-gated sodium channel blocker tetrodotoxin (TTX, 1 μM) was applied to abolish action potentials.

The fEPSP slope was determined using a linear line of best fit between the initial onset of the slope and approx. 50% maximal amplitude as calculated in WIN-WCP (see **Figure S8**).

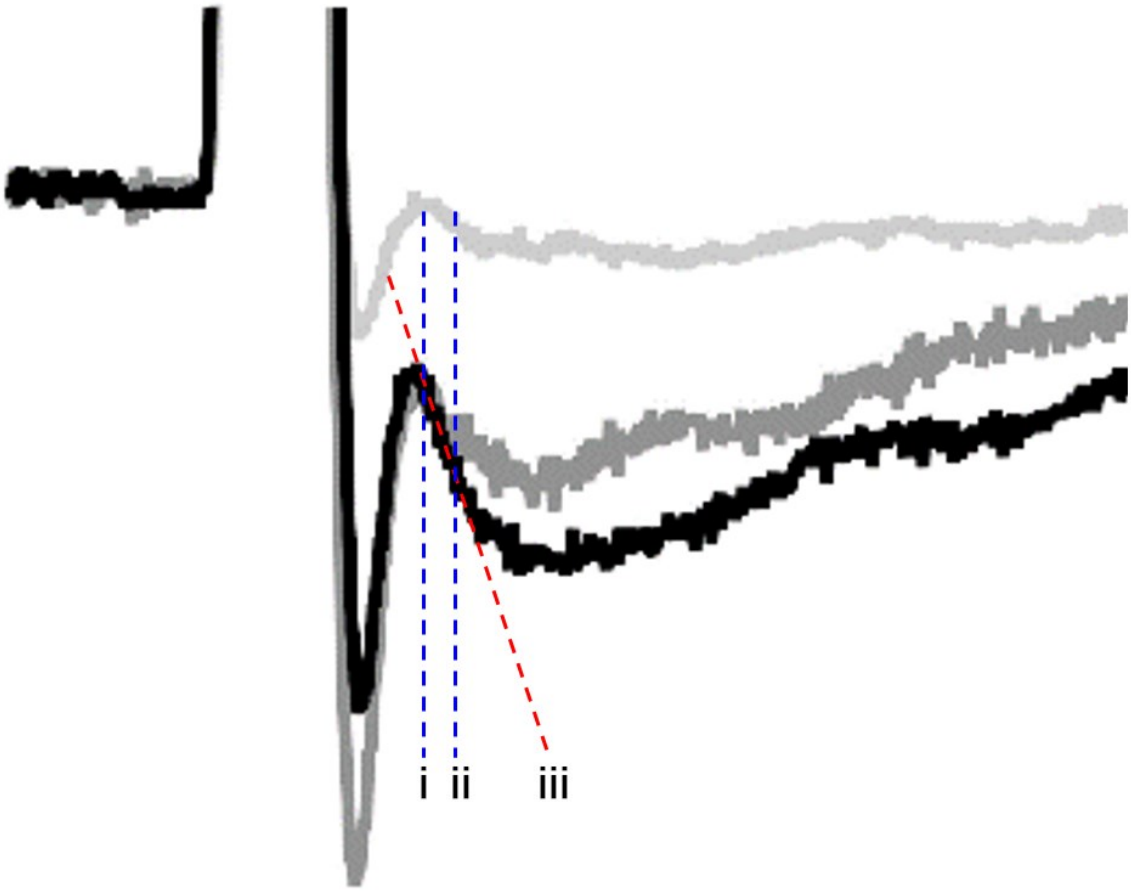

Fig. S8 Determination of the fEPSP slope using a linear line of best fit between the initial onset of the slope and approx. 50% maximal amplitude as calculated in WIN-WCP. Blue lines) represent the 'window' for the fit with **i)** at the initial steep slope, and **ii)** at approx 50% of the maximal amplitude. Red line, **iii**) is then a linear fit of the slope (to the black trace).

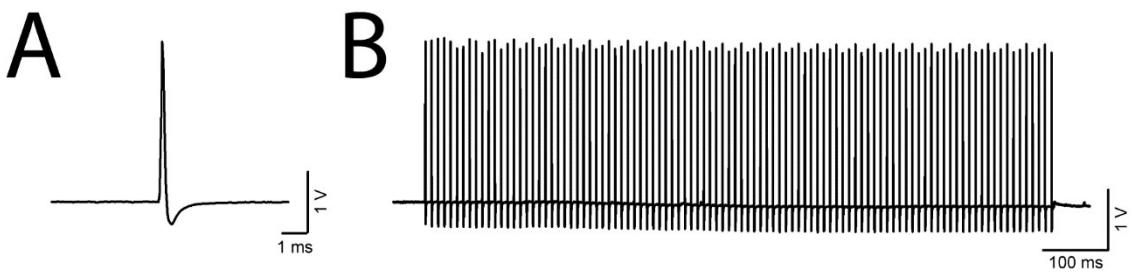

Fig. S9. Results of studies of CNTs/PDMS MEAs interaction with a slice of mouse brain ex vivo. A) A single stimulus artefact in response to a 10 V stimulus lasting 100 µs. Note the much shorter duration than the artefact obtained with the silver MEA (Fig 2) B) The CNTs/PDMS MEA was able to follow and maintain a high frequency (100Hz) train of tetanic stimuli consisting of 100 pulses, a pattern of stimulation commonly employed to induce long-term synaptic plasticity (e.g. Fig 2).