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# Nanofibrous PEDOT-Carbon Composite on Flexible Probes for Soft Neural Interfacing

Venkata Suresh Vajrala<sup>1</sup>, Valentin Saunier<sup>1</sup>, Lionel G. Nowak<sup>2</sup>, Emmanuel Flahaut<sup>3</sup>, Christian Bergaud<sup>1</sup>, Ali Maziz<sup>1\*</sup>

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### *Author contribution statement*

Venkata Suresh Vajrala: Methodology, Investigation, Formal analysis, Visualization, Validation, Writing- original draft & editing. Valentin Saunier: Investigation, Methodology, Writing - review & editing. Lionel Nowak: Conceptualization, Investigation, Formal analysis, Writing- review & editing. Emmanuel Flahaut: Conceptualization, Writing - review & editing. Christian Bergaud: Supervision, Resources, Conceptualization, Writing- review & editing. Ali Maziz: Conceptualization, Supervision, Project administration, Resources, Methodology, Formal analysis, Writing- original draft & editing.

### *Keywords*

PEDOT-Carbon<sup>1</sup>, carbon nanofibers<sup>2</sup>, Porous composite<sup>3</sup>, flexible neural interfaces<sup>4</sup>, electrophysiological recording<sup>5</sup>, neural stimulation<sup>6</sup>

### *Abstract*

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In this study, we report a flexible implantable 4-channel microelectrode probe coated with highly porous and robust nanocomposite of poly(3,4-ethylenedioxythiophene) (PEDOT) and carbon nanofiber (CNF) as a solid doping template for high-performance in vivo neuronal recording and stimulation. A simple yet well-controlled deposition strategy was developed via in situ electrochemical polymerization technique to create a porous network of PEDOT and CNFs on a flexible 4-channel gold microelectrode probe. Different morphological and electrochemical characterizations showed that they exhibit remarkable and superior electrochemical properties, yielding microelectrodes combining high surface area, low impedance ( $16.8 \pm 2 \text{ M}\Omega \cdot \mu\text{m}^2$  at 1 kHz) and elevated charge injection capabilities ( $7.6 \pm 1.3 \text{ mC/cm}^2$ ) that exceed those of pure and composite PEDOT layers. In addition, the PEDOT-CNF composite electrode exhibited extended biphasic charge cycle endurance, resulting in a negligible physical delamination or degradation for long periods of electrical stimulation. In vitro testing on mouse brain slices showed that they can record spontaneous oscillatory field potentials as well as single-unit action potentials and allow to safely deliver electrical stimulation for evoking field potentials. The combined superior electrical properties, durability and 3D microstructure topology of the PEDOT-CNF composite electrodes demonstrate outstanding potential for developing future neural surface interfacing applications.

### *Contribution to the field*

There is a need for novel materials that are capable to meet the performance criteria of a number of neural prostheses and therapies based on electrical stimulation or action potential recording. In these applications, the crucial material-dependent problem is developing microelectrodes that sense and/or stimulate neural activity from small, targeted groups of neurons with high fidelity and long-term reliability. An ideal bidirectional communication between an electrode and the neural system will require access to a low impedance soft microelectrode with a tip size comparable to individual neurons, high charge injection capability while providing smaller hardness mismatch at the electrode-tissue interface. We have combined the conductive polymer Poly(3,4-ethylenedioxythiophene) (PEDOT) with carbon nanofibers (CNFs) into an advanced porous composite material on flexible penetrating brain microelectrodes. This modification results in a strong synergetic effect between these two constituents leading to unrivalled electrochemical properties, combining low impedance, high charge injection capabilities, as well as excellent stability for sub-millisecond electrical stimulation. The combined superior electrical properties, durability and 3D microstructure topology of the PEDOT-CNF composite electrodes demonstrate outstanding potential for developing future neural surface interfacing applications.

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In review

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10 **Keywords:** PEDOT-Carbon<sup>1</sup>, carbon nanofibers<sup>2</sup>, Porous composite<sup>3</sup>, flexible neural  
11 interfaces<sup>4</sup>, electrophysiological recordings<sup>5</sup>, neural stimulation<sup>6</sup>.

12

## 13 Abstract

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15 porous and robust nanocomposite of poly(3,4-ethylenedioxythiophene) (PEDOT) and carbon  
16 nanofiber (CNF) as a solid doping template for high-performance in vivo neuronal recording and  
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23 CNF composite electrode exhibited extended biphasic charge cycle endurance and excellent  
24 performance under accelerated lifetime testing, resulting in a negligible physical delamination and/or  
25 degradation for long periods of electrical stimulation. In vitro testing on mouse brain slices showed  
26 that they can record spontaneous oscillatory field potentials as well as single-unit action potentials and  
27 allow to safely deliver electrical stimulation for evoking field potentials. The combined superior  
28 electrical properties, durability and 3D microstructure topology of the PEDOT-CNF composite  
29 electrodes demonstrate outstanding potential for developing future neural surface interfacing  
30 applications.

31 **1 INTRODUCTION**

32 Neural electrodes provide the critical interface between the nervous system and electronics. Well-  
33 defined anatomical regions from the brain can be the targets of implanted microelectrodes, enabling  
34 localized neuromodulation by either recording or delivering electrical signals at the level of individual  
35 neuron (Nicoletis et al. 2003; Spira and Hai 2013). Such capabilities have been critically important for  
36 supporting neuroscience research along with emerging clinical devices aimed at treating debilitating  
37 disorders, including deafness (Sparreboom et al. 2010), paralysis (Schultz and Kuiken 2011), blindness  
38 (Rizzo et al. 2003), Parkinson's disease (Benabid 2003), epilepsy (Theodore and Fisher 2004) and  
39 other disorders (Mayberg et al. 2005). In all of these applications, the crucial material-dependent  
40 problem is developing microelectrode array that sense and/or stimulate neural activity from small,  
41 targeted groups of neurons with high fidelity and long-term reliability (Grill et al. 2009).

42 Conventional implantable microelectrode arrays, made of silicon backbone and noble metal electrodes,  
43 such as gold (Au) platinum (Pt) or iridium (Ir) become routine in animal research and have occasionally  
44 been used in humans (Jun et al. 2017; Kellis et al. 2016; Khodagholy et al. 2015). However, they are  
45 not suitable for long-term use due to limitations regarding the electrical and mechanical mismatches  
46 with the surrounding tissue (Polikov et al. 2005). Decreasing the size of an electrode active site, to  
47 ideally target single neuron, results in low capacitance and high impedance at the electrode/tissue  
48 interface, which seriously impacts recording resolution and stimulation capabilities (Cogan 2008;  
49 Viswam et al. 2019). For neuronal recording, the electrode impedance contributes to the noise, and  
50 high impedance electrodes are expected to have a low signal-to-noise ratio (SNR) (Boehler et al. 2020).  
51 For neuronal stimulation, an ideal electrode should display a high storage capability to safely inject  
52 current pulses with minimal potential transients at the electrode/tissue interface, thus decreasing both  
53 electrode polarization and heat accumulation during stimulation (Boehler et al. 2020; Cogan 2008;  
54 Merrill et al. 2005).

55 Besides, immune reaction occurs through the mechanical mismatch between rigid electrodes and the  
56 neural tissue, triggering inflammatory responses and glial scar formation, which may lead to  
57 encapsulation of the electrodes and subsequent device failure (Jeong et al. 2015; Polikov et al. 2005).  
58 These device failures appear in the form of electrical recording degradations including increased  
59 impedance, increased noise levels and decreased signal amplitudes (Jeong et al. 2015). In this regard,  
60 there has been a demand to fabricate electrodes on flexible substrates that, by showing smaller hardness  
61 mismatch, provide a more adaptable interface to neural tissue. In addition, the electrode material

62 deposited on flexible substrate should display low electrical impedance and high charge-transfer  
63 capacity without substantially increasing the site geometric surface area (Inal et al. 2018).

64 To tackle this challenge, various types of organic electroactive materials have been employed such as  
65 conductive polymers (CPs) (Green and Abidian 2015; Maziz et al. 2021), carbon-based nanomaterials  
66 *i.e.* carbon nanotubes (CNTs) (Ansaldo et al. 2011; Hanein and Bareket-Keren 2013; Sridharan and  
67 Muthuswamy 2021), graphene (Hess et al. 2011), reduced graphene oxide (r-GO) (Luo et al. 2013) and  
68 their nanocomposites (Gerwig et al. 2012; Saunier et al. 2020b), to create much desired porosity and  
69 softness at the electrode/tissue interface. Among them, the conducting polymer Poly (3,4-  
70 ethylenedioxythiophene) (PEDOT) has been a popular choice due to its mixed electronic and ionic  
71 conductivities, high-quality electrochemical performances, together with excellent biocompatibility,  
72 softness, and ease of functionalization (Abidian et al. 2009; Fabretto et al. 2012; Green and Abidian  
73 2015; Maziz et al. 2016). Several reports showed that PEDOT coatings, doped with different counter  
74 ions such as poly(styrene sulfate) (PSS), Nafion, tosylate (Larsen et al. 2012; Maziz et al. 2017),  
75 dodecyl sulfate (Wu et al. 2015) or  $\text{ClO}_4^-$  (Maziz et al. 2014), can significantly decrease the electrode  
76 impedance (30- 250  $\text{M}\Omega/\mu\text{m}^2$ ) and increase the charge-injection capacity (1-3  $\text{mC}/\text{cm}^2$ ) as compared  
77 to flat metal sites of similar geometric area (Cogan 2008; Khodagholy et al. 2011; Venkatraman et al.  
78 2009). In addition, *In vitro* studies have demonstrated that PEDOT coating would also present a good  
79 substrate for the growth of various cell types in biological and tissue engineering areas, wherein  
80 PEDOT directly interacts with cells or tissues (Bongo et al. 2013). Despite its promising outlook,  
81 PEDOT is yet to be perfected as a coating material for neural electrodes, especially in terms of extended  
82 charge injection capabilities and long-term adhesion stability (Cui and Zhou 2007; Jan et al. 2009). For  
83 example, brain stimulation applications demand high and stable charge injection capabilities, where  
84 the electrode materials should sustain few thousands to millions of cycles of electrical stimulations  
85 pulses without corrosion, tissue damage, or delamination (Boehler et al. 2020).

86 Multiple strategies have been suggested to reinforce PEDOT coating, either by modifying the monomer  
87 itself (Ouyang et al. 2017), using adhesion promoters (Boehler et al. 2017), or by the incorporation of  
88 charged carbonaceous nanomaterials (Gerwig et al. 2012; Luo et al. 2011). In previous studies,  
89 composite materials made up of CNTs (Bhandari et al. 2009; Gerwig et al. 2012) or r-GO (Luo et al.  
90 2013), in combination with PEDOT, have been coated on metal microelectrodes to decrease the  
91 impedance and ramp-up the charge injection limits (Samba et al. 2015), even beyond the PEDOT: PSS  
92 capabilities for both acute and chronic stimulation tests (Alba et al. 2015; Kozai et al. 2016). It was



93 also reported that these mechanically strong carbonaceous materials function as reinforcing elements  
94 within the composite, preventing the PEDOT film from undergoing deformation and cracking during  
95 prolonged redox reactions (Luo et al. 2011). This excellent performance makes the combination of  
96 PEDOT with carbon-based nanomaterials a highly promising candidate material for the development  
97 of long-lasting neural interfaces. However, as of now, most of the research has focused on either the  
98 selection of near perfect electrode material with superior electrical properties or controlled 3D surface  
99 macro porous patterning of electrode to promote an intimate contact with the neural tissue. In contrast,  
100 less attention has been paid to the combination of both.

101 Recently, we have demonstrated the feasibility of a novel composite material by combining PEDOT  
102 with carbon nanofibers (CNFs) through a simple and reproducible electrodeposition method (Saunier  
103 et al. 2020b). CNFs exhibit extraordinary strength, high modulus of elasticity (940 GPa), and provide  
104 an extremely large surface area for charge transfer and cell attachment (Nguyen-Vu et al. 2006). Since,  
105 they contain basal graphite planes and edge planes, upon oxidation, their outer surface can be  
106 electrochemically functionalized with PEDOT molecules. In addition, as a result of their extremely  
107 high edge proportion with very high aspect ratios and inherent herringbone morphology, making them  
108 excellent nanoscale building block to establish interconnected, three-dimensional (3D) macroporous  
109 structures, in combination with PEDOT. We have recently shown that the combination of CNFs and  
110 PEDOT on rigid microelectrode array (MEA) resulted in as strong synergetic effect between the two  
111 components in the single composite leading to remarkable electrochemical properties, as well as a  
112 reliable *in vitro* neurotransmitter monitoring using amperometric techniques (Saunier et al. 2020b).  
113 These results suggest PEDOT-CNF composites as a most interesting electrode material for applications  
114 in neuroprostheses and neurophysiology research. In this context, to go further into the development  
115 of neural interfacing devices for *in vitro* and *in vivo* applications, we are reporting a method for  
116 preparing macroporous, stable and electrically superior PEDOT, using CNFs as a solid dopant  
117 template, on ultra-flexible penetrating neural microelectrodes. We developed a well-controlled single  
118 step deposition method and optimized it for preparing macroporous PEDOT-CNFs nanocomposite via  
119 *in situ* electrochemical polymerization technique on flexible parylene-based neural probes. This  
120 flexible substrate- provides the means to decrease the mechanical mismatch at the electrode/tissue  
121 interface. We showed that PEDOT-CNF hybrid neural microelectrodes exhibit remarkable  
122 electrochemical properties, yielding microelectrodes combining low impedance, high surface area, and  
123 elevated charge injection capabilities. This device was further tested for neural recording and

124 stimulation in the hippocampus of mouse brain slice in vitro. The obtained results opened great  
125 prospects for the development of next-generation microelectrodes for applications in brain therapies.

126

## 127 **2 MATERIALS AND METHODS**

### 128 **2.1. Fabrication of the neural implant and device packaging**

129 The photomask designs of the flexible parylene C-based probe, with four micro disk electrodes, are  
130 inspired from our previous works (Castagnola et al. 2015; Lecomte et al. 2017). The fabrication  
131 procedure is schematically illustrated in Fig. 1a. A 23  $\mu\text{m}$  thick film of Parylene C was deposited using  
132 chemical vapour deposition (Comelec C-30-S at 700 °C) on 4-inch SiP wafer. Next 50 nm thick Ti and  
133 200 nm thick Au layers were deposited and patterned, using a conventional physical vapour deposition  
134 technique followed by a lift-off with AZ-nLof 2035 (Micro chemicals). Subsequently, 1.3  $\mu\text{m}$  thick  
135 parylene C was deposited as a passivation layer, followed by an annealing step at 110°C for 16 hours  
136 under nitrogen flow to increase the adhesion between parylene C-gold sandwich layers. Next, the  
137 electrode surfaces (40  $\mu\text{m}$  diameter) and the corresponding connection pads were realized by, photo-  
138 patterning with 5  $\mu\text{m}$  thick AZ4562 photoresist, followed by the etching of the thin parylene C  
139 passivation layer using O<sub>2</sub> plasma reactive ion etching ICP-RIE (Trikon Omega 201). Later, the  
140 implants were anisotropically etched to establish smooth outlines and vertical sidewalls, using 50  $\mu\text{m}$   
141 thick BPN photoresist (Intervia BPN-65A, Dupont) and a deep reactive ion etching (ICP-DRIE) step.  
142 After that, the implants were peeled-off carefully by placing the entire wafer into DI water for at least  
143 2 hours. Later, the released implants were stripped off from the leftover photoresist using TechniStrip  
144 NF52 (Microchemicals), thoroughly washed in DI water and stored in a dry place. Finally, the implants  
145 were bonded to a customized flexible ribbon cable with golden traces (AXO-00021, pro-POWER,  
146 China) by using epoxy silver and photosensitive glue.

### 147 **2.2. Functionalization of Carbon nanofibers (CNFs)**

148 Raw CNFs (Pyrograf®-III, PR-19-XT-PS, pyrolytically stripped, platelets conical, >98% carbon basis,  
149 20-200  $\mu\text{m}$ ) were oxidized using wet chemical oxidation process where 300 mg of CNFs were placed  
150 in 200 mL of the oxidizing solution (15 M) HNO<sub>3</sub>/ (18 M) H<sub>2</sub>SO<sub>4</sub> and sonicated for 15 min, followed  
151 by 2 hours of reflux at 70 °C (Bortolamiol et al. 2014; Rasheed et al. 2007). This process helps to  
152 remove impurities (metallic particles and amorphous carbon) from the sample and make them

153 hydrophilic, so that the CNFs can be dispersed in water (Saunier et al. 2020a). Following the acid  
154 treatment, the CNF dispersion was washed with DI water to reach a neutral pH, and stored at 4°C.

### 155 **2.3. Electrochemical deposition of PEDOT-CNF composite**

156 The stock solution of oxidized CNFs was first vortexed for 10 minutes and homogenized by sonication.  
157 Next, the sample was dispersed in DI water, at a concentration of 1 mg/ml, along with 10 mM EDOT  
158 (Sigma Aldrich). Later, the CNFs-EDOT mixed suspension was incubated under vortex for 2 days at  
159 room temperature. Prior to use, the suspension containing CNFs and EDOT polymer was sonicated for  
160 2 min and vortexed again for 15 min to obtain a homogenized dispersion. The gold microelectrodes  
161 were electrochemically cleaned by cycling at 200 mV/s between -0.3 V and 1.4 V vs Ag/AgCl in 0.5  
162 M H<sub>2</sub>SO<sub>4</sub> using cyclic voltammetry (Fig. S1a). PEDOT-CNF composites were galvanostatically  
163 deposited on the clean Au electrode surface by applying the current density of 10 pA/μm<sup>2</sup> with a range  
164 of charge densities (1, 2, 4, 6 and 8 nC/μm<sup>2</sup>) (Fig. 2 and Fig. S1b).

### 165 **2.4. Optical characterization**

166 Optical microscope images of the implant, before and after the composite deposition (Fig. 1d-e and  
167 Fig. 7b) were obtained with a HIROX microscope (HI-SCOPE Advanced KH-3000). The  
168 morphological information of the PEDOT-CNF composite coatings on flexible microelectrode array  
169 (Fig. 2) and the corresponding EDX characterization (Fig. 3) were obtained by using FEG Schottky  
170 High resolution Helios 600i Dual FIB scanning electron microscope.

### 171 **2.5. Electrochemical characterization**

172 Prior to the characterization, the probes were rinsed with DI water and immersed in artificial  
173 cerebrospinal fluid (aCSF) for at least 30 min. For physiological relevance, the aCSF composition  
174 mimicked the mammalian ionic CSF composition and consisted of (in mM): NaCl 124, NaHCO<sub>3</sub> 26,  
175 KCl 3.2, MgSO<sub>4</sub> 1, NaH<sub>2</sub>PO<sub>4</sub> 0.5, CaCl<sub>2</sub> 1.1, glucose 10, and bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub> (pH  
176 7.4) (Gleizes et al. 2017). Probes were proceeded further with charge storage capacity (CSC) (Fig. 4a-  
177 b), electrochemical impedance spectroscopy (EIS) (Fig. 4c-f) and charge injection limit (CIL)  
178 measurements (Fig. 5 and 6). Electrochemical characterizations were performed in a three-electrode  
179 configuration, using a thick (2-mm diameter, ~5 mm<sup>2</sup>) Pt wire (WPI, 99.99%) as counter electrode  
180 (CE) and an Ag/AgCl reference coil electrode.

181 Impedance measurements (EIS) were performed between 10 Hz and 7 MHz, using a 10 mV AC signal  
182 at 0 V vs Ag/AgCl, whereas the cathodic charge storage capacity measurements (CSCc) were carried  
183 out by launching cyclic voltammetry on a low current potentiostat channel (BioLogic VMP3), between  
184 0.6 V and -0.8 V in aCSF at room temperature. Each electrode sample was swept for two cycles and  
185 the CSCc was calculated as the time integral of the cathodic current recorded over a potential range of  
186 0.6 V to -0.85 V in the second cycle.

187 To estimate the charge injection limit, voltage transient measurements were carried out at different  
188 input currents by applying charge-balanced biphasic current pulse waveforms at 10 Hz, with pulse  
189 durations of 1 ms (Fig. 5) and 200  $\mu$ s (Fig. 6), using a Bio-Logic VSP3 potentiostat. The negative  
190 polarization potential ( $V_p$ ) was calculated by subtracting the initial access voltage ( $V_a$ ) due to solution  
191 resistance from the total voltage ( $V_{max}$ ). The charge injection limits were calculated by multiplying the  
192 current amplitude and pulse duration at which the polarization potential reaches the water reduction  
193 limit (-1.3 V), divided by the geometric surface area of the electrode.(Boehler et al. 2020)

194 Long-term stimulation stability testing was assessed by launching at least 0.5 million continuous  
195 current pulses of 33  $\mu$ A at 10 Hz, thereby employing the charge injection capacity of 0.5 mC/cm<sup>2</sup>. The  
196 electrochemical differences before and after pulsing were measured with EIS. The electrochemical cell  
197 was sealed properly to avoid evaporation of the electrolyte during the measurements.

198 Accelerated aging of flexible probes was accomplished by immersion in 1x PBS at 57°C (Castagnola  
199 et al. 2015). At this temperature, a simulated three months (92 days) of aging in vivo is completed in  
200 23 days. EIS measurements of each electrode were taken at regular intervals at 37°C.

201

## 202 **2.6. Brain slice preparation and electrophysiological measurements**

203 All procedures were conducted in accordance with the guidelines from the French Ministry of  
204 Agriculture (décret 87/848) and from the European Community (directive 86/609) and was approved  
205 by the Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation (N° 15226-  
206 2018052417151228). Adult (>2-month-old) wild type female mice were anesthetized with isoflurane  
207 and killed by decapitation. All following procedures were made in the presence of oxygenated (95%  
208 O<sub>2</sub> and 5% CO<sub>2</sub>) and ice-cold modified, artificial cerebrospinal fluid (maCSF) whose composition was  
209 (in mM): NaCl 124, NaHCO<sub>3</sub> 26, KCl 3.2, MgSO<sub>4</sub> 1, MgCl<sub>2</sub> 9, NaH<sub>2</sub>PO<sub>4</sub> 0.5, and glucose 10 (Gleizes

210 et al. 2017). The upper part of the skull was drilled off and the whole brain was carefully removed and  
211 glued on a pedestal for slicing. 400  $\mu\text{m}$ -thick coronal brain slices were cut on a vibratome (752 M  
212 vibroslice, Campden Instrument, UK), whose chamber was filled with ice-cold oxygenated maCSF.  
213 The slices were kept at room temperature for at least one hour in an *in vivo*-like artificial cerebrospinal  
214 fluid (aCSF, composition in “electrochemical characterization” above), aerated with 95%  $\text{O}_2$  and 5%  
215  $\text{CO}_2$  (pH 7.4). For recording and stimulation, a brain slice was fixed on the mesh of a submersion type  
216 recording chamber (Scientific System Design, Mississauga, Ontario, Canada), as shown in the Fig. 8a.  
217 The recording chamber was continuously supplied in oxygenated aCSF that was gravity fed at a flow  
218 rate of 3-3.5 ml/min. The temperature was maintained at 33-34°C. The neural microelectrodes were  
219 positioned in the hippocampal regions (CA1 and CA3) of the brain slice, using a 3D micromanipulator.  
220 Tungsten-in-epoxy lite microelectrodes (FHC, 0.2–0.3  $\text{M}\Omega$ ) were also used for parallel recording and  
221 stimulation. Signals were amplified (final gain:  $\times 10^4$ ) and filtered with a NeuroLog recording system  
222 (Digitimer Ltd, UK) and digitized with a 1401plus interface (CED systems, Cambridge, UK) with a  
223 digitization rate of 20 kHz. The signals were visualized online and analysed offline using spike2  
224 software (CED) and custom scripts within Spike2 software.

225

## 226 3 RESULTS AND DISCUSSION

### 227 3.1. Morphological study of electrodeposited PEDOT-CNF composites on flexible implants

228 In this work, we used a flexible neural implant having an array of 4 gold micro-disk-electrodes (40-  
229  $\mu\text{m}$  diameter), that are sandwiched between two parylene C layers (Fig. 1a-b). A thick polymer  
230 backbone (23  $\mu\text{m}$ ) and thin passivation layers (1.3  $\mu\text{m}$ ) were opted for, such that there exists a balance  
231 between flexibility and improved long-term performance *vs* potential insulation regulation. We used  
232 this design to directly compare PEDOT-CNF and bare gold microelectrodes properties (structure,  
233 morphology, electrochemical performances and stability). The electrochemical deposition of PEDOT-  
234 CNF composite is illustrated in Fig. 1c-e. Before the electrodeposition, oxidized CNFs were  
235 synthesized for the following electrochemical synthesis of PEDOT-CNF composite (Bortolamiol et al.  
236 2014; Rasheed et al. 2007; Saunier et al. 2020b). Chemical oxidation of the CNFs leads to the formation  
237 of negatively-charged functional groups *i.e.* carboxylate and hydroxyl on the outer surface of the CNFs,  
238 which render them usable for charge-balancing anionic PEDOT dopant. Later, the PEDOT-CNF  
239 nanocomposites were deposited, on the flexible implantable electrode array, where PEDOT was  
240 galvanostatically deposited along with the entrapped oxidized CNFs within its matrix in one step (Fig.

241 1c-e). The deposition took place through a simultaneous oxidative PEDOT polymer chain propagation  
242 and CNF trapping mechanisms, resulting in a fibrous network of oxidized CNFs surrounded by  
243 PEDOT. The optimal deposition conditions were investigated by varying the surface charge densities,  
244 ranging from 1 nC/ $\mu\text{m}^2$  to 8 nC/ $\mu\text{m}^2$ , at a constant current density of 10 pA/ $\mu\text{m}^2$ .

245 SEM observations and FIB cross-sectional characterization of PEDOT-CNF composites of all  
246 deposition conditions (1 to 8 nC/ $\mu\text{m}^2$ ), showed that the entrapped CNFs were spatially distributed all  
247 over the gold electrode surface, and constitute a network of inter-connected nanofibers with variations  
248 in their aspect ratios and deposition densities (Fig. 2a-b). Since the CNFs core dictates the  
249 electrochemical growth of PEDOT, a porous and fibrous structure was a common feature among all  
250 the deposits. The deposition thickness and diameter, therefore the effective surface area, increased  
251 linearly with respect to the applied charge density (Fig. 2c-d). At the lowest charge densities (1  
252 nC/ $\mu\text{m}^2$ ), the gold electrode surface was covered by a non-uniform layer of PEDOT-CNFs. In contrast,  
253 at higher charge deposition densities, the composite film was more uniformly porous and fibrous,  
254 thereby at the same time facilitating seamless intra- and interlayer ionic/electronic transport.

255 The energy dispersive X-ray (EDX) mapping data (Fig. 3) illustrates the spatial distribution of gold  
256 (white), sulfur (blue), carbon (red) and oxygen (green) within the gold-PEDOT-CNF composite  
257 electrode at a surface charge deposition density of 8 nC/ $\mu\text{m}^2$ . Within the composite, the sulfur reflects  
258 the presence of PEDOT and the oxygen represents both PEDOT and COOH groups on oxidized CNFs.  
259 According to Fig. 3e, PEDOT is found to be densely concentrated at the gold interface, suggesting that  
260 there exists a highly ordered and less porous PEDOT-CNF composite at the gold-composite interface.  
261 In addition, the presence of sulfur was observed mostly around the walls of CNFs (Saunier et al.  
262 2020b), indicating that PEDOT is grafted around the walls of nanofibers. Overall SEM and EDX  
263 observations suggest that PEDOT is acting as a polymer chain template to trap the oxidized CNFs and  
264 propagate all around the gold electrode surface resulting a three-dimensional growth of PEDOT around  
265 the oxidized CNFs.

### 266 **3.2. Electrochemical characterization (CSCc, EIS and CIL)**

267 The electrodeposited PEDOT-CNF composite films at different deposition charge densities were  
268 electrochemically characterized *in vitro* to assess their bidirectional transduction (electrolyte/electrode)  
269 capabilities. In this regard, electrochemical impedance spectroscopy (EIS), cathodic charge storage  
270 capacity (CSCc) and charge injection limits (CIL) are the essential parameters. On the one hand, a

271 minimal impedance value is required to achieve signal noise reduction, such as thermal noise through  
 272 shunt pathways (Cogan 2008; Ghane-Motlagh and Sawan 2013; Viswam et al. 2019). On the other  
 273 hand, large charge storage capacity and maximized charge injection limit values are particularly desired  
 274 to establish safe electrical stimulation (Cogan 2008; Ghane-Motlagh and Sawan 2013).

### 275 3.2.1. CSCc and EIS measurements

276 The evaluation of the charge transfer capabilities of PEDOT-CNF composites was carried out in aCSF,  
 277 a physiologically relevant media, by sweeping a potential range between -0.85 V and 0.6 V, at a scan  
 278 rate of 200 mV/s, using cyclic voltammetry (CV). This technique provides insights regarding the  
 279 electrode interface under electrical load, the electrochemical conversion of species within the solution,  
 280 and the transient changes due to redox reactions at the electrode surface (Cogan 2008). The cathodal  
 281 CSC (CSCc) of the composite film was calculated as the time integral of the cathodal currents within  
 282 the cycled region. As indicated in Fig. 4a-b, the composite deposition on the gold microelectrode  
 283 resulted in a progressive increment in the average CSCc values, up to  $48 \pm 4$  mC/cm<sup>2</sup>, much higher  
 284 than that of the bare gold electrode ( $1 \pm 0.35$  mC/cm<sup>2</sup>). There is a linear relationship between the charge  
 285 delivered to the electrode during the deposition and charge storage capacity (Fig. 4b). This behavior is  
 286 likely due to the surface area increment that allows for the effective diffusion of electrolyte ions at the  
 287 electrode-solution interface.

288 The EIS magnitude and phase angle measurements of the composite electrodes were measured over a  
 289 range of frequencies from 10 Hz to 7 MHz (Fig. 4c-f). The phase angle measurements of the PEDOT-  
 290 CNF modified electrodes at lower frequency range (~10 Hz), revealed that the capacitive behavior was  
 291 predominant with an angle around 80° (Fig. 4f). The angle shift towards resistive charge transfer, for  
 292 a frequency range of 10 Hz to 10 kHz was proportional to the deposition charge density *i.e.* the higher  
 293 the effective surface area, the larger the phase angle shift (Kozai et al. 2016; Zhou et al. 2013). Fig. 4c  
 294 represents the bode plot displaying the impedance magnitude ( $|Z|$ ) vs. frequency. PEDOT-CNF  
 295 modified electrodes show an impedance range of 10-20 k $\Omega$ , which is at least 10 times less than that of  
 296 the bare gold electrode (200-400 k $\Omega$ ). Impedance values of the 5 deposition conditions were analyzed  
 297 using one-way ANOVA followed by post-hoc Turkey's test (n=5). ANOVA evidenced a significant  
 298 effect of deposition charge density on  $|Z|_{1\text{ kHz}}$  ( $P < 0.0001$ ), yet changes in  $|Z|_{1\text{ kHz}}$  were not proportional  
 299 to the charge density, where the values at  $|Z|_{1\text{ kHz}}$  being the most commonly used characteristic  
 300 frequency band for action potentials (Boehler et al. 2020). In comparison to the 1 nC/ $\mu\text{m}^2$  charge  
 301 density, significant lowering of  $|Z|_{1\text{ kHz}}$  was only obtained with charge densities of 4 nC/ $\mu\text{m}^2$  and 6

302  $\text{nC}/\mu\text{m}^2$  ( $P < 0.0001$  and  $P = 0.0006$  respectively, Tukey's test).  $|Z|_{1\text{ kHz}}$  obtained with charge densities  
 303 of 2 and 8  $\text{nC}/\mu\text{m}^2$  did not differ from that at 1  $\text{nC}/\mu\text{m}^2$  ( $P = 0.3$  and 0.06 respectively). The conditions  
 304 4  $\text{nC}/\mu\text{m}^2$  and 6  $\text{nC}/\mu\text{m}^2$  showed no statistically significant differences ( $P = 0.09$ ). However, the  
 305 optimal deposition condition seemed to be that at 6  $\text{nC}/\mu\text{m}^2$  as it displayed both a sharp impedance  
 306 decrement on average and the smallest variability across electrodes, with the  $|Z|_{1\text{ kHz}}$  value being  $13.4$   
 307  $\pm 2.2\text{ k}\Omega$  ( $16.8 \pm 2\text{ M}\Omega\cdot\mu\text{m}^2$ ).

308 To follow up further the investigation of the influence of the electrode surface area on the electrode  
 309 performance, Nyquist plots were made to monitor the evolution of charge transfer resistance (semi-  
 310 circle region) as a function of deposition charge on the electrode. As illustrated in Fig.4e the diameter  
 311 of the semi-circle region becomes smaller with the increase in deposition charge, where it is governed  
 312 by the electrode thickness, porosity and integrity of the Au-PEDOT-CNF composite interface (Gruet  
 313 et al. 2019; Huang et al. 2020). Among all the deposition conditions, 4  $\text{nC}/\mu\text{m}^2$  and 6  $\text{nC}/\mu\text{m}^2$  clearly  
 314 showed depressed semi-circle regions, thus reflecting the improved area and highly conductive surface  
 315 of the electrodes with lower impedance, possessing the capability of seamless bidirectional transfer of  
 316 charges/electrons.

317 In the 8  $\text{nC}/\mu\text{m}^2$  charge deposition case, even though the thickness and porosity of the electrode  
 318 improved the effective surface area, it also probably induced mechanical stress at the Au-PEDOT-CNF  
 319 interface, resulting in a slightly increased semi-circle region, therefore a charge transfer resistance and  
 320 an impedance with larger variance compared to the 6  $\text{nC}/\mu\text{m}^2$  condition. Overall, our results indicate  
 321 that a charge density of 6  $\text{nC}/\mu\text{m}^2$  optimized the deposition of PEDOT-CNF composite on the flexible  
 322 gold electrode surface resulting in a specific impedance value of  $16.8 \pm 2\text{ M}\Omega\cdot\mu\text{m}^2$  at 1 kHz. This value  
 323 is on par with the high-performance CNT fiber ( $20.5\text{ M}\Omega\cdot\mu\text{m}^2$ ) (Vitale et al. 2015) and graphene fiber  
 324 ( $9\text{-}28\text{ M}\Omega\cdot\mu\text{m}^2$ ) (Wang et al. 2019) microelectrodes, and 2 to 7 times lower, than most of the electrode  
 325 materials deposited on flexible metallic substrates reported in the literature (Fig. 5f). The corresponding  
 326 values and references are reported in Table S1.

### 327 3.2.2. Electrical stimulation

328 As shown in the Fig. 4a, CSCc measurements with respect to the deposition charge density were used  
 329 to identify the amount of charge available in the cathodic region of their respective CV sweep.  
 330 Although CV at slow scan rate provides information related to the electrochemical reactions that occur  
 331 at the electrode/electrolyte interface, it cannot reflect the amount of charge available during sub-



332 millisecond stimulation pulses. Charge balanced square wave current pulses are generally used in  
 333 electrical stimulation for electrophysiology experiments and therapies, with pulse widths ranging from  
 334 50 to 1000  $\mu\text{s}$ . A high CIL would be beneficial for these extremely fast charge-discharge processes by  
 335 preventing damages to the tissue-electrode region by reducing the incidence of irreversible Faradic  
 336 reactions (Boehler et al. 2020; Cogan 2008). To assess the charge stimulation capability of the PEDOT-  
 337 CNF composite, as a prerequisite, identifying the water electrolysis limits, especially the water  
 338 reduction limit in physiologically relevant solution is important. Fig. 5a shows that the water reduction  
 339 voltage of PEDOT-CNF modified gold electrodes is around -1.3 V. Next, the voltage excursions in  
 340 response to biphasic, cathodic first, current pulses were recorded with a 1000  $\mu\text{s}$  pulse width in aCSF  
 341 (Fig. 5b-d). Using a range of pulse current intensities, we defined the CIL as the amount of charge  
 342 injected which caused polarization ( $V_p$ ) of the electrode beyond its water hydrolysis window. From the  
 343 lowest to highest deposition charge density, the CIL values increased linearly up to the condition 6  
 344  $\text{nC}/\mu\text{m}^2$ , being the best one among all, with an average value of  $7.6 \pm 1.3 \text{ mC}/\text{cm}^2$  (Fig. 5e). This result  
 345 agreed with the corresponding impedance measurements where the 6  $\text{nC}/\mu\text{m}^2$  deposition condition  
 346 provided a low impedance value with lowest variability (Fig. 4d).

347 The optimized CIL value from this work was compared with other PEDOT based composites (Carli et  
 348 al. 2019; Kleber et al. 2019; Lee et al. 2019) and porous metals deposits (Lu et al. 2016; Nimbalkar et  
 349 al. 2018; Shin et al. 2016; Vitale et al. 2015; Wang et al. 2009) on flexible neural implants. Fig. 5f and  
 350 Table S1 shows that, among all reported electrode materials deposited on flexible metallic substrates,  
 351 the PEDOT-CNF coating displayed superior electrical properties and charge injection capabilities,  
 352 thanks to the chemical composition of the material and its physical morphology.

### 353 3.2.3. Long time performance

354 Along with the charge injection capability, it was also important to assess the biphasic charge cycling  
 355 endurance. For this purpose, the electrodes were subjected to a series of charge balanced current pulses  
 356 in physiologically relevant aCSF. Our electrodes were repeatedly pulsed over 0.5 million times using  
 357 the 200  $\mu\text{s}$  cathodic pulse width, followed by an immediate charge-compensating anodic pulse (most  
 358 commonly reported) (Boehler et al. 2020), consisting in a charge injection density of  $0.5 \text{ mC}/\text{cm}^2$   
 359 (current amplitude of 33  $\mu\text{A}$ ). Fig. 6a shows the evolution of the polarization potential ( $V_p$ ) during the  
 360 time of charge injection pulsing. A resultant  $V_p$  drift of 0.3 V, from -0.1 V to -0.4 V, was observed  
 361 after 0.5 million pulses, but remained far from the water window critical limit (-1.3 V) throughout the  
 362 stimulation. The corresponding  $|Z|_{1 \text{ kHz}}$  value was increased from an initial value of 18  $\text{k}\Omega$  to a-final

363 value of 38 k $\Omega$  after 0.5 million biphasic stimulation cycles, while still being far from the bare non-  
 364 coated Au electrode  $|Z|_{1\text{ kHz}}$  value (>200 k $\Omega$ ) (Fig. 6b). In addition, the SEM images of the composite  
 365 after the stimulation *vs* control, from the Fig. 6c, suggested that the electrodes exhibited no significant  
 366 physical delamination or degradation even after 0.5 million biphasic stimulation cycles confirming an  
 367 excellent structural control and longevity.

368 Accelerated thermal ageing tests were also carried out to evaluate the structural and electrical changes  
 369 of the PEDOT-CNF coating as a function of time which can affect an electrode in a permanent deep  
 370 brain implantation. Here, we maintained the material in PBS buffer for 23 days at 57°C, that is  
 371 considered to be equivalent to an ageing of 92 days at 37°C (Castagnola et al. 2015). Figure.7a shows  
 372 the Bode graph of the impedance before and after the ageing of the PEDOT-CNF microelectrodes on  
 373 the flexible parylene probe, with the corresponding evolution of the impedance  $|Z|_{1\text{ kHz}}$  responses *vs.*  
 374 time (Fig. 7c). After 23 days of ageing at 57°C, the PEDOT-CNF microelectrodes ( $|Z|_{1\text{ kHz}}=9.9 \pm 2.2$ )  
 375 is quite similar to the PEDOT- CNF microelectrode before ageing ( $|Z|_{1\text{ kHz}}=17.3 \pm 1.9$ ). From the  
 376 optical images, we observed that thermal ageing process does not impact the morphology of the  
 377 PEDOT-CNF deposition (Fig. 7b). This ageing tests allow us to conclude about the good mechanical  
 378 and electrical stability of the PEDOT- CNF material deposited on a flexible gold electrode surface.

379

### 380 3.3. Brain slice electrophysiological recording and stimulation

381 Electrophysiological experiments were conducted to assess the recording quality of PEDOT-CNF  
 382 electrodes and their usability as stimulating electrodes (Fig. 8a). Electrical stimulation and recordings  
 383 were performed in the hippocampus (CA1 and CA3 regions) of mouse brain slice maintained *in vitro*  
 384 (Buzsáki 2015). PEDOT-CNF modified electrodes allowed to record two types of spontaneous  
 385 neuronal activities: sharp wave-ripples (SWR) complexes and neuronal spiking activity.

386 SWR complexes constitute a mesoscopic signal that reflects synchronized activity in large population  
 387 of neurons (Buzsáki 2015). SWR complexes were recorded with minimal filtering (0.1 Hz- 3 KHz)  
 388 where two electrodes of the flexible four electrode probe were located in the cell layer of CA1 and  
 389 other two in the dendrite layer (stratum radiatum/lacunosum). As shown in the Fig. 8b, the slow  
 390 component of the SWR complex, “the sharp wave” proper, shows an inversion in polarity between the  
 391 two regions: positive in the cell layer (electrodes 3 and 2) and negative in the dendrite layer (electrodes  
 392 1 and 4), as expected given the fact that the excitatory synaptic inputs, which generate the sharp wave

393 are located on the dendrite of hippocampal pyramidal cells. The ripples correspond to the oscillatory  
394 pattern (200-250 Hz) carried by the sharp wave. The amplitude of ripples was larger in the cell body  
395 layer (electrode 3 and 2), as expected since they mostly correspond to the population spikes patterned  
396 by the local inhibitory neurons.

397 The other type of spontaneous activity that was recorded in the hippocampus corresponds to action  
398 potentials APs (spikes) generated by neurons (Buzsáki 2015; Obien et al. 2015). They were visualized  
399 between sharp wave-ripple complexes while using bandpass filtering (300 - 3000 Hz). The examples  
400 presented in Fig. 8c, were obtained with the same PEDOT-CNF modified electrodes and at the same  
401 electrode locations as in Fig. 8b. The APs are recognized as fast and mostly negative deflections of  
402 varying amplitude. To proceed further we examined whether single-unit activity, i.e., APs that can be  
403 attributed to one single neuron, could be extracted from the raw traces. First portions of trace around  
404 events were extracted using a threshold at 3xRMS of the voltage trace (dashed lines in Fig. 8c), and  
405 thereafter analyzed by PCA and clustering (not illustrated). This analysis allowed identifying constant  
406 spike shapes, which were further ascribed to single-unit activities if the inter-spike interval distribution  
407 (not illustrated) showed a clear refractory period, i.e., no interval <1 ms. In contrast, multiunit activities  
408 (black spikes in Fig. 8c) do not have a refractory period because they are produced by several  
409 independent neurons. As shown in Fig. 8c, two and three single-units (color coded) have been isolated  
410 from electrodes 3 and 2, respectively and only one single-unit was isolated with electrode 1. This is  
411 likely due to the fact that only few neuronal cell bodies, from which action potentials are generated,  
412 are to be found in the dendrite layers of the hippocampus. In summary, the resulting signal-to-noise  
413 ratio of the PEDOT-CNF electrodes facilitated the isolation of single-unit activity from neighboring  
414 neurons.

415 For comparison, the signal obtained with the non-coated bare gold electrodes is mainly composed of  
416 noise, with an amplitude comparable to that usually obtained before inserting the probe in the brain  
417 (Fig. S2). This is a consequence of the small diameter of the bare electrode active area (40  $\mu\text{m}$ ), which  
418 resulted in a high impedance. For neuronal recording, the electrode impedance contributes to the noise,  
419 and high impedance electrodes degrades the signal to noise ratio (SNR) through a combined effect of  
420 higher noise levels. On the opposite, lowering the impedance of the same electrodes with PEDOT-CNF  
421 coatings (Fig. 4c) enabled the recording of very adequate electrophysiological signals with a good  
422 SNR. From the results, the SNR ( $\pm$  SD) was calculated as the amplitude of the negative component of

423 the action potential of the isolated single unit divided by the RMS. The results show that the SNR is  
424 high enough ( $5.3 \pm 1.2$ ) ( $n = 6$ ) for proper action potentials APs (spikes) recordings.

425 In addition, the PEDOT-CNF electrodes were validated for their usability as stimulating electrodes.  
426 Electrical stimulation was applied through a PEDOT-CNF electrode positioned in the dendritic layer  
427 (stratum radiatum) to activate axons of the Schaffer collateral (the projection issued from CA3  
428 pyramidal cells and synapsing on the dendrites of CA1 pyramidal cells) (Fig. 8d-e). The extracellular  
429 recording of the local field potential was made in the cell body layer of CA1 with a conventional  
430 metallic electrode. Electrical stimulus were given in pairs (20 ms interpulse interval) and, consisted in  
431 cathodic square current pulses (200  $\mu$ s) delivered at different intensities. Pulse pairs were repeated at  
432 a frequency of 0.5 Hz to examine short-term plasticity of the synaptic responses. For suprathreshold  
433 intensities, the potential was positive due to recording in the cell layer (current sink in the dendrites),  
434 and the amplitude of the response to the second pulse of the pair was larger than that of the first one,  
435 reflecting short-term facilitation of the synaptic response (Fig. 8e). The amplitude of the potential  
436 increased in proportion to the stimulation intensity, with a saturation at around 80  $\mu$ A (Fig. 8c). This  
437 saturation suggests that all the axons implicated in the response were recruited with an intensity  $\leq 80$   
438  $\mu$ A. Thus, PEDOT-CNF microelectrodes could be used as efficient stimulating electrodes, to obtain  
439 typical evoked potentials with charge densities that remained below the water reduction limit.

440

#### 441 **4. CONCLUSION**

442 We have developed a well-controlled and versatile surface modification method for preparing  
443 macroporous PEDOT-CNFs microelectrodes on flexible implantable neural probes. We investigated  
444 by FIB and EDX the mechanism by which a macroporous nanostructure of PEDOT-CNF layer was  
445 created, where the conducting PEDOT polymer was covered uniformly and tightly around the oxidized  
446 carbon nanofibers as a solid doping template. We found that the combination of carbon nanofibers and  
447 PEDOT as a single carbonaceous composite resulted in a strong synergetic effect leading to a lower  
448 impedance, superior charge storage capacity and charge injection limit compared to bare metal and  
449 other reported organic coated flexible electrodes in electrochemical characterization. We further  
450 showed that the carbon nanofibers function as reinforcing elements within the composite material and  
451 prevent PEDOT film from undergoing delamination or cracking during long lasting electrical pulsing  
452 experiments. *In vitro* experiments on mouse brain slices showed that the PEDOT-CNFs  
453 microelectrodes can record spontaneous oscillatory field potentials as well as single-unit action

454 potentials with good signal-to-noise ratio, and allow to safely deliver electrical stimulation for evoking  
455 field potentials. These results show that these electrodes are well suited for high-performance recording  
456 and/or stimulation for applications in brain therapies.

457 Despite the potential of the PEDOT-CNFs composites that can bring, the non-toxicity properties of  
458 these nanofibrous materials in creating neural interfaces, need to be fully studied. Our preliminary in  
459 vitro cell viability experiments (MTT cell viability assay ISO 10993-5 norm) on PEDOT-CNFs coated  
460 microelectrodes showed that the nanocomposite material does not advocate any cytotoxicity, neither  
461 detachment of the CNFs from the bare electrodes (Saunier et al. 2020b). The results showed that the  
462 population of living cells increased over time in a similar fashion for controls, demonstrating that no  
463 cytotoxicity could be observed “in vitro” as viability percentage highly exceeds 75%. However, precise  
464 toxicity effects of these nanofibers when implanted in the brain need further investigation. Importantly,  
465 the risk that may arise from detaching CNF during the insertion procedure should be considered. The  
466 physical dimension characteristics is a key factor promoting the risk of toxicity to biological systems  
467 and should not be ignored. Recent studies on the effect of various carbon nanofibers and nanotubes on  
468 human epithelial cells, showed association between the physical dimensions and genotoxicity,  
469 especially those with greater lengths and larger diameters (Fraser et al. 2020). In our future works, we  
470 envision to examine the effects of the PEDTOT-CNFs through immunohistochemistry in animal  
471 models, followed by comparison of the results against existing non-coated electrodes.

472

#### 473 **4 Conflict of Interest**

474 The authors declare that the research was conducted in the absence of any commercial or financial  
475 relationships that could be construed as a potential conflict of interest.

#### 476 **5 Author Contributions**

477 Venkata Suresh Vajrala: Methodology, Investigation, Formal analysis, Visualization, Validation,  
478 Writing- original draft & editing. Valentin Saunier: Investigation, Methodology, Writing - review &  
479 editing. Lionel Nowak: Conceptualization, Investigation, Formal analysis, Writing- review & editing.  
480 Emmanuel Flahaut: Conceptualization, Writing - review & editing. Christian Bergaud: Supervision,  
481 Resources, Conceptualization, Writing- review & editing. Ali Maziz: Conceptualization, Supervision,  
482 Project administration, Resources, Methodology, Formal analysis, Writing- original draft & editing.

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## 488 **7 Supplementary Material**

489 The Supplementary Material for this article can be found online at:

490

491

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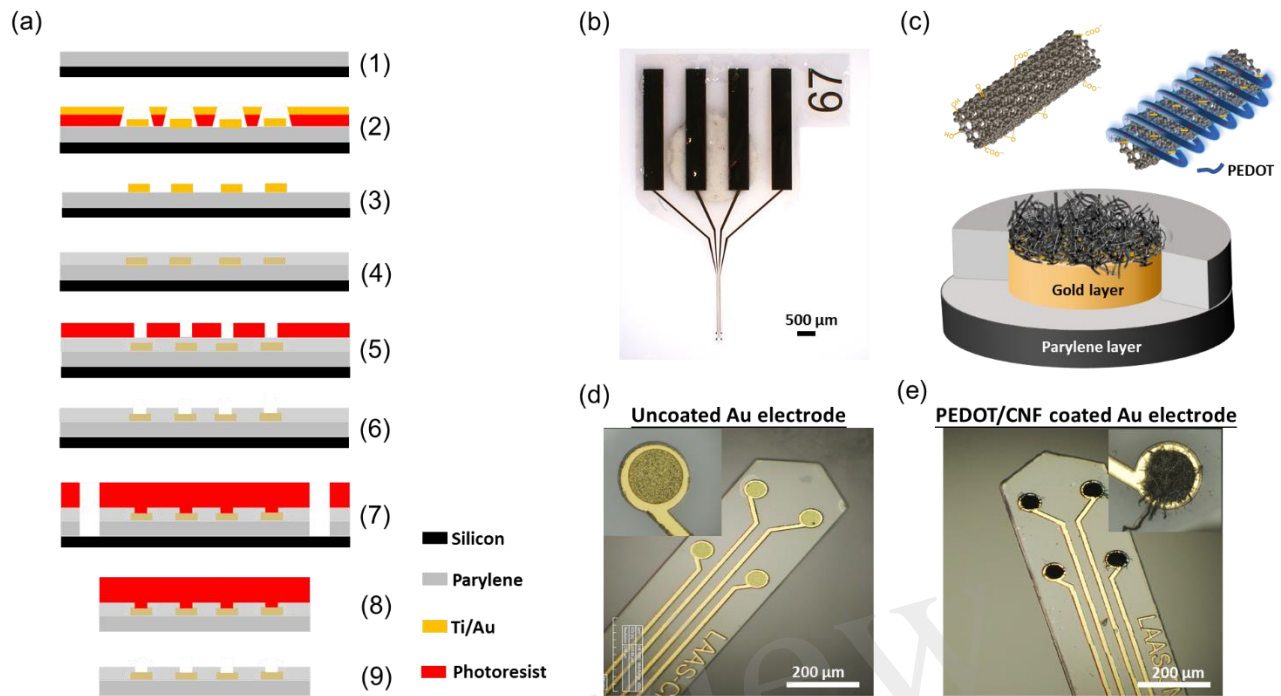
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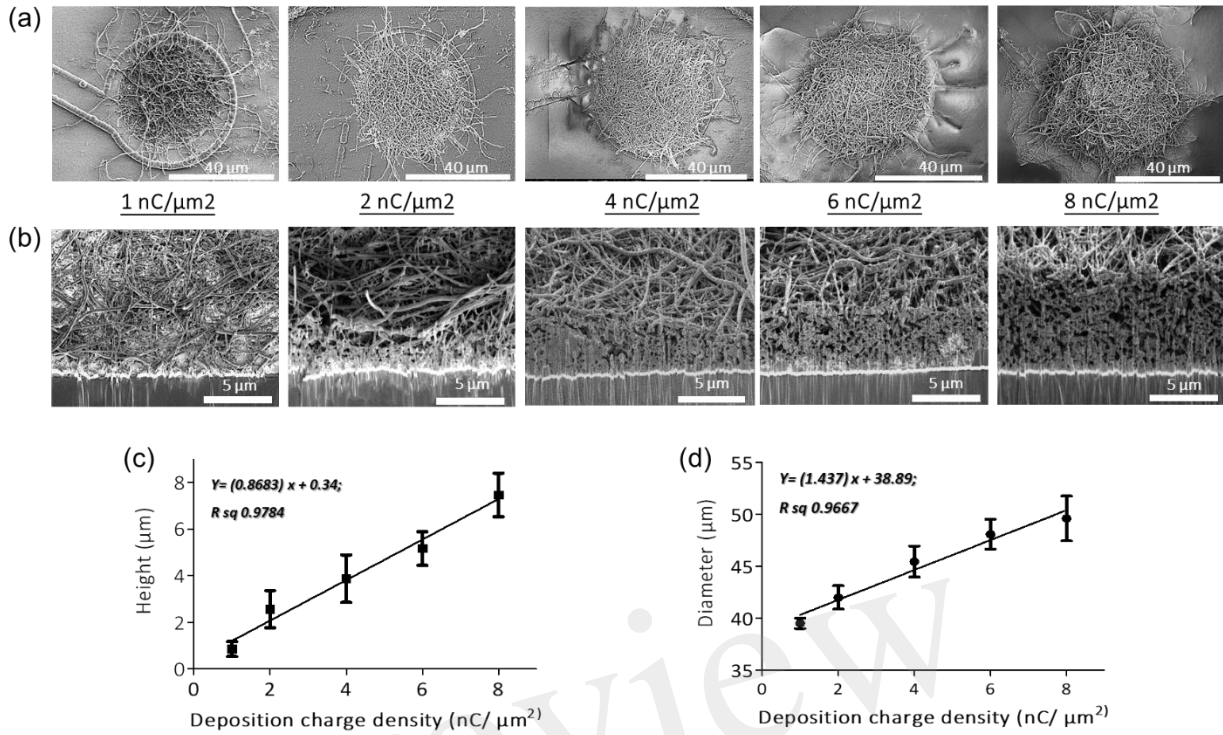
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682 **Figure 1:** Schematic illustration and optical images of neural implant. (a) Schematic overview of steps  
 683 involved in the microfabrication procedure- (1) 23 μm thick Parylene C deposition on SiP wafer; (2)  
 684 Photo-patterning of nLof and gold layer deposition (Ti/Au-50/200 nm); (3) nLof removal; (4) 2<sup>nd</sup>  
 685 Parylene C layer (1.3 μm) deposition and anneal at 110°C for 16h; (5) Spin coating of 5μm thick  
 686 AZ4562 photoresist; (6) RIE with ICP-RIE oxygen plasma; (7) Photo-patterning with BPN photoresist  
 687 and RIE with ICP-DRIE oxygen plasma; (8) Stripping of implants from SiP wafer and (9) Development  
 688 with NF52 and cleaning with DI water. (b) Optical micrograph of the neural implant. (c) Schematic  
 689 representation of the PEDOT-CNF composite deposition. (d) and (e) Optical micrographs showing the  
 690 neural microelectrodes before and after surface modification with PEDOT-CNF composite.

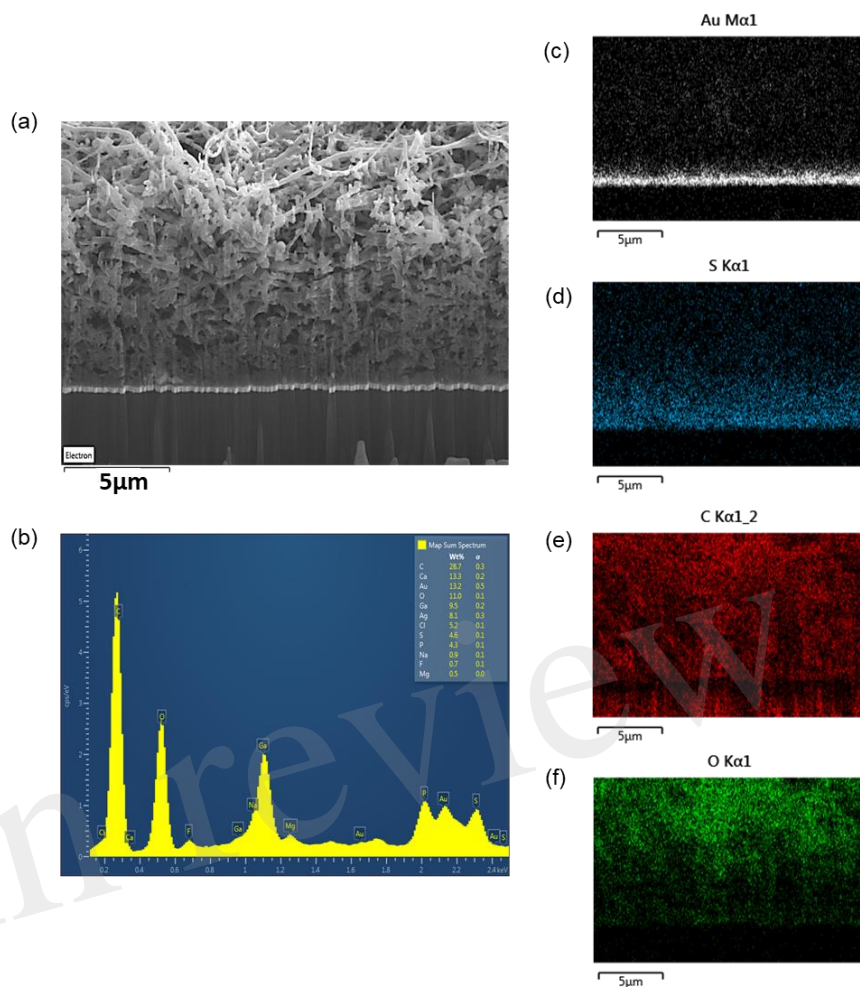
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693 **Figure 2:** SEM micrographs of (a) top views and (b) cross-sectional views of PEDOT-CNF composites  
 694 on flexible gold electrodes with different surface charge densities ranging from 1 to 8 nC/μm<sup>2</sup>. PEDOT-  
 695 CNF films were galvanostatically deposited on gold microelectrodes with a constant current density of  
 696 10 pA/μm<sup>2</sup>, using two-electrode configuration. Plots describing the evolution of deposition height (c)  
 697 and diameter (d) of the composite versus deposition charge density. The error bars represent the  
 698 standard deviation where n=6.

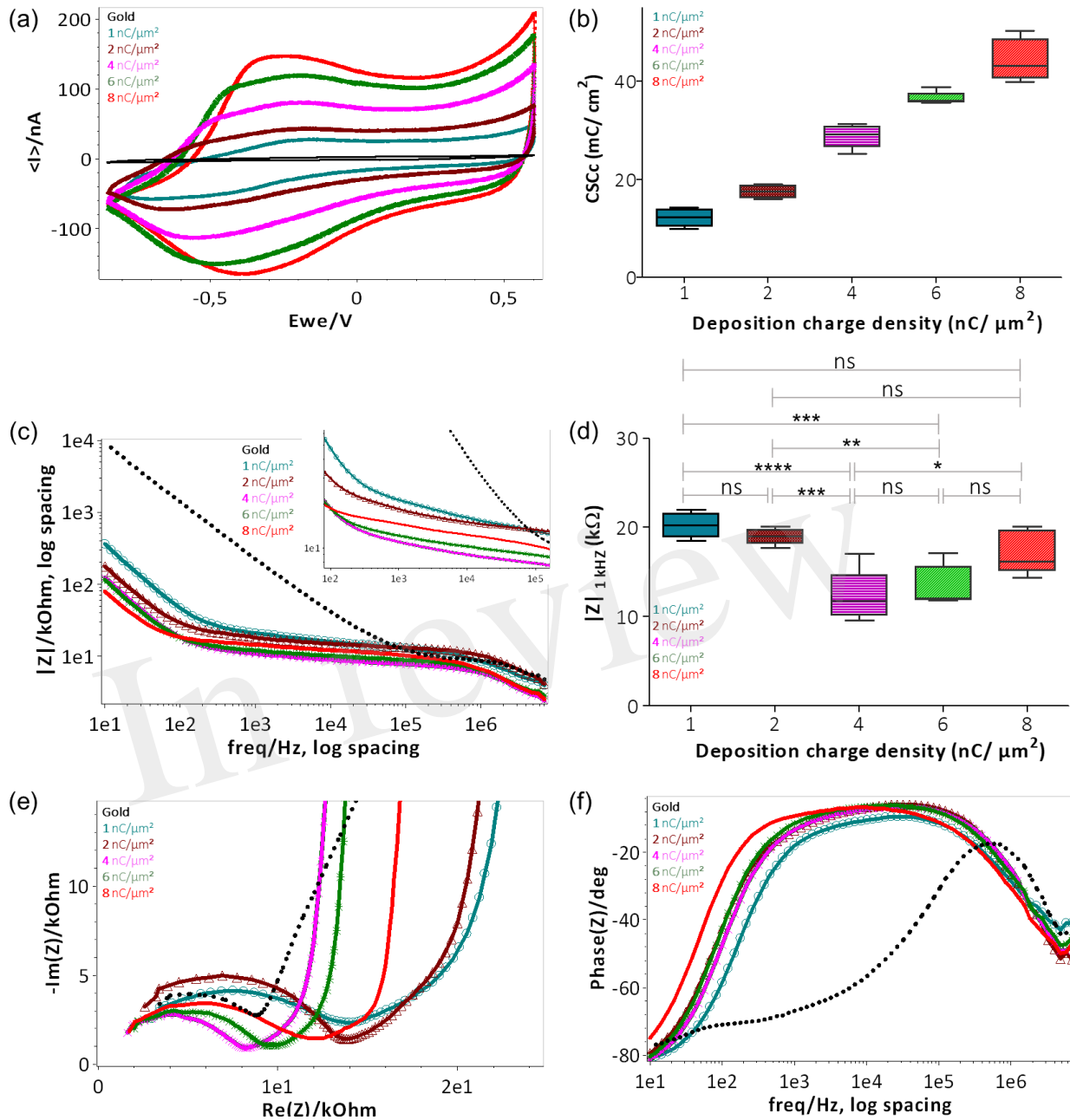
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701 **Figure 3:** (a) SEM image and (b) EDX spectrum of porous PEDOT-CNF composite ( $8 \text{ nC}/\mu\text{m}^2$ )  
 702 deposited on flexible gold electrodes. Elemental mapping images confirm the presence of (c) gold, (d)  
 703 sulfur, (e) carbon and (f) oxygen in the PEDOT-CNF composite.

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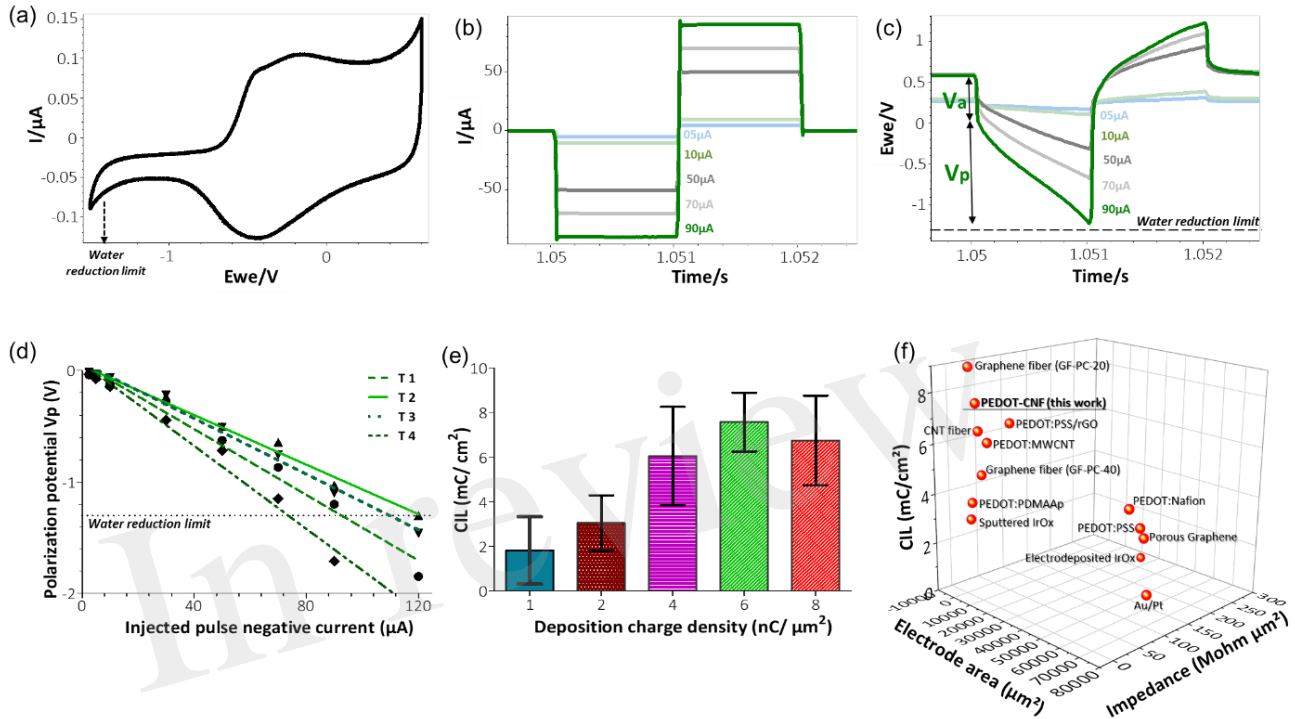


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706 **Figure 4:** Electrochemical characterization of PEDOT-CNF composite deposited on flexible gold  
 707 surface with different surface charge densities: (a) CSCc measurements by CV in aCSF at 200 mV/s  
 708 vs Ag/AgCl ref electrode; (b) Plot representing the evolution of CSCc with respect to the deposition  
 709 charge density; (c) Bode plot representing the  $|Z|$  vs frequency over a frequency range of 10 Hz to 7  
 710 kHz in aCSF at 0V vs Ag/AgCl ref electrode; (d) Plot of impedance  $|Z|_{1 \text{ kHz}}$  responses vs deposition  
 711 charge density. The statistical differences between deposition conditions were assessed by ANOVA  
 712 followed by Tukey's posthoc test, where \*\*\*\*, \*\*\*, \*\* and ns represent  $P < 0.0001$ ,  $P < 0.001$ ,  $P < 0.01$   
 713 and no significant difference, respectively ( $n=5$ ); (e) Nyquist plots and (f) phase angle measurements

714 obtained by EIS. Deposition charge density conditions were represented in different colors, along with  
 715 the plain gold electrode (black color) in the above all graphs. (For interpretation of the references to  
 716 colors in this figure legend, the reader is referred to the web version of this article).

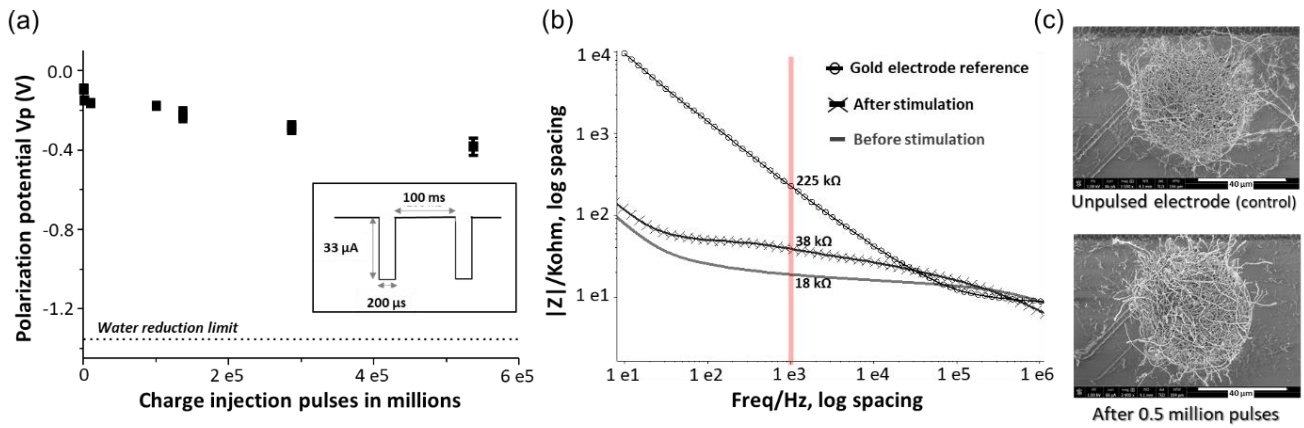
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718 **Figure 5:** *In vitro* biphasic stimulation assessment: (a) Determination of the water reduction potential  
 719 by CV of PEDOT-CNF composite in aCSF at 200 mV/s Vs Ag/AgCl. Biphasic charge-balanced  
 720 current pulses (b) and voltage responses (c) at different charge injections ranging from 5  $\mu\text{A}$  to 90  $\mu\text{A}$ ;  
 721 (d) Polarization potentials ( $V_p$ ) measured under different current pulse amplitudes. The deposition  
 722 condition of 6  $\text{nC}/\mu\text{m}^2$  was considered here and the data corresponding to 4 trials are represented; (e)  
 723 Evolution of the charge injection limit (CIL) values with respect to the deposition charge density. (f)  
 724 Comparison of the Impedance, CIL performances and geometric surface area of PEDOT-CNF  
 725 composite deposited on the flexible gold electrode surface with other flexible neural electrode arrays.  
 726 The corresponding values and references are reported in Table S1. (For interpretation of the references  
 727 to colors in this figure legend, the reader is referred to the web version of this article).

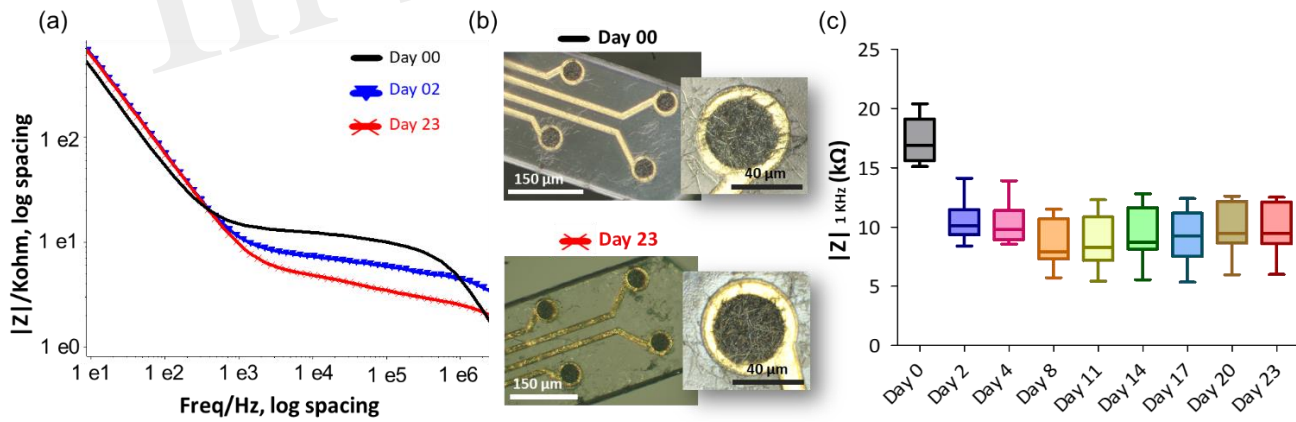
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730 **Figure. 6:** Long term stability assessment of PEDOT-CNF composite: (a) Evolution of polarization  
 731 potential over 0.5 million charge injection pulses at 0.5 mC/cm<sup>2</sup> in aCSF. Inset- Schematic  
 732 representation of the biphasic charge-balanced current waveform having an amplitude of 33  $\mu\text{A}$  and  
 733 200  $\mu\text{s}$  pulse duration at 10 Hz. (b) EIS monitoring of PEDOT-CNF composite before and after 0.5  
 734 million stimulation pulses. (c) SEM observation of the composite after the stimulation and comparison  
 735 with the unpulsed electrode (control).

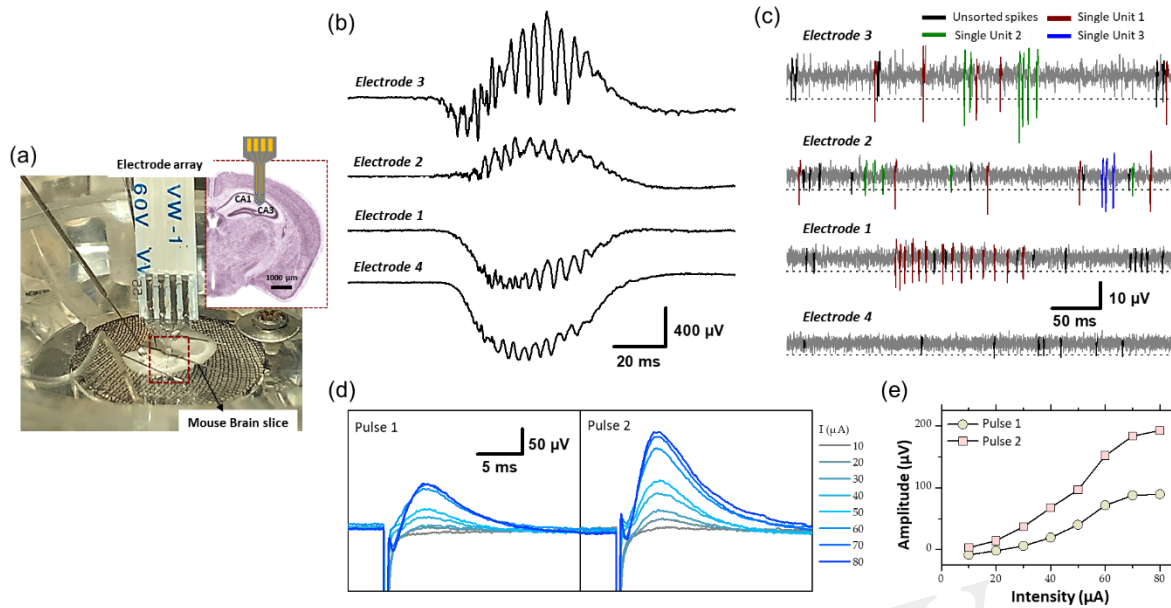


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737 **Figure. 7:** The effect of accelerated thermal aging on the PEDOT-CNF composite was examined in  
 738 PBS buffer at 57  $^{\circ}\text{C}$  for 23 days. (a) Impedance magnitude of PEDOT-CNF composite at Day 0 (black),  
 739 Day 2 (blue) and Day 23 (red). Platinum wire was used as counter and reference electrodes. (b) Hirox  
 740 microscope images of flexible probe (black) before immersion and (red) after 23 days of soaking in  
 741 PBS at 57 $^{\circ}\text{C}$ . (c) The evolution of electrode impedance at 1 KHz represented in a standard box-and-  
 742 whisker plot where the sample number n=8.

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744

745 **Figure. 8:** (a) Experimental set-up of the electrophysiological recording and stimulation, in the  
 746 hippocampal region (CA1 and CA3) of a mouse brain slice, using flexible microelectrode array  
 747 modified with PEDOT-CNF composite. Recording of spontaneous (b) sharp wave-ripples (filter: 0.1  
 748 Hz – 3 kHz) and (c) firing of action potentials from neurons (filter: 300 – 3000 Hz). Electrodes 3 and  
 749 2 were located in the pyramidal cell layer of CA1, and electrode 1 and 4 were in the dendrite layers. A  
 750 threshold of  $-3 \times \text{RMS}$  (dashed line in Fig. 8c) was used for spike sorting. Single-units were identified  
 751 using PCA and cluster analysis. Single-units are differentiated by color codes. Spikes in black  
 752 correspond to unsorted (multiunit) activity. The traces in (b) and (c) were not obtained simultaneously  
 753 but sequentially. (d) Evoked field potential recorded in the pyramidal cell layer after electrical  
 754 stimulation of the Schaffer collateral. Pairs of cathodic square current pulses (200  $\mu\text{s}$  width), with an  
 755 interpulse interval of 20 ms, were applied at 0.5 Hz. Responses evoked by the first and second stimulus  
 756 of the pairs are shown separately (pulse 1 and pulse 2). The different traces correspond to the response  
 757 obtained at different stimulation intensities (10-80  $\mu\text{A}$ ). Each trace is the average of 10-20 responses  
 758 obtained at a given intensity. (e) Field potential amplitude represented as a function of stimulation  
 759 intensity. (For interpretation of the references to colours in this figure legend, the reader is referred to  
 760 the Web version of this article).

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Figure 1.TIF

In review

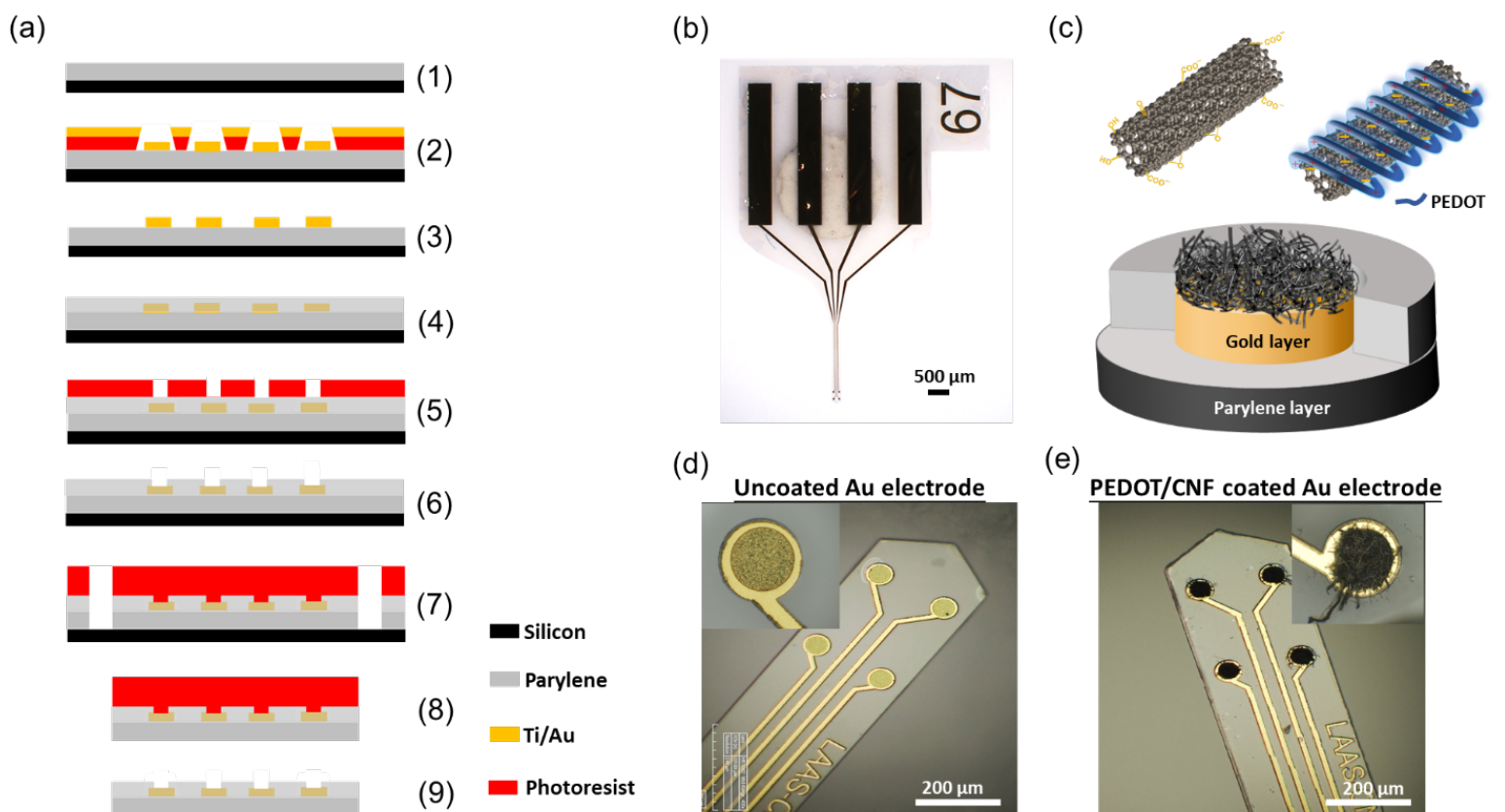


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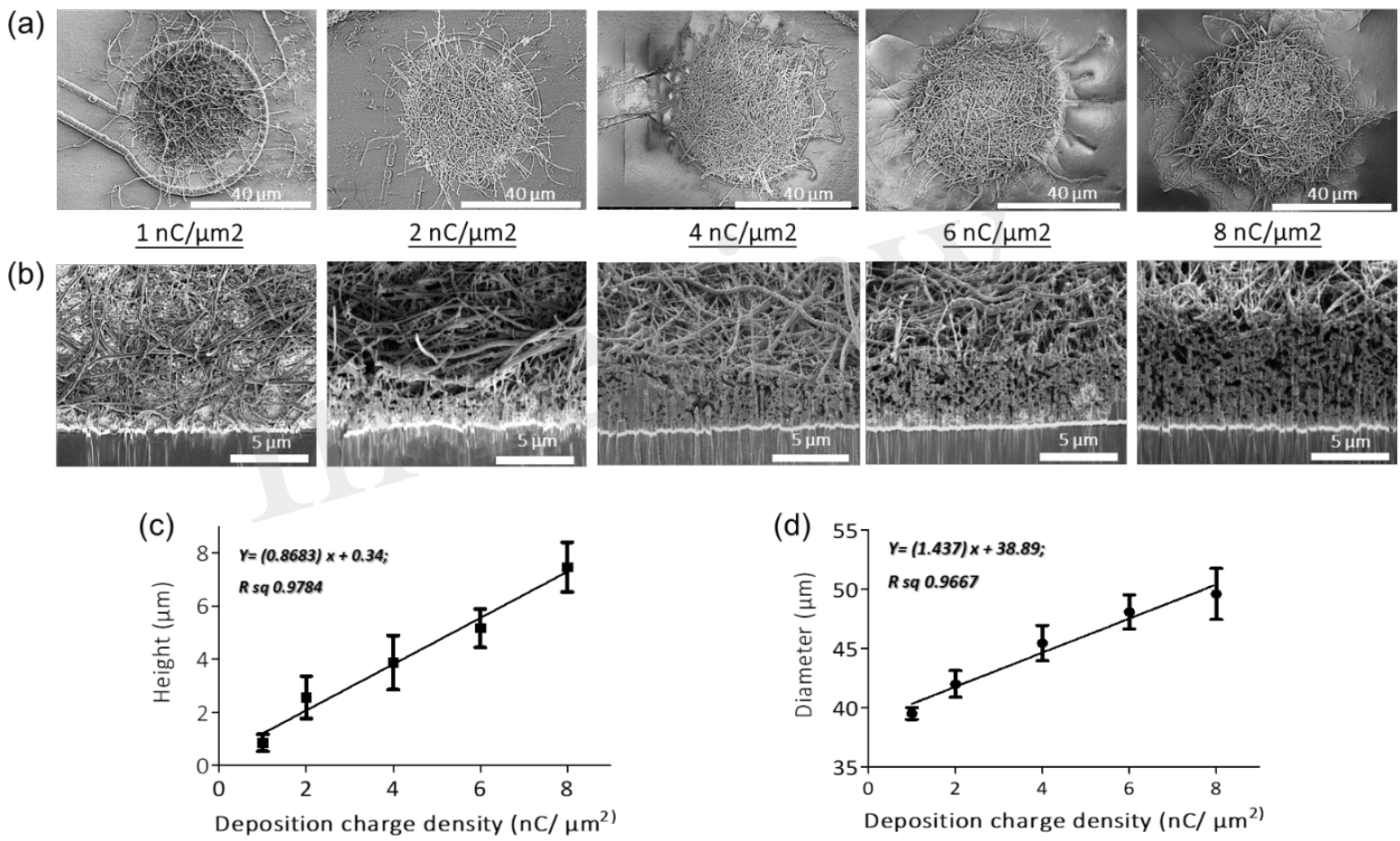
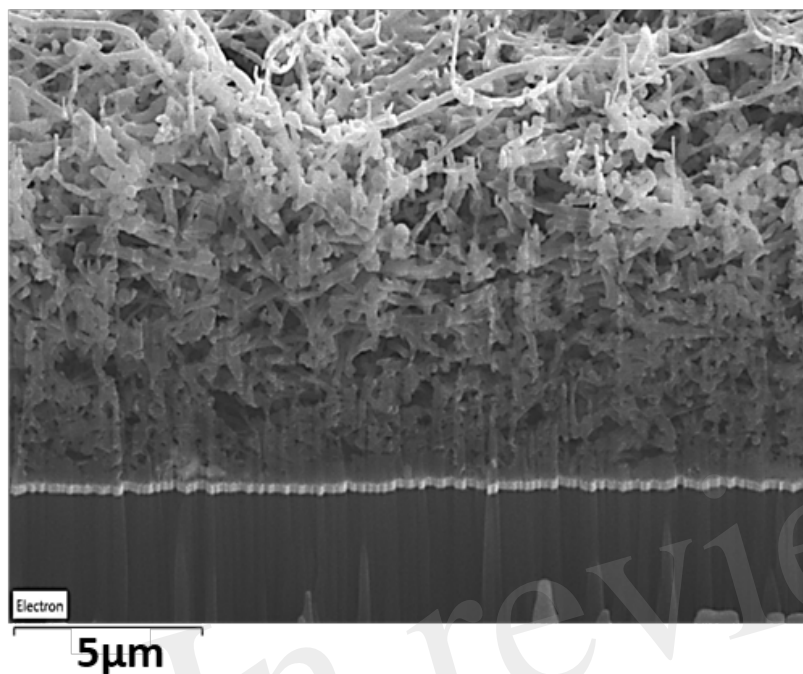
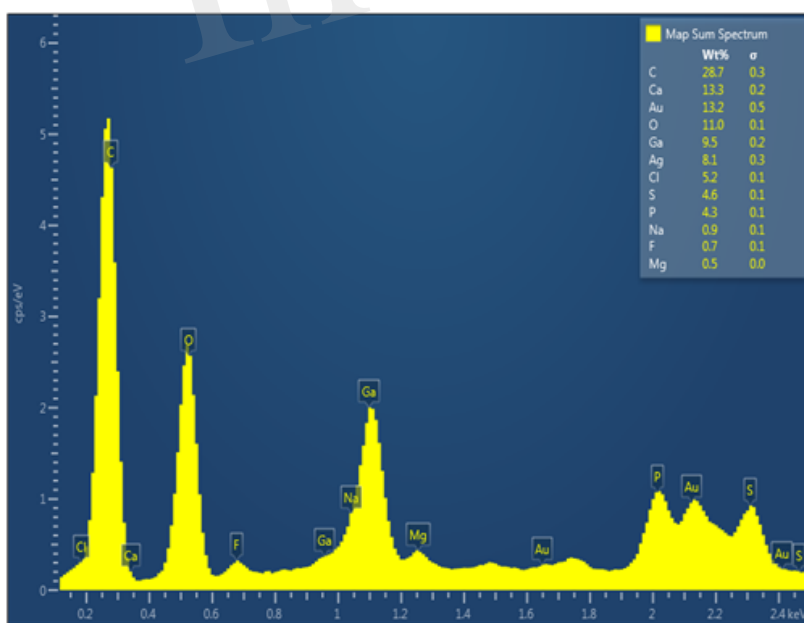


Figure 3.TIF

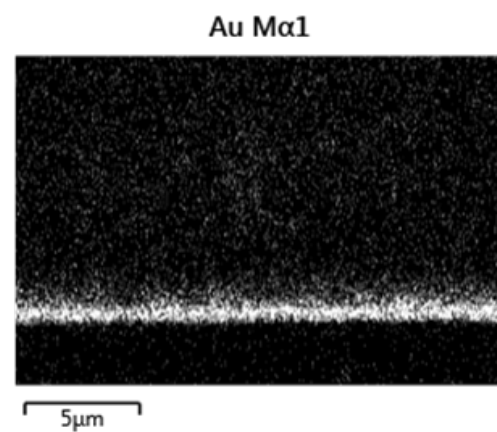
(a)



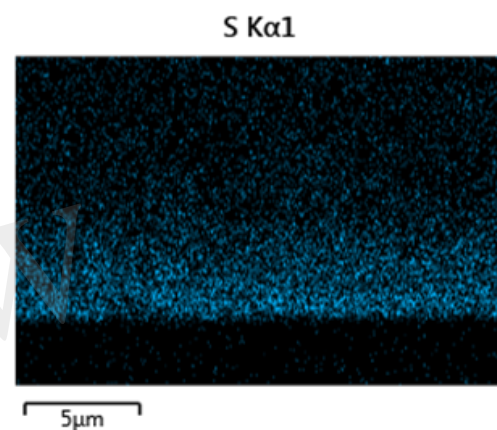
(b)



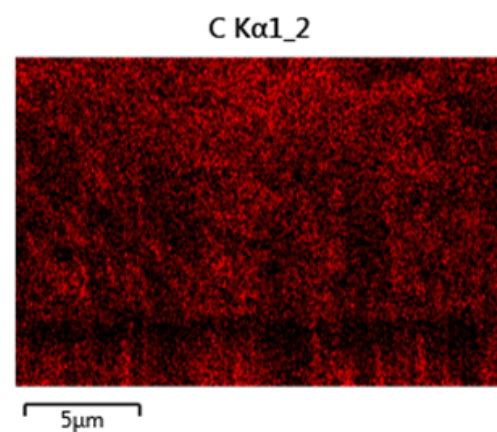
(c)



(d)



(e)



(f)

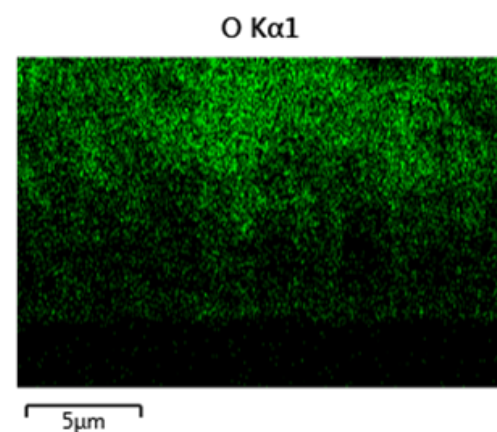




Figure 4.TIF

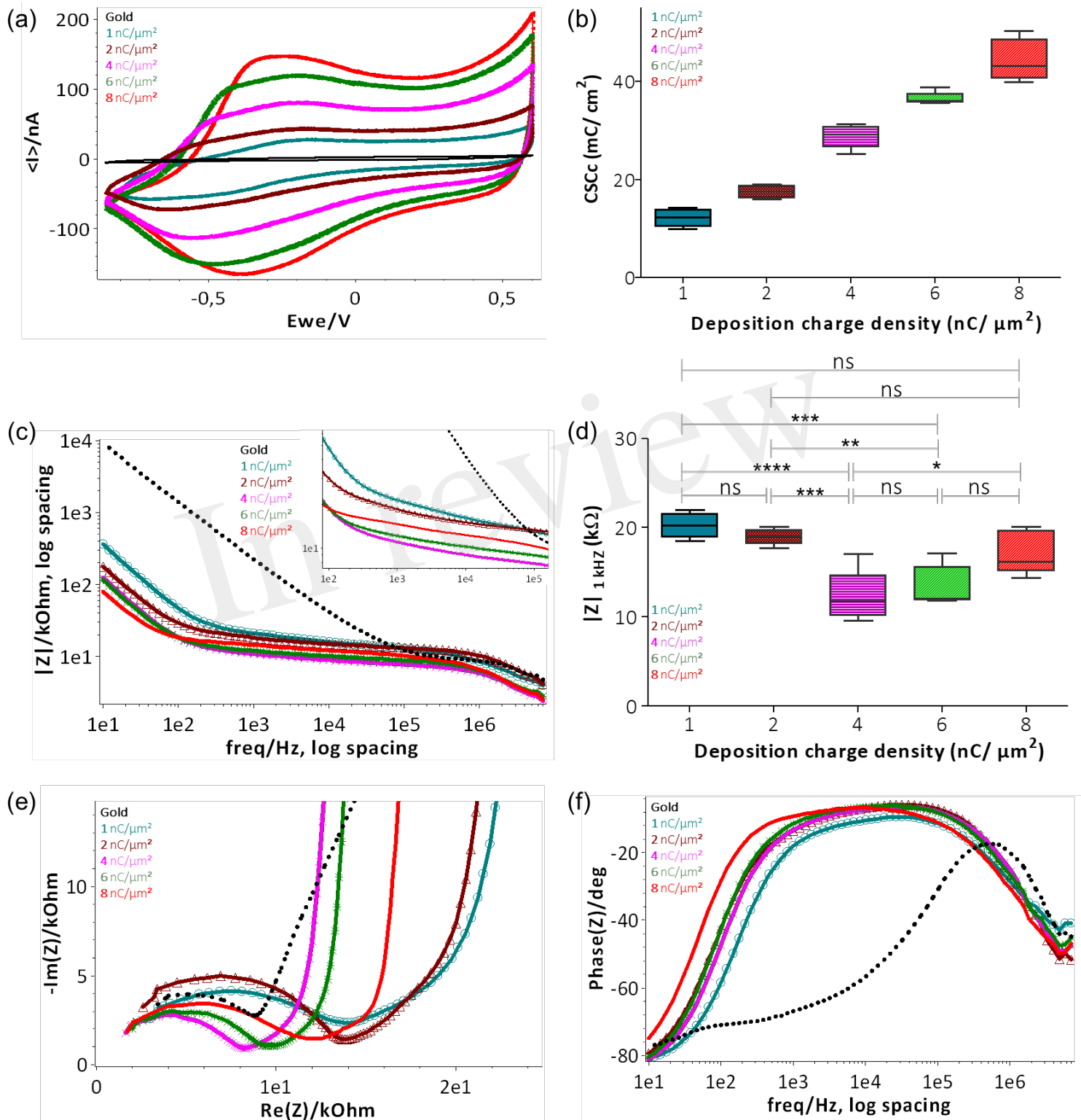


Figure 5.TIF

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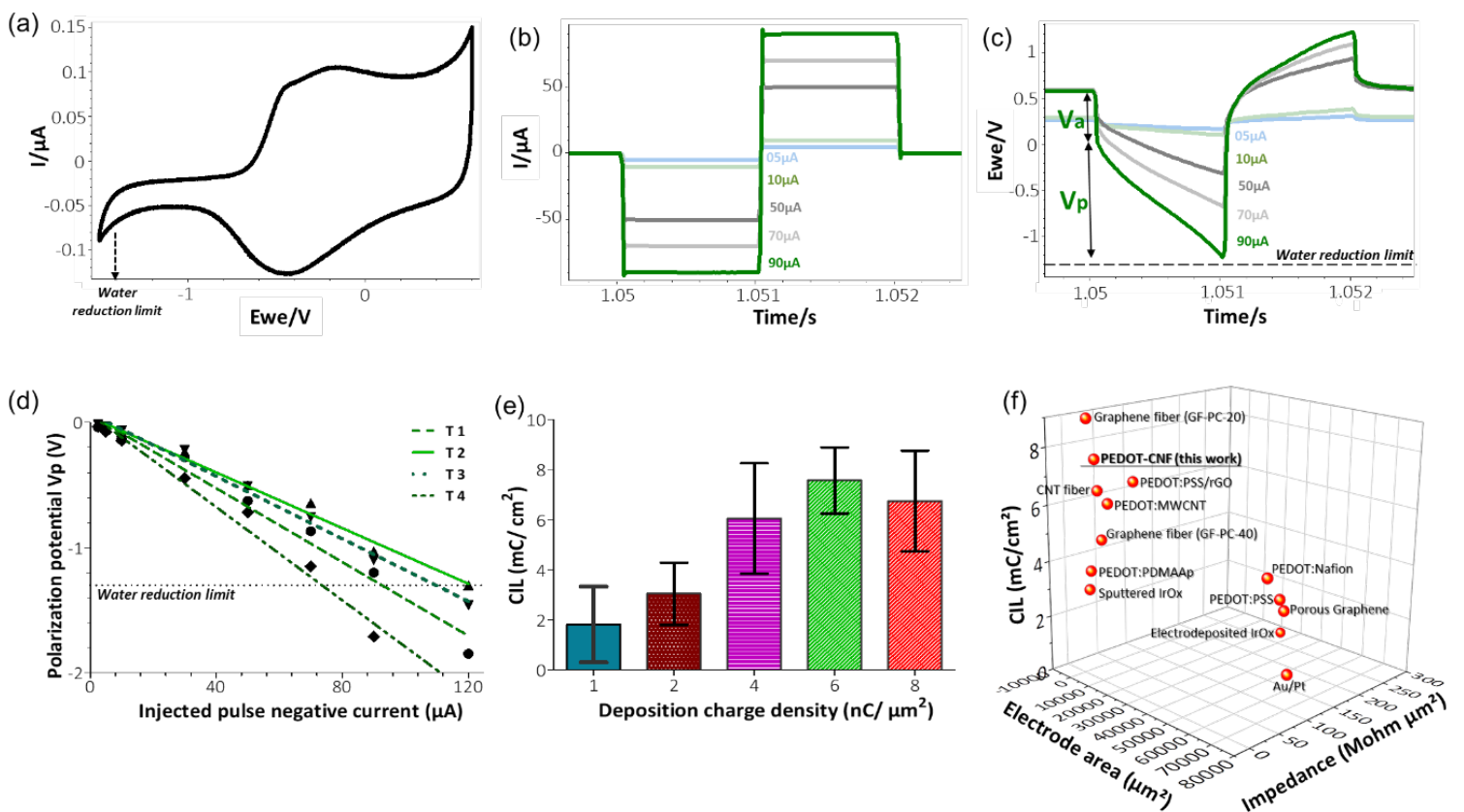


Figure 6.TIF

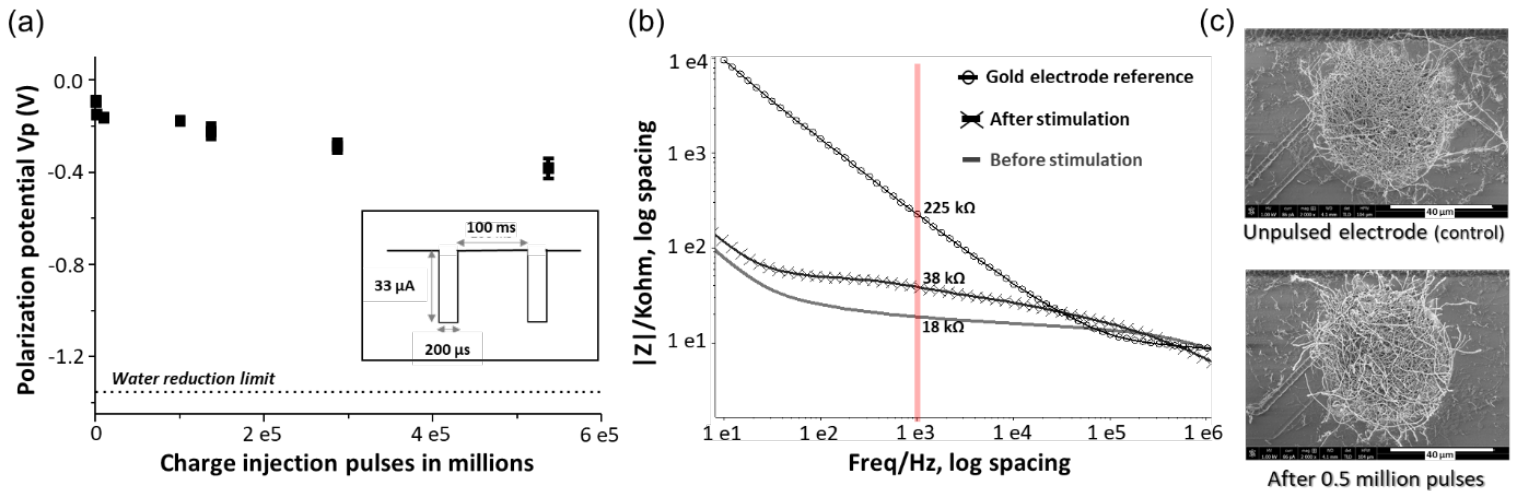


Figure 7.TIF

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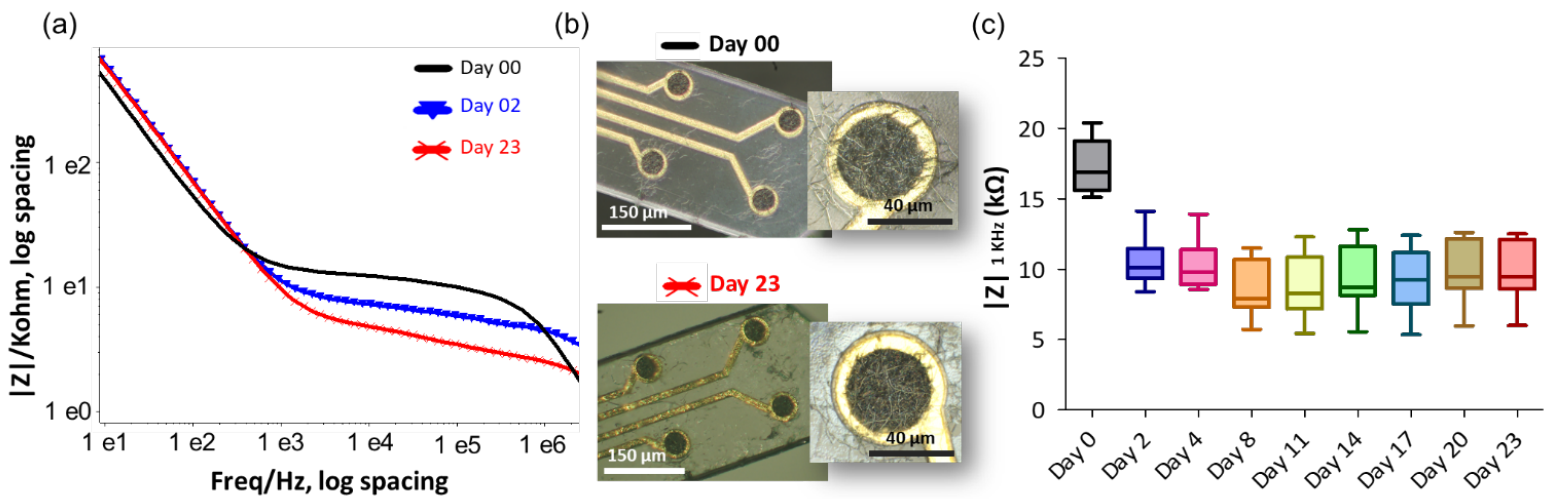




Figure 8.TIF

In review

