Diamond for neural interfacing: A review

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Abstract

The variety of materials that fall in the diamond category is vast and increasing. From single, to micro to nano-crystalline, diamond can be highly electrically insulating or synthesized to possess varying degrees of conductivity. Diamond in all its forms is without peer in terms of chemical stability exhibits excellent biocompatibility and is famously mechanically robust. Diamond is however, a difficult material with which to fabricated devices owing to its extreme hardness, lack of ductility and weldability. New synthesis and fabrication methods in recent decades have overcome some of these drawbacks and diamond has enjoyed a surge in interest as a biomedical material. In the field of neural interfaces a grand goal is permanent, high fidelity connections with neural populations. Diamond’s longevity, biocompatibility and biochemical inertness make it a highly promising material with which to achieve this goal. This review covers recent uses of diamond in the three critical areas of neural interfacing: diamond as a growth substrate, as a neurochemical sensor electrode material and as a direct neural recording and stimulation material. Looking towards the future, the enticing prospect of nano-diamonds as optically active neural interfaces is reviewed.

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1. Introduction

Human fascination with diamonds goes back millennia owing to the unique set of optical and physical properties that diamonds possess and the extreme rarity of the naturally occurring gem stone [1]. The first synthetic (High-Pressure High-Temperature (HPHT)) diamonds were produced in the 1950s leading rapidly to commercial scale diamond manufacturing in the same decade. Cheap synthetic diamonds led to an explosion in interest in the material, principally as an ultra-hard grit for cutting tools. Diamond cutting tools are now produced by a wide variety of methods and are inexpensive and common place [2]. Low pressure diamond growth also has its origins in the 1950s [3]. Low pressure growth methods however did not gain real traction until the 1980s when Japanese scientists Seiichiro Matsumoto and Nobuo Sekata optimized a number of chemical vapor deposition (CVD) methods to produce diamond [4–7]. Unlike HPHT diamonds, CVD diamond could be grown conformally on surfaces as a microcrystalline or nanocrystalline film. Such films found use as wear resistant coatings [8,9]. The very low chemical reactivity of diamond provides the added advantage that such films are also highly effective at preventing corrosion [10,11]. The first medical use of diamond was as a wear resistant coating for artificial hip joint parts [11,12]. Anticorrosion coating of cardiovascular stents and dentistry components with diamond films has also been investigated [11]. As part of the due diligence of developing diamond coated implants, the cytotoxicity of diamond and diamond like coatings has been extensively investigated. Invariably diamond and diamond like films exhibit no measurable cytotoxicity [11]. Similarly nano-diamonds, which are attractive as drug delivery vehicles [13], are generally considered to be biocompatible although the chemical surface termination has been shown to impact suitability for biomedical applications [14]. Paget et al. for instance described no cytotoxicity or genotoxicity towards a range of human cell lines for COOH terminated nano-diamond [15] whereas Marcon et al. showed marked cytotoxicity of NH2 terminated nanodiamonds towards human embryonic kidney cells [16]. Hence diamond-like coatings have been used in a variety of areas, including food packaging and even processes to coat plastic materials with diamond-like carbon films [17].

A parallel research path that arose as a result of the new ability to form diamond films was the intense study of the electronic properties of diamond. Pure diamond possesses a wide band gap and is therefore an electrical insulator. As CVD diamonds are grown from a gas mixture, the inclusion of non-carbon impurities into synthetic diamond is straight forward. The introduction of boron doping to generate electrically conducting forms of diamond increased the potential uses of diamond dramatically [18]. Boron doped diamond (BDD) is a p-type semiconductor and can exhibit a wide range of conductivities depending on the synthesis method and doping level. Superconductivity at low temperatures has even been reported [19]. Recognition that conducting diamond could be employed to interface with neural tissue swiftly followed. Compared to the general acceptance of noble metals such as platinum, the uptake of diamond in real world neuro-modulation devices has been slow, perhaps due to challenges involved in fabrication of devices from such an extremely hard material. In the past decade however interest in diamond as a neural interface material has steadily increased. Initially interest was confined to diamond as a biocompatible growth substrate for various cell types including neurons [20,21]. Invariably diamond is found to be non-cytotoxic and in some cases has been shown to improve the viability of some cell types over those cultured or stored adjacent to more traditional materials. For instance, in one study, sperm stored in diamond coated petri dishes exhibit better viability than sperm stored in conventional polystyrene petri dishes [22]. Recently prototype implantable devices featuring predominantly diamond construction or at least diamond electrodes have been described arising from the retinal prosthetic community [23,24]. Diamond has also received particular attention from the retinal prosthetic research community as a material for hermetic encapsulation of implanted electronics [25].

This review will cover research conducted during the previous ten years featuring diamond as a neural growth substrate, diamond electrodes for electrochemical sensing of neurochemicals such as dopamine and diamond for direct detection of neuron activity or for electrically stimulating neural tissue. Special attention will be focused towards devices where diamond is used substantially as a construction material for neural interfacing.

2. Synthetic diamond

2.1. Synthesis methods and products

2.1.1. High-Pressure High-Temperature

The HPHT system is inspired by the process by which diamonds form naturally within the earth. Seed diamonds are placed in a chamber with a high-purity carbon source and a metal alloy solvent. The chamber is heated to a temperature around 1400 °C, melting the metal solvent. Hydraulic pistons apply high pressure to the chamber. Carbon from the high-purity source dissolves and diffuses through the metal solvent. Dissolved carbon precipitates onto the diamond seed, as the pressure is released, adopting the crystal structure of the seed. HPHT diamonds treated by irradiation. Irradiation induces lattice defects in the diamond generating colour effects.

Graphite is thermodynamically more stable than diamond but the tetrahedral (or face centered cubic) stacking in diamond has a higher atomic density, hence diamond is favored at very high pressure. HPHT techniques are also commonly used to treat natural diamonds in order to improve clarity or change color [26]. Dopants such as boron can be included in the carbon source producing electrically conducting HPHT diamonds [27] and a variety of treatments are available to generate coloured gem stones. For further reading on HPHT see the following reference. [28].

2.1.2. Chemical vapor deposition

The CVD process, like HPHT, also employs diamond seeds upon which an activated carbon source precipitates, increasing the size of the crystal. In its simplest form, synthesis by CVD requires a diamond seed, a carbon-containing feed-gas and a method of activating the feed-gas mixture to generate reactive carbon and hydrogen species. In all CVD processes the presence of atomic hydrogen in the gas mixture is the factor that dictates the formation of diamond over graphitic forms of carbon [29,30].
hydrogen is capable of reducing the double bonds in graphitic structures; thus, under the correct conditions, graphitic material is preferentially etched and diamond growth can dominate [31]. Many different carbon sources have been used to grow CVD diamonds, from the most mundane (e.g. methane) to the highly exotic [32]. Analysis of the gases present in CVD chambers reveal that the carbon decomposition species most commonly observed are methane radicals, CH₃ [30,33,34] and acetylene, C₂H₂ [30,34] though many other carbon species have been identified [34].

The two most common methods of synthesis by CVD are hot-filament CVD and plasma enhanced CVD [29]. Hot-filament employs a thin, high melting temperature wire situated close to the seed diamonds. A feed-gas mixture is introduced over the filament. The filament is heated to 2000–2500 °C simultaneously activating the gas mixture and heating the seed diamonds. In plasma enhanced CVD an energy source such as microwave radiation is focused into the gas mixture to form a high energy plasma in close proximity to diamond seed crystals. For detailed discussion of diamond synthesis by CVD see the following references [34–36].

2.1.3. Characterization of diamond films

Synthetic diamonds are grown as standalone single crystals or as polycrystalline films or blocks. In general polycrystalline diamond materials are named according to the size of the diamonds from which the material is constructed. The grades polycrystalline diamond materials are summarized in Table 1. For further reading see CVD Diamond for Electronic Devices and Sensors, edited by Riccardo S. Sussmann [37].

Beyond the diamond size, there are a number of other properties that are often cited when characterizing diamond films. One of the more critical of these is the nature of the grain boundaries that bind the micro or nano diamonds into the matrix. Fig. 1 is a cartoon depiction of a generic PCD film indicating a diamond region and two grain boundaries regions between diamonds.

The carbon atoms in the diamond regions adopt tetrahedral, sp³ geometry but the structure of the carbon in the grain boundaries is complex and changes between film types. In high purity PCD films with large diamond crystals the percentage of non-diamond (sp²) sp³ amorphous) diamond in the grain boundaries is very low and has little impact on the properties of the film. In UNCD films however the boundaries contribute a much higher proportion of the film leading to higher sp² content. When UNCD is nitrogen treated during growth (N-UNCD), the grain boundaries become thicker and more graphitic and sp² content in the film can be as high as 65% [38–41]. The electrical conductivity in N-UNCD films is thought to occur via the grain boundaries contrasting with traditional doping such as occurs with boron inclusions in diamond [38,41].

The gas mixture in the diamond growth environment also dictates the chemical termination of the as produced diamond surface. Post processing by plasma or acid cleaning tend to radically alter the surface termination. For instance boiling in acidic solutions, electrochemical oxidation or treatment with an oxygen plasma will terminate diamond with oxygen functionalities [40,42]. The relative proportions of different oxygen functionalities (C=O, C–OH, COOH) are affected by the oxidation method employed [42]. The majority of PCD films are grown in a hydrogen rich environment and, as a result, the diamonds are hydrogen terminated from the reactor. Hydrogen terminated diamond surfaces are electrically conducting via an unusual surface transfer doping mechanism [43,44]. Conversely oxygen terminated diamond exhibits immeasurably low surface conductivity [44]. Of greater significance in terms or neuron interactions is the relative hydrophobicity and/or surface charge of the diamond. Oxygen terminated diamond is more hydrophilic than hydrogenated diamond films. In a biological environment hydrophilic films tend to be a favored substrate for cellular attachment [45].

<table>
<thead>
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<th>Table 1</th>
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<tr>
<td>Grades and typical uses of diamond and polycrystalline diamond types.</td>
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values reported at freshly polished glassy carbon for instance (25 μF cm⁻²). Fig. 2 shows Mott–Schottky plots taken from the (111) and (100) faces of single-crystal boron-doped HPHT diamond.

The slope of a Mott–Schottky plot relates to the acceptor concentration in the semiconductor and, in this instance, illustrates the preferential uptake of boron into the (100) facet of diamond during growth (2 × 10¹⁹ cm⁻² for (111) vs 4.8 × 10²¹ cm⁻² for (100) [46,49]) and illustrates the effect that dopant concentration can have on overall double layer capacitance. The very low capacitance values at ca. −0.1 V for BDD may be explained by the development of a space-charge region within the diamond, depleting the concentration of carriers at the surface [47]. The absence of sp² carbon at the surface of high-purity diamond and consequently oxygen functionalities may also be a contributing factor [47].

The low capacitance of BDD is an attractive material quality for obtaining low-noise electrodes, but equally attractive is the very wide electrochemical window that diamond exhibits in aqueous electrolyte solutions. This wide electrochemical window permits detection of analytes across a wide range of voltages without the interference of faradaic (electrochemical reactions) background current due to electrochemical oxidation and/or reduction of water. Fig. 3 shows cyclic voltammograms (CVs) conducted using a high-purity BDD electrode (black line) and a BDD electrode with considerable sp² (or non-diamond carbon) impurities (purple line) in a 0.1 M KNO₃ solution [50]. Both types of diamond exhibit a wide region where no current passes between the electrode and the solution. The purple trace contains additional features due to oxidation of sp² carbon at ≈ 1.4 V and reduction of dissolved oxygen (ORR) between −0.8 and −1.5 V. The large increases in current magnitude occurring at the extremes of the CV are due to oxidation (splitting) of water in the positive (anodic) direction and reduction of water in the negative (cathodic) direction. Cathodic water splitting and dissolved oxygen reduction are both inner sphere electrochemical reactions [51] requiring a close interaction between the respective molecules and electro catalytic sites on the electrode. Defects in the diamond caused by regions of non-diamond carbon serve as electrocatalytic sites hence reduction of water is more facile and dissolved oxygen reduction is observed. In both cases however there is still a wide region (≈ 3 V) where almost no current is measured, one of the widest electrochemical windows known. This has safety implications for neural stimulation and implications for detection of neurochemicals by oxidation where additional current from oxidation of water acts as a source of noise.
2.2.2. Nitrogen-included ultrananocrystalline diamond (N-UND)

N-UND is a material constructed of very small (≈2−5 nm) diamond crystals interspersed in a non-diamond carbon matrix. The presence of nitrogen in the CVD growth chamber increases the crystallinity of the non-diamond regions leading to graphical electrical conductivity through the network of grain boundaries. The electrical and electrochemical properties of the material are strongly influenced by the amount of nitrogen present during growth. Considering that N-UND films can contain as much as 50% non-diamond carbon [40,52] the electrochemical properties of N-UND are surprisingly diamond-like.

Fig. 4 shows CV recorded from N-UND samples grown with a high (e) and low (c) N₂ concentration in the CVD plasma. CVs were recorded before (black trace) and after (dashed trace) electrochemical oxidation by polarization above 2.5 V vs Ag/AgCl in an aqueous electrolyte [40]. Both the CVs exhibit the wide water window associated with diamond electrodes despite their estimated (by x-ray photoelectron spectroscopy) ≈50% non sp³ carbon content. Anodisation however resulted in a dramatic increase in electrochemical capacitance of the high-N₂ nanodiamond film from 25 μF cm⁻² to a value of 160 μF cm⁻². Platinum for instance, the most commonly used stimulation material typically exhibits 50–100 μF cm⁻² [53]. In this work the authors were targeting neural stimulation where high electrochemical capacitance is desired. The authors attribute the startling rise in capacitance to an increase in surface roughness and to a greater propensity of the high-N₂ diamond to form carbon-oxygen functionalities at the surface upon anodization. Fig. 5 illustrates the high nanoscale (a,b) and microscale (c,d) roughness of the films grown in this experiment and the change in structure from a granular structure at 5% N₂ (Fig. 5 (a)) to a grassy appearance (Fig. 5 (b)) at 20% N₂.

These results stand in contrast to those obtained by Pleskov et al. [54] where a maximum of 5.1 μF cm⁻² was measured on N-UND films grown with 25% N₂ in the CVD plasma. Comparison of the synthesis methods between these two papers reveal that the most notable differences in the preparation of the films was the growth time and the substrate temperature. The shorter growth time and careful seeding described by Pleskov et al. resulted in films that were considerably smoother than those of Garrett et al., a factor that would contribute directly to the lower capacitance values measured. Garrett et al. report substrate temperature in excess of 1000 °C during growth of their films compared to ≈800 °C for Pleskov, a factor that may also have altered the nature of the films. The two results are an excellent illustration of the wide variety of properties that can occur in CVD grown N-UND and the need for careful control of growth conditions in order to achieve repeatable material properties.

3. Growth of neurons on diamond

Due to its chemical and biochemical inertness, diamond is generally considered as a biocompatible material, meaning that it is chemically non-cytotoxic when in contact with biological cells. This makes diamond a material of interest for coating of medical devices, building artificial organs, and as a growth support for biological cells. Diamond has been used as a substrates for cultivation of a variety of cell types including: neurons [55] fibroblasts [56], osteoblasts [57] and many other cell lines [58]. In all cases diamond exhibited no measurable cytotoxicity and, in some cases, appears to promote cell adhesion and proliferation over conventional materials such as glass or tissue culture polystyrene.

Whether acting as a neurochemical sensor or as a recording/stimulating electrode, a healthy, non-toxic interface with the local neurons is clearly critical if the electrode is to operate successfully, in particular over extended periods. Therefore, the subject of diamond as a growth substrate is important in the context of this review. The assumption being that materials capable of promoting cultured neuron adhesion and proliferation, extended neurite growth and perhaps formation of neurite networks are more likely to maintain their electrical and chemical function in an in vivo setting.

3.1. Effects of surface properties

The surface properties of diamond vary according to different fabrication methods. Post-processing steps such as sterilization can alter the chemical composition of the surface. Therefore, it is necessary to compare the growth of neurons on diamond films with different surface characteristics. The relative sp²/sp³ content is a variable that has been shown to affect the viability and growth of some cell types on diamond like carbon (DLC) [59] including myeloblasts, HEK293 and fibroblasts. This specific question however has not to date been investigated for neurons. The effects chemical surface termination and morphology on neural adhesion, proliferation have been attracted scientific interest with conflicting results.

The as-grown diamond films are normally hydrogen terminated due to the growth environment. Hydrogen termination however is unstable in atmosphere. Oxygen termination can be attained easily by several different techniques such as acid boiling, oxygen plasma, UV and ozone irradiation. Rezek et al. developed an atomic force microscope technique to measure adhesion forces of proteins on surfaces [60] and applied that technique to show that epithelial cells exhibited changes in morphology and improved adhesion on oxygen terminated NCD compared with H terminated [61]. Ariano et al. [62] compared neuron growth on oxygen and hydrogen terminated homoepitaxial diamond films finding no significant difference, either in morphology or in survival ratios between the ciliary ganglion neurons grown on two types of diamond surface and control cultures (plastic dishes), provided that a mixture of proteic adhesion molecules (poly-L-lysine and laminin) was applied before the cell seeding for cell anchoring. Similar results were reported on NCD with different surface termination. Ariano et al. [63] used GT-17 cells without exogenous adhesion molecules and rat hippocampal neurons were cultivated by Ojovan et al. [64] with poly-L-ornithine and laminin coatings, both showing no significant difference on two types of NCD surfaces with respect to cell

Fig. 4. CVs conducted in pH 7.4 phosphate buffered saline with 0.1 M KCl supporting electrolyte. N-UND grown in the presence of 5% N₂ (a) and 20% N₂ (b) before (black trace) and after (dashed trace) electrochemical oxidation by polarization above 1.6 V vs Ag/AgCl in an aqueous electrolyte [40].
adhesion and proliferation. Neuroblastoma cells also grew equally well on unmodified (hydrogen terminated) and oxygen terminated diamond-like carbon surfaces [65]. Bendali et al. concluded overall that a protein coating was required to promote healthy growth of retinal neurons on diamond but noted that results were much more consistent when oxygen terminated diamond was used over H terminated. The authors cite the lack of stability of H terminated diamond as a possible reasons for the result [66].

In contrast, other publications report better attachment of neurons on oxygen terminated surfaces. Oxygen terminated diamond-like carbon supported rat cortical neuron growth much better than the unaltered surfaces [65]. Poly-L-lysine coating was applied to promote cell adhesion by altering the surface charge from negative to positive (cells are negatively charged). Therefore the different neuron growth may be associated with different surface hydrophilicity, which leads to different degrees of poly-L-lysine adsorption on the surface. Rat cortical neuron attachment was found to be better on N-UNCD films oxidized by hydrogen peroxide sterilization than those on the as-grown surfaces with hydrogen termination after 24-h cell culture without any additional coating, which may also be associated with their different protein adsorption capacities (unpublished). Chen et al. [67] found that neural stem cells proliferated well without additional coating on both hydrogen and oxygen terminated UNCD but slightly better on hydrogen terminated surfaces. Neuron stem cells on oxygen-terminated surfaces demonstrated a preference to differentiate into oligodendrocytes, while those on hydrogen-terminated UNCD tended to differentiate into neurons. The increased levels of neuronal differentiation are due to adsorbing fibronectin from medium [68]. Therefore, controlling the diamond surface properties has the potential to differentiate neural stem cells for different biomedical applications. Later, human fetal stem cells were also shown to attach better onto NCD films with oxidized surfaces without applying additional coatings instead of hydrogen terminated surfaces [69].

Incorporation of elements other than carbon and hydrogen is commonly used to alter diamond conductivity for fabricating biomedical electronic devices. Higher survival rates of primary cortical neurons on phosphorus doped than un-doped diamond-like carbon with poly-L-lysine was reported, suggesting that the phosphorus-doped diamond-like carbon can direct neuronal growth [65]. Though elemental boron can be mildly toxic, boron when used as a dopant in diamond is locked in the diamond lattice and therefore does not affect biocompatibility [70]. Nistor et al. [71] cultured pluripotent stem-cell-derived human neurons on diamond and found that compared with un-doped diamond, boron-doped diamond has no adverse effect on cell survival, neurite formation and the apoptosis levels of cells. When nitrogen is added into gas mixture during UNCD deposition to increase the conductivity, the number of surviving rat cortical neurons also increased and their neurite length extended [72]. The different neural interaction with UNCD and N-UNCD may be the result of different grain shapes and sizes, which further influence the protein adsorption on the diamond surfaces.

Another factor that affects neuronal growth is surface morphology. Both pluripotent stem-cell-derived human neurons [71] and primary rat cortical neurons [24] showed preference to diamond with small diamond microcrystals. Babchenko et al. [69] compared NCD films with flat, porous or nano-rods structures. When seeded without laminin coating, the neural stem cells spread over flat diamond surface homogeneously but assembled into bundles on the porous structures, and only neurospheres were...
found on the diamond nanorods, suggesting it is an inappropriate condition for neural stem cells attachment. Piret et al. [73] cultured mouse spinal cord and hippocampal cell cultures on flat and 3D-nanostructured boron-doped diamond substrates. At 8 days in vitro, cells were found to attach and extend neurites on both substrate types and no significant difference was observed.

### 3.2. Necessity of adhesion promoter coatings

There is a wealth of evidence that neurons have the ability to sense and react to the chemical and mechanical properties of biomaterials. In the natural system, neurons respond to proteins in the extra cellular matrix of adjacent neurons and function according to these interactions. Typically, during neuronal cell culture, examples of these proteins such as fibronectin, vitronectin, lysine and laminin are adsorbed to the culture substrate in order to facilitate healthy neuronal adhesion, proliferation and neurite outgrowth. There exists however some conflicting evidence regarding whether additional promoting layers such as poly-o-lysine, poly-DL-ornithine or laminin are necessary for neuron attachment and proliferation on diamond films. Similar debates exist over whether neurons can survive and form functional networks on bare diamond surfaces. Table 2 is a summary of results for neurons grown on diamond with/without the use of adhesion promoter proteins.

In essence, as with most cells, neurons prefer surfaces with features mimicking their biological environment such as the extracellular matrix they are native to. Adsorption of extracellular proteins on substrate materials is determined by properties such as surface charge, surface functional groups, surface roughness and surface curvature. For neurons grown on homoepitaxial diamond for instance, an adhesion promotion layer appears to be essential. There has been no report describing neuron survival on homoepitaxial diamond without a coating. This may well be a result of the very low roughness of homoepitaxial diamond. Again however the role of roughness on diamond is difficult to understand. In their 2013 article, Edginton et al. point out that, despite possessing similar topography and roughness, solid NCD films do not promote neuronal adhesion whereas films of isolated nanodiamonds do [74], despite the fact that the roughness of these films are below the roughness window for promotion of neuronal adhesion on silicon [75]. The conclusion Edginton et al. draw is that the important interactions are subcellular (nanoscale) interactions and possibly the size, shape and surface charge of the nanodiamonds helps offer good adhesion for proteins such as vitronectin and fibronectin expressed by the growing neurons. Are that Cultivation on homo-epitaxial diamond was published in 2004 [55] describing dissociated cortical murine neurons seeded onto a laminin patterned diamond surface. Neurons extended neurites along the laminin patterns, indicating their preference to laminin-covered surface over bare homoepitaxial diamond. Chick ciliary ganglion neurons [62] and GT1-7 cell lines [63] were also found unable to attach onto homoepitaxial diamond without any coating.

The conflicting results about the requirement of promotion layer have been focused on polycrystalline diamond, including microcrystalline (MCD), NCD and UNCD. Nistor et al. reported that for long-term survival of human pluripotent cells on MCD, it is necessary to supplement the culture medium with laminin once a week, even though laminin was coated onto the diamond surface before cell seeding [71]. Rat hippocampal neurons grown on bare NCD revealed poor adhesion and cell clustering [64]. Tong et al. used UNCD as a substrate for rat cortical neuron growth. Although the neurons attached and extended neurites on UNCD surfaces after the initial 24-h incubation [24,72], they did not survive for more than 7 days if no additional coating layer was applied.

In contrast, the studies of Chen et al. found that mouse neural stem cells did attach, proliferate and differentiate on bare UNCD surfaces for at least 9 days [67,68]. Human fetal neural stem cells were also found to survive on bare NCD films up to 8-days after cell seeding [69]. For biocompatibility studies, the required addition of neurotrophic factors to their culture could prevent neuronal degeneration, therefore the cell proliferation of neural stem cells could mask a material toxicity [66]. Cockroach neurons [76] and GT1-7 cell lines [63] attached well on UNCD and NCD without a

### Table 2: Publication list on neuron growth on diamond without additional coating.

<table>
<thead>
<tr>
<th>Diamond</th>
<th>Cell type</th>
<th>Results</th>
<th>Ref author year</th>
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<tbody>
<tr>
<td>Homoepitaxial diamond&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Murine cortical neurons</td>
<td>Neurons extended neurites along laminin patterns</td>
<td>[55] Specht 2004</td>
</tr>
<tr>
<td>Homoepitaxial diamond</td>
<td>Chick ciliary ganglion cells</td>
<td>No cell attached</td>
<td>[62] Ariano 2005</td>
</tr>
<tr>
<td>Homoepitaxial diamond</td>
<td>GT1-7 cell line</td>
<td>No cell attached</td>
<td>[63] Ariano 2009</td>
</tr>
<tr>
<td>MCD&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Human pluripotent cells</td>
<td>For long term culture (up to 150 days), the medium needs to be supplemented with laminin once a week</td>
<td>[71] Nistor 2015</td>
</tr>
<tr>
<td>NCD</td>
<td>GT1-7 cell line</td>
<td>Cells adhered and functional viability was confirmed</td>
<td>[63] Ariano 2009</td>
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<tr>
<td>NCD</td>
<td>Rat hippocampal neurons</td>
<td>No cell attached at 2 DIV</td>
<td>[77] Thalhammer 2010</td>
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<tr>
<td>NCD</td>
<td>Rat hippocampal neurons</td>
<td>Cells attached and extended neurites on both flat and nanostructured surfaces but formed clusters</td>
<td>[73] Edgington 2013</td>
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<tr>
<td>NCD</td>
<td>Rat hippocampal neurons</td>
<td>Cells revealed poor adhesion and cell clustering</td>
<td>[64] Ojovan 2014</td>
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<tr>
<td>NCD</td>
<td>Human fetal neural stem cells</td>
<td>Cells could survive but better with laminin coating</td>
<td>[69] Babchenko 2013</td>
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<tr>
<td>NCD</td>
<td>Mixed rat retinal cells and purified ganglion cells</td>
<td>Glial cells only survived on coated surfaces but neurons survived even better on bare diamond surface</td>
<td>[66] Bendali 2014</td>
</tr>
<tr>
<td>UNCD</td>
<td>Murine neural stem cells</td>
<td>Cells attached, proliferated, and differentiated on diamond for at least 9 days</td>
<td>[67] Chen 2009</td>
</tr>
<tr>
<td>UNCD</td>
<td>Cockroach neurons</td>
<td>Cells attached well on diamond surface at 2 DIV</td>
<td>[76] Voss 2012</td>
</tr>
<tr>
<td>UNCD</td>
<td>Rat cortical neurons</td>
<td>Cells attached and extended neurites at 1 DIV but could not survive at 7 DIV</td>
<td>[24,72] Ganesan 2014</td>
</tr>
<tr>
<td>UNCD</td>
<td>Rat hippocampal neurons</td>
<td>Nanodiamond coating promoted neuronal attachment with respect to neurite outgrowth, direct interaction with the substrate, long-term survival and functional properties</td>
<td>[77] Thalhammer 2010</td>
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</tbody>
</table>

Note.

<sup>a</sup> Surface coated with laminin patterns before cell seeding.

<sup>b</sup> Surface coated with poly-γ-ornithine and laminin before cell seeding.
coating. Primary mammalian neurons are more demanding than those from other animals and cell lines. Therefore, they may not be sufficient for biocompatibility assessment of materials for mammal implantations.

Primary rat hippocampal neurons were shown to attach and extend neurites on boron-doped NCD with nanostructured surfaces and the functional viability of the attached neurons at 14 days in vitro was confirmed from neural recording [73]. However, the cells were found to form clusters on the surface, indicating that cells may prefer to attach onto themselves rather than the diamond surface. Bendali et al. [67,68] reported interesting results on NCD using adult retinal cell cultures from rats. The use of a protein coating increased cell survival, particularly for glial cells, but the biopolar neurons grew in direct contact with bare diamond. Using purified adult retinal ganglion cells, the neurons survived even better on NCD without any protein coating at 6 DIV and a protein pattern on diamond cannot lead to ordered neuron growth. Thalhammer et al. [77] reported that nanodiamond powder coating increased cell survival, particularly for glial cells, but the biopolar neurons grew in direct contact with bare diamond. Using purified adult retinal ganglion cells, the neurons survived even better on NCD without any protein coating at 6 DIV and a protein pattern on diamond cannot lead to ordered neuron growth. Thalhammer et al. [77] reported that nanodiamond powder coating could promote rat hippocampal neuron growth on many different substrates, with a potency similar to the effect of a standard protein coating. In Thalhammer’s work however the cells did not survive on an NCD film in spite of the film having a similar surface topography to the nanodiamond powder film. The difference may be due to their different protein adsorption capacities of the nanodiamond powder and NCD film. The nanodiamond film may adsorb certain proteins more efficiently than NCD which in turn could promote the neuron attachment and neurite outgrowth on the surface. Also pertinent here is the work of Edgington et al. who found that nanodiamond coatings on glass universally promoted murine hippocampal neuron adhesion regardless of surface functionality and with or without adhesion promoter proteins [74]. A comparison with a continuous diamond film was not conducted in this instance.

4. Diamond electrode and diamond microelectrode array (MEA) fabrication methods

Fabrication of flat films of diamond is relatively straightforward, however for most neural interfacing applications microelectrodes or microelectrode arrays are desired. Diamond, by virtue of its extreme hardness, lack of ductility and chemical reactivity, its inability to melt and the high temperatures present during CVD synthesis is a challenging material with which to fabricate devices. Methods to produce single microelectrode and MEAs have however been developed and have been reviewed elsewhere [78,79]. For the purposes of this review a small number of examples will be chosen to illustrate the methods most commonly employed for neural interfacing.

Standalone diamond electrodes are commonly fabricated by CVD growth of diamond on a high melting temperature metal substrate. An example from Park et al. [80] is depicted in Fig. 6 showing boron-doped PCD deposited onto an electrochemically sharpened platinum wire. Tungsten wire is also a common substrate for diamond electrodes of this type [81].

Multi electrode arrays are most commonly formed by removing
material from a continuous film of conducting diamond to create discrete islands or channels of diamond following variations of the generic scheme depicted in Scheme 1 [73,82–84]. Fig. 7 shows an example from Chan et al. [85] of a device fabricated by this method.

An alternative to removal of diamond by dry etching was demonstrated by Ganesan et al. [24] who produced an N-UNCD microelectrode array in which individual electrodes were isolated by removing sections of the film with laser milling apparatus. In this instance the authors used insulating PCD as the substrate. The PCD was laser milled with a series of vias upon which the N-UNCD film was deposited. The vias acted as hermetic electrical feed-throughs for the electrode array so that the array was suitable for direct bonding to microelectronics. The same group has previously proposed a method to generate penetrating diamond electrodes by growing the diamond film in an etched silicone template to mold diamond spikes. Both electrode types are depicted in Fig. 8 [86]. An alternative laser ablation method was previously demonstrated by Pagels et al. [87]. In this method a standalone film of BDD was laser ablated through a mask generating an array of BDD columns. Insulating PCD was grown over the structure and a final polishing step revealed the BDD columns penetrating through the PCD, essentially the inverse of Ganesan et al.’s method.

Another notable alternative fabrication method is that employed by Bergonzo et al. who, rather than etching the diamond film itself, pattern the diamond seed film prior to CVD. The result is a patterned diamond film directly from the CVD chamber [23,88–90].

5. Neurochemical sensors

5.1. Electrochemical detection of neurotransmitters

The wide electrochemical window that diamond possesses coupled with low background current has made diamond an attractive material for neurochemical detection. Neurotransmitters are chemicals that transmit signals between nerve cells or between nerve and other specialized cells such as muscle cells at the neuromuscular junction. Neurotransmitters are released at synaptic sites on axons where they diffuse across the synaptic cleft and are detected by receptors on dendrites extending from the target cell. Thus the expression of neurotransmitters is an essential part of normal neural function and hence anomalies in neurotransmitter function are linked to wide range of diseases. The biogenic amines, catecholamines, dopamine, norepinephrine (noradrenaline) and epinephrine (adrenaline) along with serotonin are the most common neurotransmitter targets for electrochemical detection. The three catecholamines, all derived from the precursor 3,4-dihydroxyphenylalanine (DOPA) and are shown in Scheme 2 along with serotonin [91], another common target for electrochemical detection.

Dopamine is the most abundant of the catecholamines and, like noradrenaline and adrenaline, it is intimately involved in modulation of many aspects of brain function among other crucial physiological roles. Accurate measurement of dopamine, in particular, is highly useful because dopamine expression is linked to neurological diseases such as schizophrenia and attention deficit hyperactive disorder (ADHD) and is implicated in most diseases of addiction [91]. Serotonin, or 5-hydroxytryptamine is a dicyclic neurotransmitter biologically derived from tryptophan. Among numerous physiological roles serotonin levels are most commonly linked to depression, hence the success of the serotonin reuptake inhibitor (SRI) antidepressant drugs. All of these molecules can be detected electrochemically via oxidation of the molecule to their respective corresponding ketones according to Scheme 3.

The reactions are generally electrochemically reversible. A persistent difficulty however is the susceptibility of the oxidized forms of these molecules to nucleophilic attack by adjacent molecules. The resultant dimers are insoluble and form films on the electrode surface rapidly reducing the sensitivity of the electrodes. One of a number of possible byproducts formed by reaction of

![Fig. 7. Example of a BDD MEA developed by Chan et al. [85]. (A colour version of this figure can be viewed online.)](image-url)
sequences of CVs conducted in 10 scans compared with 90% for the CNT electrode. The degree of fouling is much reduced on the CNT electrode. Lete et al. employed BDD microelectrodes and established a detection limit of 44 nM for dopamine. Importantly they conducted this study in the presence of the common interfering molecule ascorbic acid. Kondo et al. [95] investigated the electrochemical detection of a number of catecholamines on BDD in the presence of ascorbic acid. The low detection limit was facilitated by a peak current voltage separation of 410 mV between the two analytes.

Güell et al. [93] compared the performance of smooth glassy carbon and BDD with disordered carbon nanotube (CNT) films for electrochemical detection of serotonin. They found that BDD was more sensitive than glassy carbon but less sensitive than CNT films. Detection limits of 500 nM, 2 μM and 10 nM were established respectively. BDD electrodes however were much slower to foul than CNT films. Fig. 10 shows 10 sequential cyclic voltammograms conducted in a 10 μM serotonin solution on a BDD electrode (a) and a CNT film electrode (b). The peak due to oxidation of serotonin occurs at ≈0.45 V for BDD and ≈0.38 V on CNTs indicating some degree of catalysis of the reaction on the CNT electrode. The peak magnitude on the BDD electrode however reduces by 65% over the 10 scans compared with 90% for the CNT film electrode attesting to the lower propensity of the BDD electrode to become fouled. The authors improved on this result by extending the cyclic voltammogram to −0.6 V during the detection scans. Fig. 10 (c) shows sequences of CVs conducted in 10 μM serotonin on BDD (upper) and CNT film (lower) including a cathodic excursion to −0.6 V for each CV. The degree of fouling is much reduced on the CNT film and practically eliminated on BDD.

Lete et al. employed BDD microelectrodes and established a detection limit of 44 nM for dopamine. Importantly they conducted this study in the presence of the common interfering molecule ascorbic acid [94]. The low detection limit was facilitated by a peak current voltage separation of 410 mV between the two analytes. Kondo et al. [95] investigated the electrochemical detection of a number of catecholamines on BDD in the presence of ascorbic acid and determined (in agreement with Güell et al. [93] and Patel et al. [96]) that the diamond principle advantage is due to its lack of fouling. In this work the authors report enhanced sensitivity by modifying the surface of the BDD with carboxy functionalities via a photochemical reaction with a carboxy terminated alkene. Building on earlier work [97], Tryk et al. [98] also demonstrate the importance of surface termination for the detection of dopamine at BDD electrodes. Several papers [99–101] demonstrate that N-UNCD also is highly sensitive for detection dopamine in the presence of ascorbic acid. Surface termination on diamond electrodes can also be used to covalently link molecules which can impart specific benefits. For instance covalent attachment of Tyrosinase was use to enhance BDD for electrochemical detection of small molecules including dopamine [102]. A summary of neurotransmitter detection results where a detection limit has been calculated is included in table. In a unique experiment Dankerl et al. demonstrated that a diamond field effect transistor based upon the hydrogen surface conductivity could be employed to construct a sensor for acetylcholine [103]. Table 3 shows a summary of published detection limits towards serotonin and dopamine.

5.3. Neurochemical detection in living systems

The unparalleled chemical stability and very high biocompatibility of diamond materials make them an obvious target for fabrication of long lasting implantable sensor electrodes. Several groups have demonstrated efficacy of diamond electrodes in living systems. Gosso et al. demonstrated that an array of nine BDD electrodes confined within a disc-shaped area of 20 μm diameter were effective in mapping exocytosis of catecholamines across the membrane of single mouse and bovine chromaffin cells. Expression of catecholamines from the chromaffin cells was elicited either by electrical or chemical stimulation. The nine BDD electrodes were polarized at −0.8 V vs Ag/AgCl and the current on each electrode simultaneously monitored. Exocytosis of catecholamines in the vicinity of an electrode resulted in a corresponding spike in recorded current due to oxidation of the neurotransmitters. This work

<table>
<thead>
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<th>Electrode</th>
<th>Target analyte</th>
<th>Limit of detection (nM)</th>
<th>Mixed analyte</th>
<th>Ref</th>
</tr>
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<tbody>
<tr>
<td>BDD</td>
<td>ST</td>
<td>500</td>
<td>No</td>
<td>[93]</td>
</tr>
<tr>
<td>BDD</td>
<td>DA</td>
<td>44</td>
<td>AA</td>
<td>[104]</td>
</tr>
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<td>COOH-BDD</td>
<td>DA</td>
<td>100</td>
<td>AA</td>
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<tr>
<td>N-UNCD</td>
<td>DA</td>
<td>360</td>
<td>AA</td>
<td>[99]</td>
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<td>DA</td>
<td>50</td>
<td>AA</td>
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<td>DA</td>
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<td>AA</td>
<td>[102]</td>
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Fig. 8. Example of a flat N-UNCD high density MEA isolated by laser milling (Left). Templated diamond spikes produced by Ganesan et al. (Right) [24,86]. (A colour version of this figure can be viewed online.)

Table 3
Summary of published detection limits of various diamond preparations towards serotonin and dopamine.
builds on the earlier efforts of Kisler et al. where nitrogen doped diamond-like carbon electrodes were compared with indium tin oxide (ITO) and gold electrodes for simultaneous amperometric detection of dopamine. Carbon fibre electrodes suffered from a 30% response attenuation over a 7 h period compared with just 8% for the BDD electrodes [80]. Patel and Swain et al. demonstrated in 2008 that a BDD electrode could also be used to detect nitric oxide released by neurons and smooth muscle in guinea pig ileum [107]. The BDD electrode out performed carbon fibre in terms of signal amplitude in that study. The research also stands as an example of the utility of micro-electrodes for electrochemical detection of species other than the regular catecholamine targets.

The most complete example to date of the utility of diamond for neurochemical detection in vivo is that of Yoshimi et al. [108] later highlighted by Trouillon et al. [109]. In this work the effectiveness of BDD electrodes for detection of dopamine in vivo was established in a mouse model using previously established methods [110]. The study employed side-by-side carbon fibre and BDD microelectrodes inserted into the mouse striatum. The striatum is an area of the brain associated with the reward system receiving glutamate and dopamine inputs during activity that is perceived as pleasurable. In the anaesthetized mouse model, dopamine expression was evoked by electrical stimulation with one of the electrodes whilst concurrently recording dopamine oxidation current with the other. Administration the drug nomefesine (a dopamine reuptake inhibitor) increased the amplitude of the current detected on the recording electrode thus identifying dopamine as the principle catecholamine responsible for the signal [111]. This additional experiment adds considerable strength to the work as amperometric detection is inherently a non-specific technique. The highlight of the paper however was the use of BDD electrodes to record dopamine expression in the striatum of two awake behaving Japanese monkeys. A chamber was attached to each monkey’s skull permitting access to the cortex above the striatum. The monkeys were conditioned to an orange juice reward using a Pavlovian conditioning paradigm where a light flash could be used to forecast the juice reward. BDD electrodes were inserted into the striatum and amperometric recordings taken during presentation of a sequence of light flash, no light flash, Oj reward, no Oj reward stimuli. Following the light flash indicates a strong Pavlovian response to the impending juice reward followed by a smaller current peak following the juice reward itself. Only single current peaks were observed when a juice reward was administered without a preceding light flash. The study demonstrates that BDD is an effective dopamine detection material in that all the authors report superior sensitivity exhibited by the BDD electrodes over more traditional carbon fibres.

6. Diamond devices for neural stimulation or neural recording

All nerves transmit signals to one another by rapidly exchanging ions such as sodium (Na⁺) and Calcium (Ca^{2+}) across their cell membranes. In a neuron’s resting state, Na⁺ and K⁺ are pumped across the membrane by specialized proteins generating a transmembrane electrical potential. During a natural nerve depolarization, a stimulus (i.e. a neurotransmitters arriving at a synapse) may cause a change in the permeability of a region of the cell membrane. Ions pass into the cell altering the transmembrane electrical potential in that region. If the transmembrane potential changes sufficiently, voltage gated ion channels will open en-mass, causing an axon potential travelling out from the initial segment of the axon.
and along the axons and dendrites of the cell. An action potential arriving at a synapse triggers neurotransmitters to be released and diffuse across the synaptic cleft to signal the next neuron in the chain. The sudden flux of ions occurring during a neuron depolarization causes a small perturbation in the electric field around the neuron. This perturbation can induce a corresponding perturbation in a nearby electrode which, when measured, manifests as a momentary change in electrode voltage. Conversely if a small packet of charge is delivered to the tip of an electrode, the change in the electromagnetic field around the electrode can induce a corresponding flux of ions in the adjacent tissue. If the flux of ions results in a sufficiently large change in the transmembrane potential of a local neuron, an axon potential can occur and be transmitted along a nerve. Though the two aims are clearly interrelated, neural recording and stimulation electrodes require very different properties [53].
6.1. Neural recording

In order to induce a voltage change in a recording electrode the firing neuron must first overcome the electrochemical capacitance and also the electrical impedance of the electrode solution interface. The relationship between recording quality and the various parameters available for electrodes include size, shape, material etc. is highly complex [112]. The impedance of the electrode solution interface and the size of the electrode dictate the volume of tissue that can be sampled. High impedance, small electrodes for instance sample only the closest firing neurons. Hence high impedance, small electrodes are more likely to detect the firing of single neurons, typically referred to as single unit recording. Low impedance, large electrodes are more easily affected by the wider electrical environment and therefore tend to record from a larger population of neurons. Such recordings are typically called local field potentials indicating activity of a region of neural tissue as opposed to individual cells. The majority of neural recording with diamond has been conducted with small electrodes fabricated from BDD.

Neural recordings from cultured neurons or other electrogenic cells is a common method for assessing the properties of new electrode materials [113]. Examples on diamond however are rare. Maybeck et al. elegantly demonstrated this method with an array of 64 boron doped NCD electrodes coated with HL-1 cardio myocytes [114]. They concluded that BNCD electrodes functioned as well as gold electrodes in terms of signal magnitude. In a very different approach Ariano et al. demonstrated that high purity, single crystal, hydrogen terminated diamond could be employed for neural recording [115]. Though high purity diamond is electrically insulating, hydrogen termination imparts limited conductivity to the diamond surface by a surface transfer doping mechanism [43]. Ariano et al. successfully recorded high quality action potentials from mouse hypothalamic GT1-7 cells cultured on the diamond. A particularly interesting feature of this work is that the diamond is high purity single crystal and therefore optically transparent. Therefore the technology could be easily coupled with optical techniques. Using a different technology again Dankerl et al. fabricated a diamond transistor array which they employed to record the membrane potential of HL-1 cardio myocytes and human embryonic kidney cells (HEK293) [103,116].

Halpern et al., building on earlier work [117], demonstrated a diamond electrode for neural recording in the unusual choice of a Sea Hare (Aplysia Californica), a slug like marine animal [81]. The animal has an accessible nerve (the buccal mass) which is associated with visually identifiable behaviors associated with grasping food. The electrode consisted of a hook-like stainless steel wire coated with BDD grown by hot filament CVD. The hook electrode was inserted into the buccal mass and recordings taken during normal behavior. The authors hypothesize and indeed show (though only in a single animal) that the BDD electrode had better signal to noise ratio (average spike height/random noise magnitude) and higher resistance to fouling than a stainless steel control. Stainless steel is not favored as a recording electrode due to its lack of stability compared to more typically used metals such as platinum however the results from this experiment were promising [81]. Chan et al. not only used their custom designed probe shown in Fig. 7 for neurochemical detection but also employed it to record

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Fig. 11. (a) Images taken and traces reproduced from [104] showing microscope images of a blood vessel before and after vasoconstriction induced by the stimulation electrode. Also shown (b) is the measured vessel width and corresponding current recorded at the BDD microelectrode due to oxidation of norepinephrine expressed for perivascular nerves on the blood vessel. (c) Shows the oxidation current due to dopamine release recorded at a BDD microelectrode following a light flash induced Pavlovian response and an orange juice reward response from the stratum of an awake, behaving Japanese monkey. (A colour version of this figure can be viewed online.)
directly from the auditory cortex of guinea pig [85]. In that instance however a relatively poor signal to noise ratio (≈2) was reported. The authors speculate that the recorded neurons/electrode distance and/or high impedance interconnects may have been the cause of the poor result. The result also illustrates one of the principle difficulties involved in characterizing electrodes in vivo, namely it is usually impossible to know the distance from the electrode to the recorded neuron. The recent study of Piret et al. however used a diamond MEA to record activity in an excised perfused mouse hind brain [73]. In that study they compared as-grown BDD with nanostructured BDD finding that the nanostructured variety had greatly increased capacitance (3 mF cm⁻²), decrease impedance, reduced noise level and was effective at both stimulation and recording of neurons in the mouse hind brain sample. Increasing the effective surface area of electrodes has been previously demonstrated as a highly effective method of modifying the electrochemical and hence recording and stimulation properties of neural interface electrodes [118]. The role of capacitance in recording however is not so well studied. In Piret et al.’s work the high capacitance acted as a high frequency filter, minimizing the impact of thermal noise. Thus electrode interface capacitance is a further parameter the warrants consideration when optimizing recording electrodes [119].

6.2. Neural stimulation

Diamond, prima facie, is not a promising material for neural stimulation. Simply put, like other carbon materials [120], its inherent electrochemical capacitance is too low. Effective neural stimulation requires that a sufficient amount of charge is delivered to the tip of the electrode to affect stimulation of neurons without the voltage on the electrode becoming extreme enough to cause damage to those same neurons. Extreme positive or negative voltages cause damaging electrochemical reactions to occur such as water splitting or irreversible oxidation/reduction of biomolecules. High capacitance forms of diamond have however been reported. Like other carbon materials such as carbon nanotubes or graphene, high capacitance materials can be constructed by forming micro or nanostructured aggregates with very high real surface area. Boron doped diamond when very heavily doped can exhibit high electrochemical capacitance values as demonstrated by Watanabe et al. [121] In that work it appears that non diamond inclusions in the material may be responsible for the increased capacitance.

The principle interest in diamond as a stimulation material has come from the retinal implant community. Specifically from the Bionic Vision Australia (BVA) consortium, the MEDINAS project, funded by the French National Research Agency, and the Diamond to Retina Artificial Microinterface Structures (DREAMS) project funded by the European Commission. The BVA strategy was to fabricate a rigid diamond array capable of connecting directly to and hermetically encapsulating control electronics, thus negating the need for a large cable connecting to the array from a remote controller.

The BVA device utilizes N-UNCD as the conducting form of diamond. Similar to heavily doped PCD the N-UNCD characterized by Garrett et al. [40] (Fig. 12) contained as much as 50% non-diamond carbon according to XPS analysis. Interestingly, the material did not exhibit high capacitance until a strong electrochemical oxidation of the surface of the electrode in aqueous electrolyte was conducted. They showed that this oxidation step resulted in functionalization of the surface with oxygen, a process that has been shown to drastically increase the electrochemical capacitance of carbon nanotubes [122]. The maximum capacitance reported in this first publication was 154 μF cm⁻². The authors go on to demonstrate that this materials can be used as a hermetic feedthrough and high density electrode array [24] and that the material can be safely employed to stimulate retinal ganglion cells [123]. The retinal ganglion cells in that case were in rat retina, excised and perfused. Single diamond stimulating electrodes were placed on the surface of the retina and a patch clamp recording electrode was attached to an adjacent ganglion cell to measure the effectiveness of stimulation. Three different electrodes were used in 12 experiments yielding a stimulation threshold range between 19 and 164 μF cm⁻², all well below the safe charge injection limit. In that work the capacitance of the materials when formed into single microelectrodes was improved to 300 μF cm⁻² and the authors report no electrochemical changes the electrode performance after continual pulsing at 50 Hz for one week even at the upper charge injection limit. Shivdasani et al. following year, conducted a pilot in vivo study showing that single 120 x 120 μm square N-UNCD electrodes in an array, attached retinally, could elicit a response in the visual cortex of a cat with charge injections as low as 30 μC cm⁻². Some electrodes however required over 400 μC cm⁻² to elicit a response, well over the safe limit of 300 μC cm⁻². The BVA diamond array was attached to the inner surface of the retina (epi-retinal), a notoriously difficult position to achieve both close contact and damage free retinal stimulation. A magnetic attachment method has been proposed to achieve close attachment remains challenging [124]. Close proximity of the electrode to the target neurons is pivotal in achieving low thresholds for stimulation therefore improvements in the attachment strategy could improve in vivo stimulation thresholds.

The DREAMS project, utilised an array of BDD microelectrodes transferred to a flexible substrate [23] and have successfully utilized their general strategy to fabricate a range of MEA types [89,125]. In early work the capacitance and stimulation performance was not discussed however the expected high biocompatibility of the diamond array is confirmed by extended subretinal implantation in rat. The subretinal implantation location has some significant advantages including securing the device between the retina and the choroid and providing close contact between the electrode array and the retina [73,84]. In collaborative work arising from the DREAMS project however, a high surface area form of BDD produced by growing the diamond film over a field of carbon nanotubes (Fig. 13) resulted in a capacitance (measured by cyclic voltammetry) of 3 mF cm⁻² [73], the highest yet reported for diamond. As described in section 6.1, 10 μm diameter nanostructured BDD electrodes in an array format were effective in both stimulating and recording from cultured cardiomyocytes.

7. Future prospects

Beyond the electronic and electro-chemical techniques described extensively in this review, new optical approaches using intrinsic defect centers in diamond have been proposed as a platform for multi-magnetometer arrays (MMAs). These non-invasive high resolution MMAs present an attractive proposition to image the magnetic fields arising from the transmembrane potentials generated by neuronal activity [126]. The technology is based on a particular defect center in diamond, known as the negatively-charged nitrogen-vacancy center (NV) [127]. This defect can be engineered into the diamond lattice through the introduction of nitrogen in the gas phase of CVD growth or alternatively via nitrogen ion implantation. These fabrication approaches have led to the development of 2D magnetic imaging arrays which have been used to image and reconstruct the stray magnetic fields from current carrying wires [128] and magnetic nanoparticles within living organisms [129]. The detection technology for 2D MMA imaging of neuronal activity is based on optically detected magnetic resonance (ODMR) of the NV magnetic sub levels. Using wide-field
microscopy the ODMR from diffraction limited sensing volumes (0.4 × 0.4 × 1 μm) can be imaged with millisecond timing over wide fields of view of 100 × 100 μm [130]. More recent reports have extended the field of view out to mm² at the expense of spatial resolution [131]. Since the magnetic sensors are embedded in the diamond substrate total internal reflection microscopy (TIRF) can be used to excite and report changes in the local magnetic field. This eliminates any photo-toxicity effects from the excitation light ensuring the method is non-invasive. Optical imaging provides fast and parallel signal acquisition and offers the potential to be incorporated with other optical techniques such as opto-genetics which could see the development of a fully integrated 2D solution for optical stimulation and recording. The advantages of magnetic based detection include no local charge screening as is the case with electrical recording and potentially higher spatial resolution as the extracellular magnetic signal falls away much faster than the complementary electrical signal [126]. In this regard it is critical to bring the NV magnetic sensors as close as possible to the source of the magnetic field. This can be achieved by culturing the neuronal network directly on the diamond MMA or as discussed in Section 3.2 coating the homoepitaxial diamond within a thin adhesion layer of laminin or PDL to promote growth. From the physical modelling of a hippocampal CA1 pyramidal neuron the magnetic field generated from a single axon is expected to be of order tens of nanoTelsa/ms [126], with the majority of the magnetic signal arising from intracellular ion currents. The current state-of-the-art 2D NV-based magnetometer has a DC magnetic sensitivity of 30 μT/ms [131]. From the theoretical modelling nT/ms is within reach provided the nitrogen impurity levels can be well controlled and more importantly the conversion efficiency of N to NV improves from between 1 and 5% up to 50%. Such improvements may bring to life a fundamentally new view of neuronal activity and network dynamics.

8. Conclusions

The set of materials available in the diamond stable offer an unparalleled level of choice in terms of combinations and variations in electrical and optical properties. The very high biocompatibility and chemical stability of diamond materials has always positioned them at least at the periphery of the neuromodulation research field. With modern synthesis and processing techniques however diamond materials have enjoyed a surge in popularity and the first devices substantially fabricated from diamond have appeared. The mechanical hardness, lack of ductility of diamond and difficulties connecting diamond to traditional electronics will always present fabrication challenges. Recent advances however indicate that as machining methods continue to improve, diamond materials may increase in importance in the neural interfacing field and in the biomedical research area generally. As a growth substrate diamond is clearly non-cytotoxic but claims that forms of diamond actively enhance neural growth are presently debated. In most cases treatment of diamond with an adhesion promoter protein is recommended to assist with long term survival of cultured neurons.
Diamond electrodes for electrical recording and neurotransmitter detection are notable for their low noise performance but in particular for their ability to resist fouling. Diamond electrodes for neural tissue, optical recording of magnetic particles, and biomedical implants enabled by gold active braze alloys, Biomaterials 53 (2015) 464–474.


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