All-diamond functional surface micro-electrode arrays for brain-slice neural analysis
Peer-reviewed author version


DOI: 10.1002/pssa.201532347
Handle: http://hdl.handle.net/1942/24158
Diamond-based microelectrode arrays were fabricated by using nano crystalline diamond as an insulating layer and conductive boron-doped in order to used them for analysis of brain cortical slices. MEA surface is solely composed of diamond, exposed to the cells. The impedance measurements showed negligible cross-talk between neighbouring diamond microelectrodes. Local field potentials related to neural signals were then successfully recorded from pharmacologically disinhibited rat cortical tissue slices, mechanically coupled on the surface of the MEA. The noise level of the diamond MEAs was found to be lower than commercial Pt-based MEAs, under identical measurement conditions.
<table>
<thead>
<tr>
<th>revision of your manuscript, there is an opportunity for you to provide your responses to the reviewers later; please do not add them to the cover letter.</th>
<th>We would like to thank the referee for their valuable comments and input.</th>
</tr>
</thead>
<tbody>
<tr>
<td>With the best regards, Farnoosh Vahidpour</td>
<td></td>
</tr>
</tbody>
</table>
All-diamond functional surface micro-electrode arrays for brain-slice neural analysis

Farnoosh Vahidpour1, Lowry Curley2, István Bíró2, Matthew McDonald1,2, Dieter Croux1, Paulius Pobedinskàs1, Ken Haenen1,3, Michele Giugliano2-5, Zuzana Vlčková Živcová6, Ladislav Kavan6, Milos Nesládek1,3

1Institute for Materials Research (IMO), Hasselt University, Belgium
2Theoretical Neurobiology & Neuroengineering Laboratory, Dept. Biomedical Sciences, Universiteit Antwerpen, Belgium
3IMOMEC, IMEC vzw, Belgium
4Brain Mind Institute, Swiss Federal Institute of Technology of Lausanne, Switzerland
5Department of Computer Science, University of Sheffield, United Kingdom
6Department of Electrochemical Materials, J. Heyrovsky Institute of Physical Chemistry of the AS CR, v.v.i., Czech Republic

Received ZZZ, revised ZZZ, accepted ZZZ.
Published online ZZZ. (Dates will be provided by the publisher.)

Keywords Microelectrode arrays, Surface termination, Impedance spectroscopy

Corresponding author: e-mail: farnoosh.vahidpour@uhasselt.be

Diamond-based microelectrode arrays were fabricated by using nano crystalline diamond as an insulating layer and conductive boron-doped in order to used them for analysis of brain cortical slices. MEA surface is solely composed of diamond, exposed to the cells. The impedance measurements showed negligible cross-talk between neighbouring diamond microelectrodes. Local field potentials related to neural signals were then successfully recorded from pharmacologically disinhibited rat cortical tissue slices, mechanically coupled on the surface of the MEA. The noise level of the diamond MEAs was found to be lower than commercial Pt-based MEAs, under identical measurement conditions.

1 Introduction For over a decade, academic and industrial laboratories have worked on improving the technology for electrophysiological interfacing of neural tissue to substrate-integrated microelectrode arrays (MEAs). MEAs essentially serve as neural interfaces that connect the neurons to the external measurement circuit to detect electrical potential associated with the function of excitable cells. MEAs are nowadays employed both in vivo and in vitro, used, for example, for brain research or various neuropharmacological applications1. For each of specific applications, the geometry, shape, and the material of the MEAs play critical roles. To date, MEAs are fabricated by employing a variety of conducting materials such as titanium nitride (TiN), iridium oxide (IrO), platinum (Pt), gold (Au), indium tin oxide (ITO), silicon (Si) and titanium (Ti)2-8 as the electrode material. However, the electrode material stability in biologic environment is an important issue. In particular, upon electrical extracellular stimulation, the microelectrode and interface characteristic in the contact with the tissue can change, altering the MEA response5-9,11. Most of the MEAs are formed by using a combination of different materials for electrodes and the insulating surface (such as SiN SiO2, polymers etc.), with distinctive characteristics for interaction with neural cells.

Artificial man-made, Chemical Vapour Deposition (CVD) diamond is one of very interesting thin film materials for the MEA fabrication due to its biological inertness, chemical stability and availability of both highly insulating and highly conducting B-doped thin diamond films. It has been widely reported that nano crystalline diamond (NCD) is biocompatible as deduced from both in vitro12,13 and in vivo14,15 studies, exploring imaging, drug delivery, diagnostic or treatments contexts10,16-18. Additionally diamond also exhibits reduced bio-fouling. Boron doped NCD (BNCD) is electrically conductive with a wide electrochemical potential window19. These properties make dia-
mond an attractive platform for fabrication of active bio-electronic devices.\textsuperscript{11,20–22} Thus, one immediate application of BNCD are MEAs for in vitro electrophysiological studies and pharmacological screening in neuroscience. The aim of this study is two–fold. We prepare MEAs in which the cells are exposed only to diamond surface, by using both metallicity conductive as well as isolating diamond thin films for MEAs, providing thus homogenous surface for interaction with neural cultures. We study the interaction of both the conductive and isolating diamond surfaces with cellular environment and demonstrate the MEA functionality using acute brain slices. The thin flat diamond MEA with microelectrode channels was used to monitor local responses of single neurons or populations of cells.

2 Experimental methods

2.1 Substrate preparation

49mm by 49mm fused silica substrates were employed for the fabrication of the MEAs. Fused silica was selected due to its high melting point and is stable even at high temperatures used for the diamond growth (600 – 800 °C). Another benefit of fused silica for constructing MEAs is its optical transparency, allowing optical simultaneous imaging in transmission microscopy. Before the diamond growth, fused silica substrates were cleaned according to the standard wafer cleaning procedure RCA 1 and RCA 2\textsuperscript{22} [Figure 3, step#1]. To initiate diamond growth by plasma enhanced microwave chemical vapour deposition (PE MW CVD), diamond nuclei have to be provided on the substrate surface. There are various approaches for such surface pre-treatment. One is the seeding approach with diamond nano particles by submerging the substrate into a water based particle colloid. The other is abrading the surface of the substrate using diamond powder, leaving diamond residues on the surface\textsuperscript{13}. In this study, we have applied the former approach\textsuperscript{24}. A colloid of nano diamonds (NanoAmando\textregistered B from NanoCarbon Research Institute Ltd., Nagano, Japan) has been used. These detonation nano diamonds had the average diameter of ~ 5nm. The Z potential of the particles was ~ 50 mV and the surface was predominantly sp2 terminated\textsuperscript{25}. The nano diamond colloid was ultra-sonicated prior to the seeding, to break up any large clusters of nano diamond particles to produce mono dispersion as confirmed by dynamic light scattering (DLS). The cleaned silica substrates were immersed into this colloid for a minute. Then, the samples were spun for 40 seconds until dry, using a spin coater. The seeded substrates were flushed with Milli-Q water for the first 10 seconds to remove residual nano diamonds from the surface, leaving a nano diamond monolayer.

2.2 Fabrication steps of microelectrode arrays

To fabricate diamond MEAs, diamond layers were grown on the seeded substrates. 150nm thick films of BNCD (as the conductive layer) were grown first using an ASTeX 6550 series using MW-PECVD system [Figure 3, step#2]. 375 sccm of H\textsubscript{2}, 25 sccm (5%) Methane and 100 sccm (8000ppm) of Trimethylboron (TMB) were used as gas mixture for the MW-PECVD growth. TMB was used in Hydrogen mixture. 8000 ppm TMB leads to the boron concentration of about 3 E 21 cm\textsuperscript{-3}. The substrate temperature of 600 °C was used for the diamond growth, selected to obtain relatively smooth surface whilst keeping sufficiently high boron incorporation. The substrate temperature was controlled using a Williamson Pro 92-38 infrared pyrometer. The growth conditions were optimized to achieve a high sp\textsuperscript{3} carbon purity\textsuperscript{19} and high B-doping level (>10\textsuperscript{20}cm\textsuperscript{-3}) leading to a sheet resistance (of about 500Ω/square) of the film grown under the conditions: pressure 22 Torr and MW power 3500 Watt. Once the BNCD film growth was completed, a photolithography step was performed [Figure 3, step#3] using negative photoresist NR9-3000PY (Futurrex, inc.) and a photolithography mask, as shown in Figure 1.

Subsequently, a thin metal layer was deposited on the surface for negative lift-off lithographic processing and used as a mask for definition of electrodes [Figure 3, step#4]. For the purpose of masking, different metals were studied. Because subsequently an un-doped diamond layer is grown on top of patterned metallic films, one has to be cautious in the metal selection to achieve optimal adhesion to diamond. In case of chromium, the adhesion of diamond to Cr was not sufficient. Titanium made a good adhesion to diamond, but it was not suitable for the wet HF etching that attacks the quartz substrate. Therefore, tungsten was used as a protection mask, exhibiting a good adhesion to diamond, also resisted the diamond growth process, and the final etching step (by H\textsubscript{2}O\textsubscript{2}, 30%) is not harmful for the substrate. Tungsten has a relatively low resistivity: 10\textsuperscript{-4}Ωcm (compared to ~ 10\textsuperscript{2}Ωcm for BNCD) and can be therefore used for enhancing the conductivity on top of BNCD layer, sandwiched between BNCD and NCD. Our experiences indicate only ~ 20 nm of W is sufficient to achieve good masking. Tungsten is deposited by magnetron sputtering using a tungsten target, 50 sccm of argon and 100 Watts power under working pressure of few milli Torr. Further, an acetone-assisted lift off of the metal was carried out to define the conductive microelectrodes (where cells are later exposed to BNCD surface for recording or stimulation), the conductive tracks and the connection pads [see Figure 1, 150nm thick film of diamond residues were removed in 

Figure 1: Left: Design of the Lithography mask for conductive areas. Right: The zoomed center of the mask showing the channels.
also Figure 3, step#5). The BNCD film with the mask was exposed to reactive ion etching (RIE) using O2 plasma under the conditions of 300 Watt, 30 sccm O2 and a working pressure of a few m Torr to etch the BNCD layer outside the intended electrode area [Figure 3, step#6]. Next, a ~120nm thick NCD film was grown on top of the structure as an insulating layer on the MEA structure [Figure 3, step#7]. The tungsten layer was kept on the BNCD surface and used later on as an etch-stop layer to protect the BNCD in diamond etching steps. The growth conditions for the NCD film were 495 sccm of H2, 5 sccm (1%) of methane and 4000 Watt power at a gas pressure of 20 Torr. NCD layer was grown in a temperature between 650 to 700 °C. After NCD growth, another step of photolithography and metal mask (chromium) deposition was applied [Figure 3, step#8] using the insulating photolithographic mask [Figure 2] to mask the insulating areas, subsequently remove the NCD on microelectrodes and to open NCD by RIE down to BNCD [Figure 3, steps# 9,10,11]. The chromium layer was deposited by applying argon plasma and a chromium target. 100 Watts of power and 50 sccm argon was used under the working pressure of few m Torr to coat the NCD surface.

![Figure 2: Left: Design of the Lithography mask for NCD (insulating) areas. Right: The zoomed centre of the mask showing channels with 20μm diameter and 200μm spacing.](image)

After RIE processing, chromium mask was removed by wet etching, by using standard Cr etchant (Sigma Aldrich, Belgium) [Figure 3, step#12]. Finally, tungsten layer was etched by H2O2 solution (30%, Sigma Aldrich, Belgium) [Figure 3, step#13]. Once the metallic masks were removed, the diamond microelectrode array was produced according to the plan shown in Figure 3. The surfaces available for interaction with neurons remained entirely made of diamond.

![Figure 3: Schematic of complete plan steps for fabrication of diamond MEA:1)Fused Silica wafer, 2) BNCD growth, 3)Photolithography, 4) Metal-1 deposition, 5)Lift off, 6)BNCD etching, 7)NCD grown, 8)Metal-2 deposition and Photolithography, 9)Metal-2 mask etching, 10)Photoresist acetone-cleaning, 11)NCD etching, 12)Metal-2 mask removal, 13)Metal-1 mask removal.](image)

### 2.3 Surface termination and wettability of diamond

To study the wettability of both conductive and isolating diamond surfaces in cell experiments, two sets of fused silica substrates were prepared on which NCD and BNCD thin films were subsequently grown. One set of the NCD and BNCD coated substrates were hydrogen terminated; using hydrogen plasma exposure in the ASTeX reactor (800°C, 10 minutes, cooling in hydrogen). For the other set of substrates, the surfaces were oxygen terminated using UV induced ozone treatment in a PSD series digital UV-ozone system (Novascan Technologies, Inc.) for 30 minutes to create a homogeneously oxidized surface. The surface wettability by measuring the water droplet contact using OCA15EC Video Based Optical Contact angle Measurement Instrument.

To study the impact of the biological environment on the diamond surface wettability, the same set of samples were later treated with 1:1 Dulbecco’s Modified Eagle’s Medium (DMEM) and nutrient mixture F-12 from Sigma (Taufkirchen, Germany) containing 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin. The changes in diamond surface wettability due to physisorption of molecular or
ionic species from the cell medium were monitored using the same setup. To investigate this effect, the incubation was carried out and for a short (1-2 hours) and longer period of time (19 hours) in medium with FBS. After washing the surface in Milli-Q water, the contact angles were re-measured and compared.

2.4 Electrical characterization of diamond electrodes

The impedance spectroscopy was employed to characterize the diamond MEA and to evaluate the cross talk between the electrodes in dry condition (i.e. through the diamond layers), which is important to be minimized. For cross talk measurement, HP 4194a Impedance/Gain-Phase analyser from Keysight technologies was employed. The impedance for the cross talk evaluation was measured between two microelectrodes using a 50 mV peak-to-peak excitation signal which was applied by the signal generator. The impedance was assessed in two conditions: wet (i.e., in phosphate-buffered saline, PBS) and dry.

For impedance measurement of the electrodes, Solartron 1260 Impedance/Gain-Phase analyser was employed. The same setup was used to evaluate the signal to noise ratio of the MEA. An input AC voltage of 3V was applied and the output voltage was measured across the frequency range of 1 MHz to 1 Hz.

2.5 Brain tissue slices preparation and MEA electrophysiology

Tissue preparation was performed by standard methods, closely following the guidelines of the Ethics Committee of the University of Antwerp. Briefly, 21 days old Wistar rats were anaesthetized with Isoflurane (IsoFlo, Abbott, USA), decapitated, and their brains excised. 300 μm thick tissue slices (parasagittal) of the somatosensory cortex were cut by a vibratome (VT1000 S, Leica Microsystems, Diegem, Belgium) in ice-cold Artificial Cerebro-Spinal Fluid (ACSF). The ACSF contained (in mM) 125 NaCl, 25 NaHCO₃, 2.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂, 25 glucose, balanced by 95% O₂, 5% CO₂ and adjusted to pH 7.3. The same solution was also employed after cut, to incubate slices at 36°C for at least 45 min, during slice storage at room temperature, as well as during the electrophysiological recordings, performed at room temperature. All chemicals were obtained from Sigma-Aldrich (Diegem, Belgium). Slices were trimmed, to a width of ~5-6mm, and gently placed over the active surface of the diamond MEAs, upon previous treatment with cellulose nitrate (Protran, Fisher Scientific, Belgium; 0.14mg/ml in 100% Methanol). MEAs replaced the chamber of an upright microscope. MEA microelectrodes were then employed to monitor non-invasively the extracellular electrical field of neuronal microcircuits in proximity with the substrate. An ad hoc commercial amplifier was employed - 1060BC, Multichannel Systems GmbH, Reutlingen (Germany) to acquire spontaneous electrical activity at 25 kHz/channel, after 1200x amplification. MC Rack software (Multichannel Systems, Reutlingen, Germany) was employed for disk storage of the digitized data. An acquisition computer with a PCI A/D board (MC Card, 64 channels A/D, 4 DIO, 16bits Multichannel Systems, Reutlingen, Germany), was employed in this experiment.

3 Results and discussion

3.1 Surface characterization of MEAs

Figure 5 shows the images of functional diamond MEAs fabricated on fused silica substrates. Microscope and SEM images confirmed the successful patterning of diamond and presence of diamond on the well-defined active sites.

To prepare the MEA for measurement in PBS, a ring was attached to the MEA to function as the PBS holder [Figure 6].

Figure 6: Flexible reusable cell culture silicone chamber (Greiner BioOne) were attached to MEA, creating a bath chamber required for electrochemical impedance measurement under PBS.
3.2 Surface termination and wettability of diamond

The contact angle measurements showed that the
Oxygen terminated diamond surfaces (BNCD and NCD)
yielded the contact angle of 20°-30° which is lower than
that of hydrogen terminated diamond surfaces (BNCD and
NCD) with larger contact angles ~ 80°, representing hy-
drophobic and hydrophobic surfaces, respectively. This con-
forms the known effect that the wettability of the surface
increases by oxygen treating the diamond[27,28].

The contact angles of the substrates after treatment with
medium with 10% FBS changed dramatically [Figure 7].
For the H-treated, originally hydrophobic samples, the con-
tact angle decreased to 60-65° and for the O-treated, origi-
inally hydrophilic, samples, it increased to 55-60° after me-
dium treatment. The results indicated that the wettability of
the surfaces is significantly modified by the interaction of
diamond surface with the cell medium containing 10% FBS.
Since the surface of hydrogen or oxygen terminated
diamond exhibits different surface band bending respec-
tively[29], the electrical charge close to the diamond surface
can be compensated by ionic species or biomolecules such
as protein cocktail leading to wettability change. Interest-
ingly, we see identical change of contact angle for both
BNCD and NCD (one would aspects lower band bending
for BNCD due to high acceptor concentration of >10²⁵ cm⁻³).
This confirms that BNCD and NCD surfaces interact by
the same way with the cellular cultures and NCD and
BNCD provide a homogeneous interface to neural cultures.

Figure 7: Contact angle measurement across differently terminat-
ed diamond surfaces, before and after (1-19)h incubation in me-
dium with FBS.

3.3 Electrical characterization of diamond MEA

Electrochemical impedance spectroscopy was used to
characterize the conductive diamond MEAs.
Impedance cross-talk data were plotted for a representative
pair of conducting electrode lines [Figure 8]. Impedance of
the diamond electrodes is measured in dry and in PBS
buffer in a frequency range of 1MHz to 100 Hz.

The cross talk in dry condition (without PBS) shows high
impedance. The capacitive and resistive cross talk imped-
ance through the diamond MEA (dry condition) is ~ 10⁶
Ohms. In case of measurement in PBS, the impedance be-
tween two adjacent electrodes is lowered due to conduc-
tion between the adjacent electrodes through the electrolyte.
When neurons are cultured on the MEA or brain slices are
placed on the electrodes the impedance to the electrolyte
will increase due to sealing the active electrode surface by
neurons.

The electrochemical impedance spectrum measured in PBS
buffer was fitted using equivalent circuit on Figure 9. In
this circuit the Ohmic resistance $R_s$ of the electrolyte solu-
tion, electrodes, contacts etc. is in series with the parallel
combination of the space charge capacitance (BNCD/electrolyte interface) represented by constant
phase element (CPE) and its associated resistance ($R_1$) in
series with diffusion impedance $Z_w$, the so-called Warburg
element[30]. This equivalent circuit with diffusion controlled
process provided the best fit to our experimental spectrum.

Figure 8: Cross-talk measurement, comparing dry and wet condi-
tions

Figure 9: Equivalent circuit used to fit the electrochemical im-
pedance spectrum for diamond-based microelectrode array
(MEA), where $R_s$ is Ohmic serial resistance, $CPE$ is constant
phase element, $R_1$ is the associated charge transfer resistance and
$Z_w$ is the Warburg element.

The Nyquist plot of diamond-based microelectrode meas-
ured in PBS solution [Figure 10] shows at high frequencies
a semicircle corresponds to the electron transfer-limited
process followed by a straight line at the lower frequencies
range represents the diffusion limited electro-chemical
process[31].
3.4 Acute brain slice experiment on diamond MEAs

The diamond MEAs were further used for standard acute brain slice electrophysiological experiments as described in the Methods, under “submerged” conditions. Pharmacological disinhibition was then obtained by bath-applying 20µM of GABAzine (i.e., SR-95531) in ACSF, as a competitive antagonist of GABA$_A$ receptors. This resulted in spontaneous episodic electrical (epileptiform) activity *in vitro*.

An A/D converter and a 60 channels analogue amplifier are used for recording the local electrical field potentials, corresponding to the excitable activity of neighbouring neurons. Raw electrical voltages were recorded under a monopolar configuration, i.e., with reference to a synthesized chlorinated silver pellet electrode, immersed in the bath chamber. Waveforms were acquired and plotted using MATLAB, showing Local Field Potentials during an epileptiform epoch [Figure 12], reflecting the synchronous activation of local subset of pyramidal neurons, spatially summated in close proximity of the MEA microelectrodes. Pharmacological disinhibition was necessary as somatosensory cortical slices display very low spontaneous activity.

The overall level of background signal associated to the diamond MEAs when emerged to the Artificial CerebroSpinal Fluid (ACSF) without the brain slice has been measured by the same recording setup, under the identical conditions using the 1060BC Multichannel Systems GmbH, Reutlingen (Germany). This background signal recorded from all the channels at 1 kHz is shown in Figure 13. This background signal is composed of the noise of the amplifier alone (also plotted in Figure 13) and the system noise that is composed of noise due to the exposure of MEA to electrochemical environment (impedance is measured with respect to the ground electrode immersed in the electrolyte) at 1 kHz. In general the noise was found to be generally lower for diamond microelectrodes, compared to the...
4 Conclusions  Diamond arrays of microelectrodes were successfully fabricated. The diamond MEAs have a single monolithic surface composed only of diamond. The wettability of BNCD and NCD differently terminated diamond surfaces was examined. The treated BNCD and NCD surfaces when exposed to a medium with FBS, showed a change in hydrophobicity, regardless of the prior oxygen or hydrogen treatment. The electrochemical impedance spectroscopy of diamond microelectrodes was analysed and the signal cross-talk in dry conditions between the neighbouring microelectrodes was found negligible. The diamond MEAs allowed detection of the local field potentials for acute rat brain slices, with an overall noise level of the diamond MEA (~2.3 μV at 1000Hz) generally lower than conventional MEAs (~2.8 μV at 1000Hz). This all indicates that diamond MEAs have the potential to represent an advanced technology for long term electrical interfacing with neuronal systems.

Acknowledgements  We are grateful to M. Wijnants for technical assistance. This work was financially supported by ECFP7 projects (n. 280778-2 NMP-MERIDIAN and n. 264872, ITN-NAMASEN), by the Research Foundation – Flanders (FWO, grant n. G088812N), by the Belgian Science Policy (grant IAP-P7/20), by the Grant Agency of the Czech Republic (contract No. 13-31783S) and by Hasselt University (BOF). PP is a Postdoctoral Fellow of the Research Foundation – Flanders (FWO).

5 References


