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Strategy towards independent electrical stimulation from cochlear implants: Guided auditory neuron growth on topographically modified nanocrystalline diamond

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ABSTRACT

Cochlear implants (CI) have been used for several decades to treat patients with profound hearing loss. Nevertheless, results vary between individuals, and fine hearing is generally poor due to the lack of discrete neural stimulation from the individual receptor hair cells. A major problem is the deliverance of independent stimulation signals to individual auditory neurons. Fine hearing requires significantly more stimulation contacts with intimate neuron/electrode interphases from ordered axonal re-growth, something current CI technology cannot provide.

Here, we demonstrate the potential application of micro-textured nanocrystalline diamond (NCD) surfaces on CI electrode arrays. Such textured NCD surfaces consist of micrometer-sized nail-head-shaped pillars (size 5 × 5 μm^2) made with sequences of micro/nano-fabrication processes, including sputtering, photolithography and plasma etching.

The results show that human and murine inner-ear ganglion neurites and, potentially, neural progenitor cells can attach to patterned NCD surfaces without an extracellular matrix coating. Microscopic methods revealed adhesion and neural growth, specifically along the nail-head-shaped NCD pillars in an ordered manner, rather than in non-textured areas. This pattern was established when the inter-NCD pillar distance varied between 4 and 9 μm.

The findings demonstrate that regenerating auditory neurons show a strong affinity to the NCD pillars, and the technique could be used for neural guidance and the creation of new neural networks. Together with the NCD's unique anti-bacterial and electrical properties, patterned NCD surfaces could provide designed neural/electrode interfaces to create independent electrical stimulation signals in CI electrode arrays for the neural population.

Statement of Significance

Cochlear implant is currently a successful way to treat sensorineural hearing loss and deafness especially in children. Although clinically successful, patients' fine hearing cannot be completely restored. One problem is the amount of the electrodes; 12-20 electrodes are used to replace the function of 3400 inner hair cells. Intense research is ongoing aiming to increase the number of electrodes. This study demonstrates the use of nanocrystalline diamond as a potential nerve-electrode interface. Micrometer-sized nanocrystalline diamond pillars showed high affinity to regenerated human neurons, which grew into a pre-defined network based on the pillar design. Our findings are of particular interest since they can be applied on any silicon-based implant to increase electrode count and to achieve individual neuron stimulation patterns.

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1. Introduction

The human auditory nerve contains around 35,000 neurons, 95% of which are so-called type I neurons innervating approximately 3400 inner hair cells (IHC) in the cochlea. These receptor cells are strictly ordered along the three turns of the cochlea, constituting the primary sensory receptors that convey afferent signals to the brain. Patients lacking these mechanoreceptors suffer from profound sensorineural hearing loss and may benefit from cochlear implant (CI) treatment. To date, there are over 400,000 CI users worldwide, and even more would benefit from this treatment. Despite excellent results over the past 30 years, there is still a need for improvement. Remaining problems include low sound quality, poor speech discrimination and a lack of pitch perception [1]. In addition, there is on-going research to improve the neural potential using stem cell-based therapy.

One major problem is the inability to deliver independent stimulation to individual or similarly tuned neurons, due to the relatively high current spread from the CI electrodes. In fact, this has been the bottleneck for further improvement in CIs [1,2]. There is a significant overlap in the activated neurons by each electrode [2,3], resulting in poor electrode selectivity influencing frequency discrimination and speech perception [4]. This is not surprising, since a CI employs approximately 12–20 electrodes to replace the function of 3400 hair cells. The use of phased array channels could focus the currents and reduce the stimulation overlap. However, this method requires a higher total stimulation current, significantly more electrodes, a higher density of axon-electrode contacts and closer contacts. The current CIs are unable to provide this [5]. Another biological obstacle is that regenerated axons lack organization in contrast to the normal cochlea [6]. It is therefore necessary to find new tools or materials to guide and organize regenerated axons for better individual stimulation of the neurons.

To regulate the cell-substratum functions, it is essential to create functionally engineered tissues and medical implants [7–9]. Nanocrystalline diamond (NCD) is a unique material possessing extraordinary mechanical properties (low friction coefficient, smooth surface and high toughness), chemical stability and biocompatibility [10]. It is particularly favorable for use in biomedical applications, such as dental, orthopedic and ophthalmological implants and surgical instruments [11–22]. Moreover, NCD is highly resistant to bacterial colonization when compared with steel and titanium [23].

Notably, NCD can be doped with boron to yield excellent electrochemical properties, including a wide working potential window and low background current. Doped NCD is also stable and highly suitable for bio-sensing applications [24–31]. Moreover, recently ultra-nanocrystalline diamond was grown in the presence of nitrogen (N-UNCD), showing high charge injection capacity [32]. Thus, safe electrical neural stimulations can be achieved [33] and N-UNCD is also considered to be non-cytotoxic [34].

For developing better biological performance in solid biomaterials, surface properties are more dependent on the modification methods of both the chemical and topographical aspects [35–38]. Ito and Khan et al. summarized the various methods of surface modification to regulate cell functions [39,40]. Basically, two different types of strategies can be employed to guide cellular adhesion and growth on cell-substratum surfaces: chemical modifications and topographical modifications. During the last years, several studies concerning the chemical modifications of the NCD or diamond-related surfaces have been made. Most of them reported the viability to chemically functionalize the NCD surface into hydrogen, oxygen and ammonia terminated by utilizing plasma treatments, UV and chemical-vapor-deposition (CVD) related techniques [41–44]. However, from a topographical perspective, there only exist a few studies investigating the NCD or other diamond related materials as substrates to regulate cell functions.

One significant issue with making topographical modifications of the diamond is that such a surface is extremely stable. Lately, Kalbacova et al. cultured SAOS-2 osteoblast-like cells on different types of NCD films grown on a flat silicon substrate, and on silicon substrates with a higher surface roughness. The roughness was in the nanometer region for all of the silicon substrates, and was created by mechanical lapping and chemical–mechanical polishing. They showed that growing an NCD film on a flat silicon wafer, yielding a root mean-square (rms) roughness value of 20 nm for the NCD film, enhanced osteoblast adhesion when compared to a polystyrene surface. For higher surface roughness in the NCD film, this effect was not as strong [45].

Prior to that, Specht et al. described the ordered growth of mouse cortical neurons on unstructured single crystalline diamond, and created high-resolution laminin patterns on the pretreated hydrophilic diamond surfaces with the proteins by utilizing a micro-contact printing technique [46]. It has also been shown that neurons can form functional networks on nanodiamond-coated substrates (with a resulting surface rms roughness between 10 and 20 nm) without the need for extracellular matrix (ECM) protein coating, demonstrating the potential for using diamond as the neuron-electrode interface. However, the formed neuron network was disorganized and the nanodiamond coating technique could not provide a guidance effect towards the neurons [47].

Very recent work on using nanostructured boron-doped diamond surfaces as microelectrodes has shown that neural interfacing with good performance, with potential use in long-term neural implants, could be accomplished [48]. Two research groups have demonstrated the use of nanostructured micrometer sized silicon-pillars for culturing neurons [49,50]. One group made the microstructured silicon-pillars (which had nano-roughness on the pillar sidewalls) superhydrophobic via the deposition of a thin Teflon-like material [49], while the other group used bare silicon-pillars, with different sizes and distances between the pillars [50]. Both substrates obtained superhydrophobic properties [49] and silicon micro-pillars [50].

Here, we studied inner-ear neuron growth of both human and mouse origins on micrometer-sized pillars in the NCD. The aim was to test whether human inner ear neurons and human neural progenitor cells may be more efficiently expanded using these devices, and if these inner ear neurons can be both electrically stimulated and functionally evaluated after maturation. As a first step, human inner ear ganglion explants were used. The NCD pillar surfaces, fabricated using lithographic and inductively coupled plasma (ICP) etching techniques, were set in four different spacings between the pillars. The interaction between the textured surfaces and the neuron growth was monitored by various types of microscopic techniques. Finally, the affinity between the regenerating neurons and different surfaces was compared, and the physiological function of the regenerating neurons was assessed.

2. Experimental methods

2.1. Materials and characterization

NCD with a thickness of 1 μm was deposited on a 4-inch silicon wafer by hot filament CVD methods (rho-BeSt coating GmbH, Austria). The NCD film was characterized with a confocal dispersive Raman microspectrometer (Horiba Jobin Yvon, Labram HR800 UV). An argon ion laser running at 514 nm with an output power of about 5 mW was focused on the NCD surface through a 100× objective (NA = 0.9, WD = 0.21 mm), while the same objective