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Nitrogen-incorporated ultrananocrystalline diamond microneedle arrays for electrochemical biosensing

Shelby A. Skoog [a], Philip R. Miller [a,b], Ryan D. Boehm [a], Anirudha V. Sumant [c], Ronen Polsky [b], Roger J. Narayan [a,*]

[a] Joint Dept. of Biomedical Engineering, UNC-Chapel Hill and NC State University, Raleigh, NC, USA
[b] Department of Biosensors and Nanomaterials, Sandia National Laboratories, Albuquerque, NM, USA
[c] Center for Nanoscale Materials, Argonne National Laboratory, Argonne, IL, USA

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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.

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 the use of doped NCD thin films for several electrical and electrochemical applications [12]. Doped diamond electrodes have demonstrated superior electrochemical properties with low background

* Corresponding author at: Joint Department of Biomedical Engineering, University of North Carolina and North Carolina State University, 911 Oval Drive, Box 7115, Raleigh, NC 27695-7715, USA. Tel.: +1 919 696 8488; fax: +1 509 696 8481.
E-mail address: roger_narayan@msn.com (R.J. Narayan).

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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 acetaminophen [19], adenosine [20], ascorbic acid [19,21], caffeine [22], chlorpromazine [21], cysteine [23], dopamine [24,25], glutathione [26], histamine [27], nicotinamide adenine dinucleotide (NADH) [24], oxalic acid [28], 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 facilitate 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 min 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 1 h; 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 °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 50× 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 × 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) (Fig. 3A). Using double-sided adhesive tape, a single microneedle array was fixed to the top platen of the instrument (Fig. 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 min 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-6390HV 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 min 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 (3 M NaCl) electrode was used as the reference electrode. The N-UNCD