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Optimizing growth and post treatment of diamond for high capacitance neural interfaces

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ABSTRACT

Electrochemical and biological properties are two crucial criteria in the selection of the materials to be used as electrodes for neural interfaces. For neural stimulation, materials are required to exhibit high capacitance and to form intimate contact with neurons for eliciting effective neural responses at acceptably low voltages. Here we report on a new high capacitance material fabricated using nitrogen included ultrananocrystalline diamond (N-UNCD). After exposure to oxygen plasma for 3 h, the activated N-UNCD exhibited extremely high electrochemical capacitance greater than 1 mF/cm^2, which originates from the special hybrid sp^2/sp^3 structure of N-UNCD. The *in vitro* biocompatibility of the activated N-UNCD was then assessed using rat cortical neurons and surface roughness was found to be critical for healthy neuron growth, with best results observed on surfaces with a roughness of approximately 20 nm. Therefore, by using oxygen plasma activated N-UNCD with appropriate surface roughness, and considering the chemical and mechanical stability of diamond, the fabricated neural interfaces are expected to exhibit high efficacy, long-term stability and a healthy neuron/electrode interface.

1. Introduction

Neural interfaces, also called brain-computer interfaces, which are formed at the interface of neural prosthesis and the neural tissues, hold the potential to treat or assist people with disabilities of neural function. Neural interfaces have the potential to restore or enhance the function of neural tissues which might be damaged or lost due to disease or injuries. Common targets for such devices are hearing loss [1], vision impairment [2,3] and paralysis [4,5]. Beyond medical applications, the emergence of neural interfaces offers the promise of technologies that formerly resided only in the realm of scientific fiction. For instance, the emergence of technologies that permit control of machines with our mind potentially frees us from the physical limitations of our bodies and could greatly increase our capabilities. Neural interfaces also offer a unique means to gather insight into our basic understanding of neurosciences, in particular insights concerning brain function and the mechanisms behind neural diseases.

The generation of action potentials by electrical stimulation or transduction of action potentials by electrical recording forms the basis of neural prosthetic interfaces [6]. The properties of electrodes at the interfaces for neural stimulation and recording are critical to the success of the devices. The selection of electrode material directly determines the efficacy, reliability and lifetime of the neural interfaces. Apart from the requirements of chemical and biochemical stability, it is necessary for the interfacing materials to be able to be processed into devices, exhibit robust mechanical stability and maintain electrochemical functionality within the harsh biological environment over the long term [7]. The biocompatibility of the interface material is another critical prerequisite in that any increase in distance formed between the electrodes and the target neural cells due to acute or chronic inflammation resulting in neuron loss may have adverse health outcomes and decreased neural functionality. These processes will ultimately

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significantly degrade the performance of the electrodes [8,9]. For stimulation applications, any increase in the electrode to neuron distance will increase the current required for excitation [10] and when used for recording, the distance between the active neurons and the electrode is inversely related to the amplitude of the signals obtained [6]. In addition, meeting the requirements for the electrochemical properties of the electrodes is also pivotal. The capacitance and impedance at the electrode-tissue interface directly impacts the safety and efficacy of stimulation [11,12] and the quality of recording [13,14]; electrochemical reactions at the interface can lead to electrode dissolution [15] and production of chemical species that may be damaging to tissues [6,12]. Therefore, it is difficult to find a material to fulfill all these requirements, viz. biocompatibility, high charge injection capacity, low impedance, chemical and electrochemical stability, simultaneously.

Conventionally, neural electrodes have been fabricated using materials such as platinum [16], gold [17] and conducting polymers [14]. These have been demonstrated to record or stimulate neural activity both *in vitro* and *in vivo* [13,14,16,17]. There are however concerns when using these materials. For example, metals such as platinum and gold exhibit low charge injection limits and high impedance, which can be unsuitable for effective electrical stimulation and high quality recording, particularly when very small electrodes are employed [13]. In the case of conducting polymers, their mechanical properties are not ideal, as they are quite brittle [18,19]. Recently developed polymers, named "shape-memory polymers (SMPs), can change mechanical properties *in vivo* to improve the modulus and geometry match with body tissue after rigid insertion, thus reducing the damage to neural cells as can happen when implanting electrodes of stiff materials such as platinum and other metals [20,21]. However, the lack of electrochemical stability is another limiting problem when using conducting polymers for long-term neural interfaces [22]. Therefore, it is desirable to explore other materials with a combination of better intrinsic properties for the development of neural interfaces.

In comparison with the conventional materials used for neural interfaces, diamond possesses a number of desirable properties for advanced biomedical applications [23,24]. Diamond exhibits outstanding biochemical stability, chemical inertness and mechanical stability. Therefore devices fabricated from diamond are expected to operate steadily for longer periods of time than many other materials. Studies have shown that diamond produced by chemical vapor deposition (CVD) is highly biocompatible, eliciting a minimal immune response comparable to that of titanium [25] or medical grade silicone [26]. Diamond can promote the adhesion, proliferation and differentiation of various types of cells, including fibroblasts [27,28], osteoblasts [29] and many other cell lines [30,31]. By incorporating non-carbon contaminants such as boron and nitrogen, the fabricated diamond can be rendered highly conductive [32,33] therefore attractive to be utilized for neural interfaces.

One drawback of using diamond for neural interfacing is that it typically exhibits very low electrochemical capacitance. Diamond has long been shown to exhibit a large water window but the small electrochemical capacitance of conventional diamond materials, and the corresponding low charge injection capacity results in a material that is generally not suitable for neural stimulation. Modified versions of diamond however have been found to be suitable for neural stimulation. For example, a device fabricated by The Bionic Vision Australia (BVA) consortium for restoring the sight of blind people utilizes nitrogen included ultrananocrystalline diamond (N-UNCD) as the conducting form of diamond for retinal stimulation, attached to the inner surface of the retina (epi-retinal) using a magnetic attachment method [34]. Previous results have reported the maximum charge injection capacity for the N-UNCD electrodes on the BVA device was 160 $\mu C/cm^2$ (after electrochemical activation [35]), a value that was later improved to 300 $\mu C/cm^2$ [36]. An *In vivo* study was subsequently conducted by Shivdasani et al. and they showed that a single 120 × 120 μm^2 square N-UNCD electrodes as part of an array, attached retinally, could elicit a response in the visual cortex of a cat with charge injections as low as 30 $\mu C/cm^2$ [37]. Some electrodes however required over 400 $\mu C/cm^2$ to elicit a response, higher than the safe charge injection capacity of the diamond (300 $\mu C/cm^2$). The term "charge injection capacity" is defined as the amount of charge that can be injected into the electrode before the voltage of the electrode exceeds a predetermined safe limit. Mathematically this is the equivalent of multiplying the electrochemical capacitance of the electrode by the maximum safe voltage. We have previously determined the safe voltage window for diamond as lying between −1.8 and 1.1 V [36] therefore the lower of these limits (1.1 V) is used to determine the charge injection capacity. Therefore, for diamond, charge injection capacity can be derived from capacitance by multiplying by 1.1 V.

In this paper, we report an increase in the electrochemical capacitance and therefore charge injection capacity of N-UNCD from 17 $\mu F/cm^2$ for the as-grown material, to more than 1000 $\mu F/cm^2$ after 3 h of treatment with low energy (50 W, 25% O_2 in argon) oxygen plasma, resulting in a charge injection capacity of more than 1 mC/cm^2. The origin for this increase, and the role of hybrid sp^2/sp^3 carbon in N-UNCD is one foci of this paper. We hypothesize that the increase in capacitance is due to the incorporation of oxygen functionalities followed by etching of the electrochemically active graphitic grain boundaries in the nanocrystalline diamond film. The *in vitro* biocompatibility of activated diamond was then assessed using primary rat cortical neurons and the surface morphology was found to be critical for neuron attachment and neurite outgrowth. The study not only provides a simple and efficient method for improving both the electrochemical and biological properties of N-UNCD films, it might also shed light on improving other forms of carbon materials for neural interface applications. Significantly the results provide a method of producing a diamond electrode that is optimized for both high charge injection capacity and biocompatibility.

2. Materials and methods

2.1. Diamond preparation

Synthesis of N-UNCD films: N-UNCD films were deposited onto 10 × 10 mm n-type Si (100) substrates (MMRC Pty Ltd) using an Iplas microwave plasma-assisted chemical vapor deposition system, as described previously [35]. Briefly, silicon substrates were seeded before deposition with nanodiamond (NanoArmor) by ultrasound in ~5 nm nanodiamond/methanol solution. A gas mixture of 79% argon, 20% nitrogen and 1% methane (All gases, BOC Australia, purity 99.999%) was used. During the growth, the microwave power was maintained at 1000 W, gas pressure at 90 Torr, and stage temperature at 900 °C. All the N-UNCD films were grown for 15 h, resulting in a film thickness of 6–8 μm and a conductivity (two-point measurement) of 46 S/cm.

Unless specifically mentioned, the experiments were conducted using the grown surfaces of the N-UNCD films without removing the silicon substrates. Some N-UNCD surfaces with low roughness were obtained by dissolving away the silicon substrates with an etchant solution (HF: HNO_3: CH_3COOH = 10:10:1) revealing the 'seeding' side of the diamond films which in general is smoother than the growth side.

After the N-UNCD synthesis, samples were placed in a Diener Femto plasma cleaner using a 3:1 argon: oxygen plasma at 45 sccm