Nitrogen-incorporated Ultrananocrystalline Diamond Microneedle Arrays for Electrochemical Biosensing

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Abstract

Microneedles are minimally invasive transdermal medical devices that are utilized for various applications, including drug delivery, fluid sampling, micro-dialysis, and electrochemical sensing. These devices are associated with less pain and tissue damage as compared with conventional hypodermic needle-based devices. In this study, we demonstrate fabrication of titanium alloy microneedle arrays with nitrogen-incorporated ultrananocrystalline diamond (N-UNCD) coatings. Microneedles were micromachined from ASTM F136 ELI Ti-6Al-4V alloy, a widely used medical-grade titanium alloy. N-UNCD coatings were deposited on the microneedles using microwave plasma enhanced chemical vapor deposition to enhance mechanical strength, increase hardness, improve biocompatibility, and provide an electrochemically stable surface. The structural and chemical properties of the N-UNCD titanium alloy microneedle arrays were evaluated using scanning electron microscopy and Raman spectroscopy. The mechanical robustness and skin penetration capability of the devices were demonstrated using cadaveric porcine skin. Finally, the electrochemical properties of the N-UNCD electrodes were evaluated; *in vitro* electrochemical detection of uric acid and dopamine was demonstrated using unmodified N-UNCD electrodes. These results demonstrate the application potential of N-UNCD-coated titanium alloy microneedles for transdermal electrochemical biosensing applications.

Keywords

nanocrystalline diamond, microneedles, electrochemistry, biosensor

1. Introduction

In vivo biosensors enable real-time and continuous detection of physiologically relevant molecules for monitoring patient health; as such, these devices are of significant interest to the biomedical community.¹ The development of electrochemical microneedle-based sensors may allow for minimally invasive transdermal sensing of biomolecules with reduced tissue damage and minimized pain.² These devices use microneedles to access biological fluids and integrated electrodes for highly sensitive, selective electrochemical detection of biomolecules.

Although more extensively evaluated for drug delivery applications, microneedle arrays have been successfully integrated with electrochemical biosensors for analytical sensing of physiologically relevant molecules. For example, glucose sensors have been developed that contain microneedles in conjunction with sampling mechanisms and enzyme-based sensing substrates.³⁻⁶ Hollow microneedle arrays fabricated by stereolithography have been integrated with carbon fiber and carbon paste electrodes for detection of ascorbic acid, hydrogen peroxide, glucose, glutamate, and lactate.^{1,2,7,8} For example, Windmiller et al. used pyramidal-shaped hollow microneedle arrays and carbon paste electrodes for selective monitoring of lactate, demonstrating lactate detection in the presence of common physiological interferents such as ascorbic acid, uric acid, and acetaminophen.⁷ Miller et al. developed a multiplexed, hollow microneedle-based biosensor array for simultaneous detection of pH, glucose, and lactate to monitor exercise-induced metabolic acidosis and tumor microenvironments.⁸ These microneedle-based sensors provide an attractive platform for minimally invasive and real-time biosensing of several analytes. Clinical translation of these microdevices for *in vivo* physiological monitoring will require ease of microneedle fabrication, mechanically robust and biocompatible sensor components, as well as electrochemically sensitive and stable electrodes.

Diamond is an extremely stable material that exhibits excellent biocompatibility, chemical inertness, and mechanical robustness.⁹ Advances in thin film technology have enabled deposition of nanocrystalline diamond thin films, which have been considered for use as a biointerface in many types of medical devices.⁹⁻¹¹ Synthesis of highly conducting NCD thin films through doping has led to use of doped NCD thin films for several electrical and electrochemical applications.¹² Doped diamond electrodes have demonstrated superior electrochemical properties with low background currents, wide potential windows, high current density electrolysis, and high over potential

for oxygen evolution.¹³⁻¹⁷ For biosensing applications, diamond electrodes have exhibited low detection limits as well as excellent precision and response stability for detection of numerous analytes without surface modification.^{16,18} Non-functionalized diamond electrodes have been applied for electrochemical detection of numerous physiologically relevant molecules, including acetaminophen¹⁹, adenosine²⁰, ascorbic acid^{19,21}, caffeine²², chlorpromazine²¹, cysteine²³, dopamine²⁴⁻²⁵, glutathione²⁶, histamine²⁷, nicotinamide adenine dinucleotide (NADH)²⁴, oxalic acid²⁸, and serotonin.^{27,29}

In addition to their superior electrochemical properties, conductive diamond electrodes may function in the harsh, electrolytic physiological environment without degradation, unlike other microelectronic materials such as silicon, SiO_x, and gold.³⁰ The chemical stability and inertness of diamond facilitates its use for biological applications. Furthermore, low adsorption of proteins and contaminants by diamond reduces fouling, enabling extended *in vivo* use of diamond electrodes.¹⁶

In this study, we demonstrate fabrication of titanium alloy microneedle arrays with nitrogen-incorporated ultrananocrystalline diamond (N-UNCD) coatings. The integration of conductive nanocrystalline diamond on a microneedle platform enables minimally invasive, highly stable electrochemical biosensing of transdermal fluids. Microneedle arrays were machined from a medical-grade titanium alloy and coated with nitrogen-incorporated ultrananocrystalline diamond using microwave plasma chemical vapor deposition. The N-UNCD coating provides a biocompatible interface with an electrochemically stable surface. The structural and chemical properties of the N-UNCD-coated titanium alloy microneedle arrays were examined using scanning electron microscopy and Raman spectroscopy. The microneedle arrays were evaluated for their mechanical robustness and skin penetration capabilities using a mechanical testing instrument. Finally, the electrochemical properties of the N-UNCD electrodes were evaluated; *in vitro* electrochemical detection of uric acid and dopamine at physiologically relevant concentrations was examined using unmodified N-UNCD electrodes. To our knowledge, this is the first development of nitrogen-incorporated ultrananocrystalline diamond microneedle arrays for transdermal electrochemical biosensing applications. The results suggest that N-UNCD microneedle-based device may serve as an attractive platform for minimally invasive, continuous monitoring of physiologically relevant molecules.

2. Materials & Methods

2.1 Fabrication of Nitrogen-incorporated Ultrananocrystalline Diamond-coated Titanium Alloy Microneedle Arrays

Microneedle arrays were micromachined from a medical-grade ASTM F136 ELI Ti-6Al-4V alloy bar (Allegheny Technologies, Pittsburgh, PA) using 3-axis computer numerical control (CNC) milling. The titanium microneedle arrays were fabricated with 7 microneedles in a staggered orientation. The solid microneedles were conical in shape and exhibited heights of approximately 340 μm and base diameters of approximately 230 μm . Titanium alloy disks with diameters of 1.6 cm and thicknesses of 2.5 mm were also fabricated to provide flat substrates for electrochemical testing.

Nitrogen-incorporated ultrananocrystalline diamond was deposited on the titanium alloy microneedle arrays and titanium alloy disks using a 915 MHz microwave plasma chemical vapor deposition (MPCVD) (Lambda Technologies, Raleigh, NC) facility located at Argonne National Laboratory. To clean the titanium alloy substrates, samples were sonicated in acetone and subsequently rinsed in methanol to remove debris and contaminants. Prior to N-UNCD deposition, the titanium alloy substrates underwent nucleation pretreatment via sonication in nanodiamond suspension in methanol for 20 minutes with subsequent rinsing steps in acetone, isopropanol, and methanol. The titanium alloy samples were then adhered to a silicon wafer with minimal silver paste before loading into the CVD chamber. N-UNCD deposition was conducted over a deposition time of one hour; a gas mixture of argon:methane:nitrogen at a ratio of 160 sccm:3 sccm:40 sccm was used in this study. The deposition was conducted at a substrate temperature of 850 $^{\circ}$ C, a working pressure of 80 mbar, and an input power of 2500 W.

2.2 Physico-Chemical Characterization of Uncoated and N-UNCD-Coated Microneedles and Disks

Uncoated and N-UNCD-coated titanium alloy microneedle arrays and disks were evaluated using scanning electron microscopy (SEM). The surface morphology and microneedle structure were examined using a Nova 600 NanoLab dual-beam scanning electron microscope/focused ion beam instrument (FEI, Hillsboro, OR). Image acquisition was performed using an acceleration voltage of 20 keV and a current of 0.62 nA.

Confocal Raman microscopy was conducted on the N-UNCD coatings with a Renishaw inVia Raman spectrometer (Renishaw, Gloucestershire, UK) in extended mode and a He-Ne laser with a wavelength of 633 nm.

The laser was introduced to the sample through a 50X objective lens. For microneedle arrays, Raman spectra were obtained at both the microneedle tip and the flat base of the substrate.

2.3 Porcine Skin Penetration Testing of N-UNCD Microneedles

Microneedle arrays were imaged with a Leica EZ4 D stereo microscope (Leica Microsystems, Wetzlar, Germany) before and after insertion into cadaveric porcine skin. Excised, full-thickness cadaveric porcine skin was obtained from a local abattoir and cut to 4.5 cm x 4.5 cm. The cadaveric porcine skin was inserted into a customized tissue holder to maintain tension in the tissue during penetration testing. The customized tissue holder was screwed into the base fixture of a Bose Electroforce® 3100 mechanical testing instrument (Bose Corporation, Framingham, MA) (Figure 3A). Using double-sided adhesive tape, a single microneedle array was fixed to the top platen of the instrument (Figure 3A). The microneedle array was placed in contact with the porcine skin to a load of 0.2 N prior to testing. The microneedle array was lowered onto the skin at a rate of 0.1 mm/s to a load limit of 7 N.

After withdrawal of the microneedle array, the cadaveric porcine skin was dyed with a few droplets of 1 mg/mL methylene blue dye for 20 minutes to stain the penetrated tissue. Excess dye was then removed by gently wiping the skin surface with sterile saline swabs followed by ethanol swabs. Subsequently, the stained tissue was observed under a Leica EZ4 D stereo microscope and the penetration holes were imaged.

Following porcine skin penetration testing, the N-UNCD microneedle arrays were imaged using SEM to determine if the microneedles were damaged during insertion. Secondary electron images (SEI) of the microneedle arrays were obtained using a JEOL JSM-6390 HV scanning electron microscope (JEOL, Tokyo, Japan). An acceleration voltage of 15 keV and a working distance of 11 mm were used for image acquisition.

2.4 Electrochemical Characterization of N-UNCD Electrodes

Nitrogen-incorporated ultrananocrystalline diamond-coated titanium and uncoated titanium substrates were cleaned prior to electrochemical measurements by sonicating in ethanol for 10 minutes with subsequent rinsing in nanopure DI water and drying with nitrogen. Initial electrochemical characterization of the electrodes was performed using cyclic voltammograms in 5 mM potassium ferricyanide in 0.1 M KCl aqueous solution on a Model 273A Potentiostat/Galvanostat (Princeton Applied Research, Oak Ridge, TN). A platinum wire auxiliary electrode

served as the counter electrode and an external Ag/AgCl (3M NaCl) electrode was used as the reference electrode.

The N-UNCD coated titanium alloy disk working electrode was covered with polyethylene cleanroom tape (ITW ALMATM, ThermoFisher Scientific, Waltham, MA) containing a 1/8 inch diameter exposed active area (8 mm²).

Cyclic voltammetric scans were performed from -0.1 V to 0.6 V at scan rates ranging from 10-300 mV/s.

2.5 Electrochemical Detection of Uric Acid and Dopamine Using Unmodified N-UNCD Electrodes

For electrochemical detection of uric acid and dopamine, the N-UNCD working electrodes were cleaned as described above. The working area of the electrode was controlled by laser cutting (Universal Laser Systems, Scottsdale, AZ) 1/8 inch diameter circles into electroplating tape and applying the tape to the N-UNCD electrodes by hand. Patterns for the working area were drawn in CorelDraw (Corel Corporation, Ottawa, Canada) and imported into the Universal Laser Systems software with laser cutting parameters of 100% power, 75% write speed, 300 DPI, and 0.0 inch stage height. Detection of uric acid and dopamine was made using linear sweep voltammetry (LSV) with a commercial potentiostat (DropSens, Llanera, Spain). Scans were conducted from -0.2 V to 0.8 V at a scan rate of 10 mV/s. Dopamine concentrations were varied from 1 μ M to 30 μ M and uric acid concentrations were varied from 25 μ M to 200 μ M. All electrochemical measurements were made against an Ag/AgCl reference and platinum wire counter electrode.

3. Results & Discussion

3.1 Fabrication and Characterization of N-UNCD coated Titanium Alloy Microneedles

The development of a microneedle-based electrode would enable minimally invasive transdermal detection of biomolecules. These devices use microscale needles to access interstitial fluid, which contains biomolecules that can be used to monitor patient health and/or diagnose medical conditions; for example, continuous glucose sensing may be used to improve control over blood glucose levels in diabetes mellitus patients. Microneedle-based platforms eliminate the stress and pain associated with application of conventional hypothermic needles and do not require a trained medical professional for use.² Furthermore, microneedles can access biological fluids without the interference of perspiration or skin irritation, which may serve as limitations for noninvasive transdermal sensors³¹. Microneedle arrays may also be created that enable simultaneous detection of a variety of analytes (i.e., multiplexed biosensing).⁸ As such, microneedle-based electrodes may be utilized for real-

time and continuous detection of physiologically relevant molecules for evaluating patient health in a rapid and straightforward manner.

Ti-6Al-4V is a titanium alloy that is widely used in biomedical applications, including orthopedic implants, dental screws, dental implants, and cardiovascular devices.³² ASTM F136 ELI Ti-6Al-4V alloy was chosen as the microneedle substrate material due to its biocompatibility, mechanical robustness, and ease of fabrication. Furthermore, Ti-6Al-4V has been previously shown to be a suitable substrate material for deposition of nanocrystalline diamond thin films.³³

Titanium alloy microneedle arrays were successfully fabricated in a reproducible manner using 3-axis CNC milling. The uncoated titanium alloy microneedle arrays showed minimal variation in microneedle dimensions and in spatial orientation. The microneedles were arranged in two rows in a staggered orientation with horizontal center-to-center spacing of 1.4 mm and diagonal center-to-center spacing of 1 mm. The conical microneedles exhibited heights of approximately 340 μm and base diameters of approximately 230 μm , giving an aspect ratio of approximately 1.5. The microneedle tip diameters measured approximately 10-20 μm . Microneedle heights of a few hundred micrometers were chosen to enable penetration of the epidermis of human skin (50-150 μm) but preclude interaction with nerve receptors in the deeper layers of the tissue.³⁴ High magnification SEM images show no significant defects; the rough surface topology and the visible striations, grooves, and pits on the surface were attributed to the milling process (Figure 1 A-C).

Nanocrystalline diamond was chosen as the electrode material since it is a stable and chemically inert substrate that exhibits mechanical robustness and excellent biocompatibility. Nanocrystalline diamond has been widely considered for use in electrochemical biosensing applications due to its advantageous electrochemical properties such as a wide band gap and a large electrochemical potential window.³⁵⁻³⁶ The surface chemistry of nanocrystalline diamond enables functionalization to produce an exceptionally tunable biomolecular interface.³⁷ A comparison of DNA-modified UNCD with commonly used substrates for biological functionalization (e.g., gold, glass, glassy carbon, and silicon) demonstrated that UNCD provides higher stability and sensitivity than the other substrates, making it an ideal substrate for biological modification and use in biosensing.³⁸ Furthermore, the mechanical robustness and chemical resilience of nanocrystalline diamond makes nanocrystalline diamond

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4 electrodes less susceptible to physical and chemical degradation in the harsh physiological environment,
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6 permitting use in long-term sensing applications.
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8 To synthesize an electrically conductive diamond film, a dopant is incorporated within the diamond film
9 during the CVD deposition process. Nanocrystalline diamond may be doped with boron to create a p-type
10 semiconductor or nitrogen to create a n-type semiconductor.³⁹ Nitrogen incorporation during growth leads to
11 enhanced sp²-bonded carbon regions at the grain boundaries of the UNCD film, resulting in improved electrical
12 conductivity.⁴⁰⁻⁴¹ Compared to boron-doped diamond, nitrogen-doped nanocrystalline diamond shows a wide
13 working potential window and a minimized background current.¹⁰ N-UNCD films have been used for
14 electrochemical sensing of several important physiologically relevant molecules, such as glucose¹⁰ and
15 dopamine.^{25,42} Xu et al. used nitrogen-doped diamond electrodes for amperometric detection of glucose,
16 demonstrating glucose detection with good temperature stability and with long-term stability.¹⁰ Shalini et al.
17 demonstrated detection of dopamine in the presence of common biological interferences (e.g., ascorbic acid and
18 uric acid).^{25,42}
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32 In our study, nitrogen-incorporated ultrananocrystalline diamond films were deposited on the titanium
33 alloy microneedle arrays and titanium alloy disks using MPCVD. The thickness of the electrically conductive N-
34 UNCD film that was deposited for one hour was approximately 350 nm. SEM micrographs of the N-UNCD coating
35 on the titanium substrates show a uniform, conformal N-UNCD coating with small grain sizes (Figure 1D-F). The N-
36 UNCD film deposited was densely packed and continuous, even on the microneedle tips that contained grooves
37 and pits.
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45 Confocal Raman spectra of the N-UNCD coatings are shown in Figure 2. Raman spectra were obtained on
46 the N-UNCD coated microneedles at the microneedle tip and at the flat base of substrate to confirm the chemical
47 bonding at these locations. Raman spectra were also obtained on the N-UNCD coated titanium alloy disks to
48 ensure the chemical bonding of the films on titanium alloy disks was similar that on the microneedles. Raman
49 spectra of the microneedle tip, flat substrate of the microneedle, and the N-UNCD coated disk all exhibited the
50 same three primary peaks. The broad shoulder observed at 1140 cm⁻¹ is associated with transpolyacetylene at the
51 grain boundaries of the nanocrystalline diamond film.⁴³ The D-band peak observed at 1340 cm⁻¹ and the G-band
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peak observed around 1580 cm^{-1} are both attributed to sp^2 -bonded carbon. Typically, the G-band peak is observed closer to 1550 cm^{-1} ; however, a shift in the position of the G-band to higher wavenumbers has been observed in nitrogen-incorporated UNCD films due to a slight variation in the configuration of the sp^2 -bonded carbon at the grain boundaries.⁴⁴⁻⁴⁵ The flat base of the microneedle substrate and the coated disk exhibit similar ratios between the D-band peak and the G-band peak. This ratio is slightly higher than that for the microneedle tip, potentially due to differences in film growth from temperature variation within the device during deposition. Birrell et al. observed an increase in this ratio as increasing concentrations of nitrogen were incorporated into the plasma during NCD deposition.⁴⁵

3.2 Porcine Skin Penetration Testing of N-UNCD Microneedles

Microneedle arrays were applied to cadaveric porcine skin at a rate of 0.1 mm/s to a load limit of 7 N using a Bose Electroforce® 3100 mechanical test instrument, as shown in Figure 3A. Assuming an equal distribution of force within the device, a 7 N load limit was chosen to provide 1 N of load per microneedle within the 7-microneedle array. A previous study by Davis et al. on the penetration force required for microneedle tissue penetration showed an approximately linear relationship between the insertion force and the interfacial area of the microneedle tip, with *in vivo* testing on human skin requiring insertion forces of $0.1\text{--}3\text{ N}$ for a single microneedle.⁴⁶

In our study, the microneedle arrays demonstrated penetration of the cadaveric porcine skin, as evidenced by methylene blue staining of the tissue following microneedle withdrawal. Methylene blue cannot penetrate skin with an intact stratum corneum layer. On the other hand, damaged skin (with a discontinuous stratum corneum layer) allows for diffusion of methylene blue; as such, a marked color change may be observed at the site of tissue damage.⁴⁷ Optical images of the methylene blue-stained tissue show three distinct puncture marks; two potential puncture marks were noted below the distinct puncture marks (Figure 3B). Two of the top puncture marks are approximately $1360\text{ }\mu\text{m}$ apart; this measurement closely corresponds with the $1400\text{ }\mu\text{m}$ microneedle-to-microneedle spacing of the microneedle design. The increased spacing between the other puncture marks is possibly due to the creases within the skin and the elastic nature of the tissue. The porcine hairs shown in the image potentially obstructed the other microneedles within the array, preventing penetration of the

tissue by all of the microneedles within the array. Porcine tissue that was treated with methylene blue without microneedle application showed no distinct puncture marks; however, some discoloration of the tissue due to the dye was noted.

Evaluation of the microneedle structure and N-UNCD coating after porcine skin penetration testing was conducted using the stereomicroscope and SEM. SEM images of N-UNCD titanium microneedle array after insertion into cadaveric porcine skin show the microneedle tips remained fully intact with no N-UNCD film delamination or fracture (Figure 3C). The results suggest that the microneedle arrays are suitably robust for skin penetration and that the N-UNCD coating has sufficient adhesion to the titanium alloy substrate for skin penetration.

3.3 Electrochemical Characterization

N-UNCD coated titanium substrates and uncoated titanium substrates were electrochemically characterized in potassium ferricyanide using cyclic voltammograms (CV's) as seen in Figure 4A. Uncoated Ti-6Al-4V alloy chips and N-UNCD coated chips run at 10 mV/s scan rate in potassium ferricyanide showed that the uncoated titanium alloy substrates possess no redox activity towards the ferricyanide solution, with no visible oxidation or reduction peaks (data not shown). The N-UNCD coated sample, run using the same parameters, showed reversible oxidative and reductive peaks.

For further characterization of the N-UNCD coatings, CV's were run from -0.1 to 0.6 V at scan rates varying from 10 to 300 mV/s. The N-UNCD CV's showed reversible oxidative and reductive peaks, $\Delta E_p = 180$ mV at 100 mV/s, indicating facile electron transfer at the diamond surface and stability over three cycles. Plots of the reduction and oxidation peak currents versus square root of the scan rate are seen in Figure 4B. A linear relationship was seen for both the reduction and oxidation peaks to the scan rate, indicating diffusion-controlled reaction, as described by the Cottrell Equation.⁴⁸

These results are similar to the results of other studies that involve use of nitrogen-doped diamond electrodes for electrochemical sensing. Zhang et al. evaluated the electrochemical properties of nitrogen-doped, boron-doped, and undoped diamond. The study demonstrated characteristic CV behavior for a ferricyanide redox couple on the nitrogen-doped diamond films¹². Shalini et al. also demonstrated a quasireversible response of

nitrogen-doped diamond films for ferricyanide, exhibiting ΔE_p values of 90-175 mV for nitrogen-doped diamond deposited at several temperatures.²⁵ This study compared the electrochemical characteristics of the nitrogen-doped diamond to those of boron-doped diamond and glassy carbon; these materials demonstrated ΔE_p values of 98 mV and 210 mV, respectively.²⁵

3. 4 In Vitro Electrochemical Detection of Uric Acid and Dopamine using N-UNCD Electrode

In vivo sensors enable real-time detection of physiologically relevant molecules that may aid in diagnosis, monitoring of patient health, and development of therapeutic treatments. Uric acid is produced through metabolic breakdown of purine nucleotides and is present in human blood serum and urine. At high levels, uric acid is associated with various medical conditions including gout, arthritis, diabetes, hypercholesterolemia, as well as cardiovascular and kidney diseases.⁴⁹ Dopamine is a primary neurotransmitter molecule that is present in the central and peripheral nervous system. The neurotransmitter functions in the nervous system as well as the renal, hormonal, and cardiovascular systems; it has been used for investigation of neural disorders such as Parkinson's and schizophrenia.²⁵ Both biomolecules are of considerable importance for patient health, requiring reliable and sensitive detection mechanisms.

In this study, linear scanning voltammetry was used to detect uric acid and dopamine by running scans from -0.2V to 0.8V at 10 mV/sec (Figure 5). Uric acid concentrations varying from 0 to 200 μ M were evaluated. A distinct oxidation peak was observed at a potential of 0.48 V (Figure 5A). A linear relationship was observed between the uric acid concentration and the peak current throughout the detection range (Figure 5B). Oxidation peak potentials were consistent across the range of tested concentrations. Dopamine concentrations varying from 0-30 μ M were detected at an oxidation peak potential of 0.65 V (Figure 6A). The small oxidation peak at 0.3 V for the 30 μ M concentration was due to experimental error, with no additional peaks visible on other scans. Varying concentrations of dopamine were tested from 0 to 30 μ M with a linear relationship seen across this range (Figure 6B). Detection above this value resulted in inaccurate readings with variable peak currents. The oxidative peaks between the two analytes were separated by $\sim$ 200 mV when tested separately; however, attempts to detect the

two analytes simultaneously were unsuccessful and only a single peaks was observed due to overlapping of the individual signals. Previous studies have demonstrated simultaneous detection of uric acid and dopamine using nitrogen-incorporated UNCD electrodes.²⁵ Shalini et al. demonstrated simultaneous detection of dopamine, uric acid, and ascorbic acid with significant peak separation using nitrogen-doped diamond electrodes.^{25,42} They noted that the nitrogen-doped diamond electrodes demonstrated superior peak separation compared to the boron-doped diamond, graphite, and glass carbon electrodes.^{25,42} A detection limit for dopamine of 0.36 μ M in the presence of ascorbic acid was demonstrated.²⁵ Their results show the sensitivity and high selectivity of nitrogen-doped diamond for simultaneous detection of physiologically relevant molecules. Future studies involving nitrogen-doped diamond coated microneedles will investigate varying deposition parameters and detection procedures to improve the N-UNCD electrode and further resolve the two peaks.

Although unmodified doped diamond electrodes have shown excellent capabilities for detection of physiologically relevant small molecules, the major advantage of using diamond-based electrodes lies in the potential of these electrodes for bio-functionalization. The surface of nanocrystalline diamond may be chemically functionalized to produce exceptionally tunable biomolecular interfaces.⁵⁰ Developments in chemical and photochemical processes for covalent attachment of functional biomolecules have further broadened the use of NCD electrodes in advanced biosensors. Future studies will investigate the use of N-UNCD microneedle electrodes with immobilized biomolecules (e.g., enzymes and antibodies) for sensitive and highly selective electrochemical detection of biomolecules.

4. Conclusions

We have demonstrated successful fabrication of nitrogen-incorporated ultrananocrystalline diamond-coated titanium alloy microneedle arrays that are capable of electrochemical detection of dopamine and uric acid. The N-UNCD coated microneedle arrays were fabricated in a straightforward and reproducible manner; minimal microneedle-to-microneedle variation in microneedle dimensions was noted. The N-UNCD coating deposited by CVD was continuous with small grain sizes and exhibited the typical carbon bonding signatures representative of N-UNCD thin films. Diamond-coated titanium microneedle arrays penetrated full-thickness cadaveric porcine skin without microneedle fracture or diamond film delamination, demonstrating suitable robustness for transdermal

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4 applications. Electrochemical analysis of the N-UNCD coatings on the titanium alloy show suitable electron transfer
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6 rates and reversibility for electrochemical biosensing applications. *In vitro* electrochemical detection of uric acid
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8 and dopamine using unmodified N-UNCD electrodes were observed across physiologically relevant concentrations
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10 for each analyte. By utilizing the superior electrochemical properties of N-UNCD as well as its potential for
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12 biofunctionalization, we envision the use of these N-UNCD-coated titanium alloy microneedle arrays for many
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14 types of transdermal electrochemical biosensing applications.
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Figure Captions

Figure 1. SEM images of uncoated titanium alloy microneedle arrays (A-C) and N-UNCD coated arrays (D-F). High magnification images show the surface morphologies of the uncoated titanium alloy microneedles (C) and the N-UNCD coated microneedles (F).

Figure 2. Raman spectra of the N-UNCD coating on titanium alloy disk, microneedle tip (MN tip), and flat base (MN flat) of microneedle array.

Figure 3. Skin penetration test setup (A). Optical micrographs of full-thickness cadaveric porcine skin stained with methylene blue after penetration by a microneedle array (B). SEM micrograph of N-UNCD titanium microneedle array tip after insertion into cadaveric porcine skin (C)

Figure 4. Cyclic voltammetric scans of potassium ferricyanide in 1X PBS pH 7.4 versus a Ag/AgCl reference and Pt wire counter electrode at varying scan rates from 10-300 mV/s (A) and plots of the reduction and oxidation peak currents versus square root of the scan rate (B).

Figure 5. Linear sweep voltammograms of N-UNCD electrodes in a 1X PBS pH 7.4 solutions containing uric acid concentrations ranging from 0 to 200 μ M versus an Ag/AgCl reference and Pt wire counter electrode (A) and a plot of uric acid oxidation peak currents at various concentrations (B).

Figure 6. Linear sweep voltammograms of N-UNCD electrodes in a 1X PBS solution containing dopamine concentrations ranging from 0 to 30 μ M versus an Ag/AgCl reference and Pt wire counter electrode (A) and a plot of the dopamine oxidation peak currents at various concentrations (B).

Figure 1.

Figure 2

Figure 3

Figure 4

Figure 5

B

Figure 6

B