Metal Nanoparticle Modified Carbon-Fiber Microelectrodes Enhance Adenosine Triphosphate Surface Interactions with Fast-Scan Cyclic Voltammetry

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ABSTRACT: Adenosine triphosphate (ATP) is an important rapid signaling molecule involved in a host of pathologies in the body. Historically, ATP is difficult to directly detect electrochemically with fast-scan cyclic voltammetry (FSCV) due to limited interactions at bare carbon-fibers. Systematic investigations of how ATP interacts at electrode surfaces is necessary for developing more sensitive electrochemical detection methods. Here, we have developed gold nanoparticle (AuNP), and platinum nanoparticle (PtNP) modified carbon-fiber microelectrodes coupled to FSCV to measure the extent to which ATP interacts at metal nanoparticle-modified surfaces and to improve the sensitivity of direct electrochemical detection. AuNP and PtNPs were electrodeposited on the carbon-fiber surface by scanning from −1.2 to 1.5 V for 30 s in 0.5 mg/mL HAuCl₄ or 0.5 mg/mL K₂PtCl₆. Overall, we demonstrate an average 4.1 ± 1.0-fold increase in oxidative ATP current at AuNP-modified and a 3.5 ± 0.3-fold increase at PtNP-modified electrodes. Metal nanoparticle-modified surfaces promoted improved electrocatalytic conversion of ATP oxidation products at the surface, facilitated enhanced adsorption strength and surface coverage, and significantly improved sensitivity. ATP was successfully detected within living murine lymph node tissue following exogenous application. Overall, this study demonstrates a detailed characterization of ATP oxidation at metal nanoparticle surfaces and a significantly improved method for direct electrochemical detection of ATP in tissue.

KEYWORDS: electrochemistry, purines, ATP, dopamine, voltammetry

1. INTRODUCTION

Adenosine-5′-triphosphate (ATP) is an important purine extracellular signaling molecule and has an essential role in a variety of neurological and immunological disorders. The ability to monitor ATP signaling on rapid time scales is important for elucidating the mechanism of action and quantitating the availability of ATP to interact at nearby receptors and transporters during both health and disease. Many methods have been developed to detect ATP including electrochemical aptamer-based sensors, enzyme sensors and surface-modified electrodes, and fluorescence, however, many of these methods suffer from slow temporal resolution or the inability to quantitate rapid concentration changes over time. Fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes is an electroanalytical technique which enables millisecond sensing of electroactive neurochemicals; however, direct electrochemical detection of ATP is difficult due to limited interactions at carbon-fiber surfaces. Recently, our lab developed a method to functionalize the surface of carbon-fiber with amines to enable significant improvements in direct ATP detection as facilitated by an increase in electrostatic interactions at the surface. Prior work from our lab has also suggested that ATP interaction at carbon surfaces is directly impacted by the surface topology and functionality. Despite these advancements, ATP detection on carbon surfaces is challenging, and an in-depth characterization of ATP interaction at other electrode surfaces is necessary to advance understanding of ATP electrochemistry. Here, we developed a method to electrodeposit both Au and Pt nanoparticles on carbon-fiber surfaces to provide a critical analysis of ATP interaction at metal nanoparticle-modified surfaces. We show that Au and Pt nanoparticles significantly enhance ATP oxidative current, the catalytic conversion of ATP oxidation products at the surface, facilitate enhanced adsorption strength and surface coverage, and promote adsorption-controlled interactions. We demonstrate that metal nanoparticle-modified electrodes enable subsecond detection
of ATP in the lymph node which will provide a platform for future work to investigate sympathetic ATP modulation of immunity, a critically understudied area in immunomodulation.12–15

Metal nanoparticles (NPs) have unique catalytic and electronic properties that have made them attractive for electrochemical sensors.16 A few reports have demonstrated AuNP-modified carbon-fibers for improved dopamine detection with FSCV and amperometry.16,17 The high surface energy of Au and Pt can promote adsorption with amines, carboxyls, and other metal ions.18 Adsorption of small molecules on metal nanoparticle surfaces can positively impact electrochemical sensing. The catalytic behavior of both Au and Pt leads to faster redox reactions and enables expansion to difficult targets.19–22 A recent study has shown that ATP adsorption onto AuNPs competes with binding to aptamers on Au surfaces providing evidence that ATP can interact with Au-based electrodes. The high catalytic activities and strong binding affinity of AuNP and PtNP facilitate conjugation with biomolecules. To further understand and quantify ATP adsorption onto metal nanoparticles, we compare and calculate the extent to which Au and Pt nanoparticle-modified carbon-fiber microelectrodes affect adsorption strength, surface coverage, and electrocatalytic behavior to inform the development of improved methods to directly oxidize ATP at electrode surfaces.

Here, we optimized the electrodeposition of metal nanoparticles on carbon-fiber surfaces to investigate the extent to which ATP interacts at metal-modified surfaces to enable significant improvements in direct real-time ATP detection with FSCV. Careful examination of how metal surfaces modify the interactions of ATP at the surface provided a detailed understanding of optimal methods for direct electrochemical detection. Overall, we provide an easy and robust method to significantly improve direct real-time ATP detection in tissue, which will have far-reaching impacts on probing ATP signaling.

2. EXPERIMENTAL SECTION

2.1. Reagents

All chemicals were purchased from Fisher Scientific (USA) unless otherwise noted. A Tris buffer (15 mM Tris, 1.25 mM NaH2PO4, 2.0 mM Na2SO4, 3.25 mM KCl, 140 mM NaCl, 1.2 mM CaCl2, dehydrate, and 1.2 mM MgCl2) at pH 7.4 was used in all injection analysis experiments. Dopamine (DA), Norepinephrine (NE), and ATP were dissolved in 0.1 M HCl solution to make 10 mM DA, 10 mM NE, and 10 mM ATP stock solutions (Sigma-Aldrich, St. Louis, MO) and were stored at 4 °C. Stock solutions were diluted daily in Tris buffer for experiments. Chlorauric acid (HAuCl4) and potassium hexachloroplatinate (K2PtCl6) were purchased from Sigma-Aldrich. All aqueous solutions and electrodeposition were made from deionized water (Milli-Q, Millipore, Billerica MA). For biological experiments in the lymph node, a bicarbonate-buffer was used (130 mM NaCl, 2.5 mM KCl, 1.3 mM NaH2PO4, 26 mM NaHCO3, 1 mM MgCl2, 2 mM CaCl2, and 10 mM glucose at pH 7.4).

2.2. Electrode Fabrication and Modification

Cylindrical carbon-fiber microelectrodes were made from 7-μm in diameter T650 carbon-fibers (gift from Mitsubishi Chemical Carbon Fiber and Composites Inc., Sacramento, CA). Carbon-fibers were vacuum aspirated into a capillary glass tube (1.2 mm × 0.68 mm, A-M Systems, Sequim, WA) and pulled into two using a vertical micropipet puller (Narishige PE-22, Tokyo, Japan). To create a cylinder electrode, fibers were trimmed to approximately 50–100 μm from the glass seal using a microscope (Fisher Education). Electrodes were prepared prior to modification. Electrodes were cleaned with isopropyl alcohol (IPA) and water to remove salt and allowed to dry prior to modification. To modify electrodes, either 0.5 mg/mL chlorauric acid (HAuCl4) or 0.5 mg/mL potassium hexachloroplatinate was electrodeposited on the carbon-fiber surface and this electrodeposition created formation of metal nanoparticles on the surface. Electrodeposition was done by applying a potential sweep to the surface scanning from −1.2 to 1.5 V at a rate of 5 V/s against a Ag/AgCl reference electrode for 30 s. See Figure S-1 for electrodeposition optimization data.

2.3. Fast-Scan Cyclic Voltammetry (FSCV)

Fast-scan cyclic voltammograms were collected using the WaveNeuro potentiostat with a 1 MΩ headstage (Pine Instruments, Durham, NC). Data was collected using a National Instruments PCIe-6363 interface board (Austin, TX) and HDCV software (UNC-Chapel Hill, Mark Wightman). Cyclic voltammograms (CVs) were background subtracted to remove nonfaradaic currents. The electrode was scanned from 0.4 to 1.45 V (vs Ag/AgCl) and back with a 400 V/s scan rate and a repetition rate of 10 Hz. The electrode was equilibrated for 10 min prior to testing, and the average current for each analyte was recorded. Electrodes were tested using a home-built flow injection analysis system using a six-port HPLC actuator (Valco Instruments, Houston, TX). A syringe pump (Chemyx, Stafford, TX) set to a flow rate of 1 mL min−1 was used to deliver buffer and sample to the electrode. All experiments were performed at room temperature.

2.4. Surface Characterization

The physical and chemical properties of unmodified and metal nanoparticle-modified carbon-fiber surfaces were analyzed. To visualize and qualitatively assess the electrode, scanning electron micrographs (SEM) were collected using an FEI XL30 SEM coupled to an EDAX detector for energy dispersive spectroscopy (EDS). SEM images were gathered at an accelerating voltage of 5.00 kV and 610 mm away. ImageJ Fiji was used to analyze the metal nanoparticles size after electrodeposition on the carbon-fiber surface.

2.5. Exogenous Addition of ATP to Lymph Node Slices

All the animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Cincinnati. Female C57BL/6j mice (Jackson Laboratories, Bar Harbor, ME; Research Resource Identifiers, RRID: IMSR_JAX:000664) between the ages of 8–12 weeks were used in the experiments. Mice were group housed in the vivarium at the Laboratory Animal Medical Services (LAMS) Department at the University of Cincinnati, where food and water was given ad libitum, with 12-h light/dark cycles. For the experiment, mice were anesthetized with isoflurane (Henry Shrein) and euthanized by cervical dislocation. Lymph nodes were harvested, sliced, and collected as previously reported.28–30 Briefly, mesenteric lymph nodes from the gut were harvested near the intestinal ileum and immediately placed in ice-cold 1× DPBS (without calcium and magnesium, Gibco) with 2% heat-inactivated fetal bovine serum (premium grade FBS, VWR ≤ 20 EU/mL) for 2 min. Lymph nodes were embedded in 6% low melting point agarose (Lonza, NJ) prepared in DPBS, and were allowed to harden on ice. A 10 mm tissue punch was used to remove each lymph node embedded in the agarose (Robbins Instrument, NJ). The agarose block was mounted with superglue onto a specimen disc and placed in the buffer tray with slightly oxygenated (5% CO2 with 95% O2) ice-cold bicarbonate buffer. The agarose blocks containing lymph nodes were sliced at 300-μm thickness using a Leica VT1000S vibratome (Chicago, IL). The slices were immediately transferred using a camel-hair paint brush (TedPella, CA) into a 6-well culture plate. Each well contained 3 mL of complete RPMI culture media including RPMI (Hyclone) with 10% FBS, 1×t-glutamine (Gibco), 50 U/mL of Pen/Strep (Gibco), 50 μM beta-mercaptoethanol (Gibco), 1 mM sodium pyruvate (Hyclone), 1× nonessential amino acids (Hyclone), and 20 mM HEPES (Gibco). Immediately after slicing, the culture plate was placed in a sterile incubator set to 37 °C with 5% CO2. The
tissue slices were allowed to recover for approximately an hour prior to the experiment.

During testing, slices were placed in a Warner Instruments standard perfusion chamber (Hamden, CT) held at 37 °C and perfused with oxygenated bicarbonate buffer at a rate of 2 mL min−1 using an Ismatec Reglo ICC 4-channel digital peristaltic pump (Cole-Parmer, Vernon Hills, IL). A metal-modified electrode was then implanted within the T-cell zone of the lymph node, approximately 75 μm beneath the surface, using an MM-3 micromanipulator (Narishige) and allowed to equilibrate. A Parker Hannifin Picospritzer III (Hollis, NH) was used for exogenous delivery. ATP (150 μM) was administered approximately 100 μm away from the working electrode using a glass micropipet with a 15 m opening at the tip, similar to previous reports.6,31 The picospritzer pressure was set to 30 psi with 800 ms ejections. Exogenous ATP was successfully detected in tissue using both AuNP and PtNP-modified electrodes.

To test the extent to which the electrodes experience biofouling, we harvested lymph nodes from mice and homogenized the tissue samples in bicarbonate buffer using a tissue probe sonicator (QSonica, LLC model Q55, Newtown, CT). Electrodes (bare carbon-fiber, AuNP- and PtNP-modified carbon-fiber) were pretested for both 1 μM DA and 5 μM ATP. Electrodes were then soaked in the homogenized tissue solution for 1 h, followed by immediate post-testing. The ratio of post-tested oxidative current to pretested oxidative current for each analyte was determined to quantify the impact of biofouling on the electrode surface.

2.6. Statistics
All data were analyzed with GraphPad Prism V. 9.0 (GraphPad Software Inc., La Jolla, CA). Statistical p-values were significant at the 95% confidence level (p < 0.05). Values are reported as the mean ± standard error of the mean (SEM), and n represents the number of electrodes or slices.

3. RESULTS AND DISCUSSION
3.1. ATP Detection at Unmodified Carbon-Fiber Microelectrodes
ATP undergoes three two-electron/two-proton oxidation reactions; however, only the first two oxidation products are ever detected with FSCV due to slow electron transfer kinetics.6,32-34 To observe ATP oxidation peaks with FSCV, an electrochemical waveform scanning from −0.4 to 1.45 V and back at a rate of 400 V/s is used. ATP’s primary oxidation peak is observed at 1.4 V on the cathodic scan due to slowed electron transfer, and the secondary peak is observed at 0.8 V11,32 and is always significantly smaller than the primary oxidation peak. The tertiary oxidation peak is rarely observed on bare carbon-fibers. Detection of ATP at carbon-fiber microelectrodes is significantly more challenging than detecting dopamine (DA), a popular analyte detected with FSCV (Figure 1).35-37 Figure 1 shows an example of false-color plots and cyclic voltammograms (CVs) for 5 μM ATP (Figure 1A,C) and 1 μM DA (Figure 1B,D) collected on the same electrode to demonstrate a significant difference in observed oxidative current. Despite ATP being 5-fold higher in concentration, an approximately 6-fold decrease in oxidative current for ATP is detected compared to DA. This has been well documented in the literature and is mostly attributed to ATP’s negative charge inhibiting interaction at oxide-rich carbon surfaces and ATP’s slow electron transfer kinetics.6,32-34 Because of this, it is essential to develop electrode surfaces which are specifically tuned to improve ATP detection. Fundamental understanding of direct ATP interaction at the electrode surfaces is limited but is crucial for developing sensitive ATP detection methods.

3.2. Metal Nanoparticle Carbon-Fiber Microelectrode Modification Procedure
The concentration of metal nanoparticles and the duration of electrodeposition were optimized to modify carbon-fiber microelectrodes for detection with FSCV. Previously, the Zestos lab showed that AuNP-modified carbon-fiber microelectrodes increase DA’s oxidative current approximately 2-fold and was stable for 4 h.17 Here, we further optimized the concentration and deposition time and used an extended deposition waveform, at faster scan rates than what was previously demonstrated, to measure the extent to which AuNP deposition procedures impact ATP detection, with DA serving as a “control”. All optimization procedures were completed using AuNPs, and this method was later used for PtNP modification. The waveform for electrodeposition scans from −1.2 to 1.5 V, at a rate of 5 V/s. Deposition times for 0.5 mg/mL AuNPs ranging from 15 s to 5 min were tested (Figure S-1A,B, n = 10–12) for both 1 μM DA and 5 μM ATP. The
ratio of oxidative current postmodification to premodification was calculated and plotted as a function of deposition time. A ratio above 1.0 indicates an increase in current due to modification, whereas a ratio below 1.0 indicates a decrease in current. Overall, the oxidative current for DA minimally changed at AuNP-modified electrodes; however, oxidative current for DA was significantly higher at 30 s compared to 3 min deposition times (one-way ANOVA F(4,27) = 2.852 and \( p = 0.043 \), Bonferroni post-test \( p = 0.0195, n = 7–12 \) (Figure S-1A). Despite the significant difference between 30 s and 3 min deposition, improvements in dopamine current never increased above 1.7-fold. Our observed change in dopamine oxidative current is slightly lower than prior reports; however, the deposition waveform and time of deposition are different making these results difficult to accurately compare. Overall, our results indicate that AuNP-modified surfaces increase DA interactions but in general, changes are minimal. Additionally, prior work has demonstrated that Au is less sensitive to DA with FSCV.\(^{38,39}\) Conversely, the oxidative current for ATP increased at all deposition times, and 30 s was significantly different compared to 5 min deposition (one-way ANOVA \( F(4,35) = 1.943 \) and \( p = 0.021 \), Bonferroni post-test \( p = 0.0195, n = 7–12 \) (Figure S-1B). Much larger increases in oxidative current was observed for ATP compared to DA. Based on these results, a deposition time of 30 s was chosen as optimal for ATP detection. Deposition times greater than 1 min produced measurable noise at the electrode, with less improvements in oxidative current, likely due to agglomeration of AuNPs on the surface.

The concentration of metal nanoparticles was optimized by keeping the deposition time constant at 30 s while varying the HAuCl\(_4\) concentration from 0.1 mg/mL, 0.5 mg/mL, and 1 mg/mL (Figure S-1C,D, \( n = 10–12 \)). Small increases in DA current were observed at all concentrations tested; however, no significant differences between concentrations were observed (one-way ANOVA \( F(4,26) = 0.534 \) and \( p = 0.593 \)). On average, the ATP oxidative current increased between 3 and 4-fold at all concentrations tested. Again, no significant differences between concentrations were observed (one-way ANOVA \( F(4,27) = 0.804, p = 0.923 \)). Despite this result, it was qualitatively observed that larger concentrations resulted in an increase in the variance in the data set with decreases in the signal/noise (S/N). Overall, 0.5 mg/mL was chosen as optimal. At the optimized procedure, DA increased on average \( 1.7 \pm 0.2 \)-fold and ATP increased \( 4.1 \pm 1.0 \)-fold at AuNP-modified electrodes (Figure 2, \( n = 7–12 \)). The improvements in detected current after AuNP-modification are significantly different between DA and ATP (unpaired \( t \) test, \( p < 0.01 \)). As a control, we tested whether applying the electrodeposition waveform alone (no metal nanoparticles in solution) could increase observed oxidative current for DA and ATP due to electrooxidation of the surface at high potentials. On average, we observed \( 1.3 \pm 0.1 \)- and \( 1.5 \pm 0.2 \)-fold increases for DA and ATP, respectively, demonstrating that the majority of the changes in ATP current arises from the presence of metal nanoparticles on the electrode surface, whereas the increases observed for DA were likely due solely to the electrodeposition waveform (\( n = 6 \), Figure S-2). The electrodeposition waveform could be exposing more surface oxides on the carbon surface due to the expanded potential window applied.\(^{40}\)

![Figure 2](https://doi.org/10.1021/acsmearsciu.1c00026)

**Figure 2.** ATP oxidative current increases at both Au and Pt nanoparticle modified carbon-fiber microelectrodes. Electrodes were electrodeposited with either Au or Pt nanoparticles by applying a waveform that scans from \(-1.2 \) to \(1.5 \) at \(5 \) V/s. Significantly higher increases in current are observed for ATP compared to DA at AuNP (A) and PtNP (B) modified electrodes (\( n = 7–12 \)). Representative CVs are shown for bare carbon (CF, black), AuNP-modified (orange), and PtNP-modified (blue) for ATP (C) and DA (D).

### 3.3. Comparison of Oxidative Current Increases at AuNP and PtNP-Modified Carbon-Fiber Microelectrodes

Electrodeposition of 0.5 mg/mL potassium hexachloroplatinate resulted in significantly larger increases in ATP current compared to DA (Figure 2B, paired \( t \) test, \( p < 0.0001, n = 7–12 \)). Electrodes were tested prior to and after deposition. The ratio of postmodification current to premodification/bare electrode current (\( i_{pp}/i_b \)) was calculated and plotted as a function of the analyte (Figure 2). On average, the DA oxidative current increased \( 1.7 \pm 0.1 \)-fold and \( 1.8 \pm 0.1 \)-fold for AuNP and PtNP-modified electrodes, and ATP increased \( 4.1 \pm 0.3 \)-fold and \( 3.5 \pm 0.3 \)-fold for AuNP and PtNP-modified electrodes, respectively (\( n = 7–12 \)). Example representative CVs for ATP (Figure 2C) and DA (Figure 2D) comparing each electrode type (bare carbon fiber, AuNP, and PtNP-modified) show that metal nanoparticle modification is beneficial for improving ATP detection with FSCV; whereas, minimal improvements are observed for DA detection. Similar increases in oxidative current were observed for both AuNP and PtNP-modified electrodes. Additionally, we validate that electrodeposition of metal nanoparticles on the surface does not impact the temporal resolution of the technique by comparing the shapes of the current vs time traces before and after modification (Figure S3). No change in peak shape was observed.

### 3.4. AuNP and PtNP-Modified Electrodes Increase Electrocatalytic Behavior for ATP

Detection of ATP’s secondary and tertiary oxidation peaks are difficult to detect on bare carbon-fiber electrodes due to slow kinetics and minimal interaction at the electrode surface. We observed increases in current for ATP’s secondary and even tertiary oxidation peaks at metal-nanoparticle modified electrodes providing evidence that metal surfaces improve the electrocatalytic activity of ATP at the surface (Figure 3). ATP’s oxidation scheme is represented in Figure 3A. Figure 3 shows example color plots and CVs for 5 \( \mu \)M ATP at bare
carbon-fiber (Figure 3B), AuNP-modified carbon-fiber (Figure 3C), and PtNP-modified carbon-fiber (Figure 3D). ATP was manually injected at approximately 5 s and washed away at approximately 9 s. Dashed lines indicate at different time points (time points 1, 2, and 3) CVs were analyzed to visualize the oxidation reaction over time, and the oxidation peaks are labeled on the CVs. At line 1 (5 s, black line), only the primary oxidation product is observed. By line 2 (7 s, red line), the secondary oxidation product is observed, and by line 3 (9 s, purple line), the tertiary oxidation product is clearly observed. The representative CVs at each of these lines (1, 2, and 3) demonstrate oxidation peaks growing over time.

Figure 3. Metal nanoparticle electrode modification enhances the electrocatalytic conversion of ATP oxidation products. The oxidation scheme for ATP (A) and a comparison of the color plots and representative CVs of 5 μM ATP over time are shown for bare carbon-fiber (B), AuNP-modified carbon-fiber (C), and PtNP-modified carbon-fiber (D). ATP was manually injected at approximately 5 s and washed away at approximately 9 s. Dashed lines indicate at different time points (time points 1, 2, and 3) CVs were analyzed to visualize the oxidation reaction over time, and the oxidation peaks are labeled on the CVs. At line 1 (5 s, black line), only the primary oxidation product is observed. By line 2 (7 s, red line), the secondary oxidation product is observed, and by line 3 (9 s, purple line), the tertiary oxidation product is clearly observed. The representative CVs at each of these lines (1, 2, and 3) demonstrate oxidation peaks growing over time.
significantly impact the extent to which it interacts at the electrode surface.\textsuperscript{41} Guanine-based purines are easier to oxidize\textsuperscript{41} than adenine-based purines and may be less impacted by surface modifications. This provides additional evidence which supports many prior reports that the finite interactions at the electrode surface are most definitely controlled by not only the electrode surface but also the analyte structure.

3.5. Comparison of Detection Stability at Bare and Metal Nanoparticle Modified Carbon-Fibers

The stability of detection over time is important for monitoring neurochemicals in tissue and to ensure that the modification procedure is stable. Purines have been shown to polymerize on the electrode surface under specific circumstances.\textsuperscript{42,43} We demonstrate that ATP detection is not stable over time on bare carbon-fiber microelectrodes (Figure 4) by repeatedly injecting 5 \( \mu \)M ATP at the electrode 25 times. The current was normalized to the first injection, and deviation from 100\% indicates instability of detection. ATP current decreased by 54\% by the 25th injection at bare carbon-fiber microelectrodes (open circles), with only a 29\% and 13\% decrease in ATP current at AuNP (orange squares) and PtNP (blue circles), respectively. (\( n = 5 − 8 \)).

![Figure 4. Metal nanoparticle-modified electrodes are stable with repeated injections of ATP. 5 \( \mu \)M ATP was repeatedly injected at the electrode 25 times. The current for the primary oxidation peak for ATP was normalized to the first injection and compared over time. Deviation from 100\% indicates instability of detection. ATP detection stability with FSCV monitoring neurochemicals in tissue and to ensure that the modification procedure is stable. Purines have been shown to polymerize on the electrode surface under specific circumstances.\textsuperscript{42,43} We demonstrate that ATP detection is not stable over time on bare carbon-fiber microelectrodes (Figure 4) by repeatedly injecting 5 \( \mu \)M ATP at the electrode 25 times. The current was normalized to the first injection, and deviation from 100\% indicates instability of detection. ATP current decreased by 54\% by the 25th injection at bare carbon-fiber microelectrodes (open circles), with only a 29\% and 13\% decrease in ATP current at AuNP (orange squares) and PtNP (blue circles), respectively. (\( n = 5 − 8 \)).](https://pubs.acs.org/doi/10.1021/acs.measurementau.1c00036/fig/si/05)

![Figure 5. Scanning electron microscopy (SEM) images of bare and metal nanoparticle modified carbon-fibers. (A) Bare carbon fiber, (B) 0.5 mg/mL AuNP-modified carbon-fibers (30 s deposition), and (C) 0.5 mg/mL PtNP-modified carbon-fibers (30 s deposition). Images on the right are at higher magnification to reveal NPs on the surface. Scale bars are shown on the image.](https://pubs.acs.org/doi/10.1021/acs.measurementau.1c00036/fig/si/05)

3.7. Adsorption of ATP at Metal Nanoparticle-Modified Surfaces

Clear differences in surface topology compared to bare carbon-fibers were observed due to the presence of nanoparticles on the surface. Varying topologies can affect the surface adsorption behavior.\textsuperscript{6} Likewise, the nanoparticle can become a nanoelectrode upon contact with the electrode substrate.\textsuperscript{46} In this study, we observe that changes in analyte structure (DA vs ATP) can directly impact adsorption behavior on the metal surface.\textsuperscript{47–50} Many studies suggest that Au and Pt surfaces offer high sensitivity for dopamine detection; however, we did not observe significant differences in oxidative current improvements between these two metals.\textsuperscript{38,45} We confirmed the presence of Au and Pt on the surface by also collecting EDS spectra of the surface (Figure S-7).

3.6. Surface Characterization

Scanning electron microscopy (SEM) with EDAX was used to compare qualitatively and quantitatively the surface of the bare and modified electrodes. SEM images of a bare carbon-fiber (Figure 5A), AuNP-modified carbon-fiber (Figure 5B), and PtNP-modified carbon-fiber (Figure 5C) demonstrate differences in the particle size between Au and Pt. SEM images were taken for four to six electrodes per group, and Figure 5 shows examples for each condition. Topographical differences are evident between the traditional CFME and metal nanoparticles treated CFME. The average nanoparticle diameter for AuNPs was calculated to be 39.8 ± 0.45 nm and 27.4 ± 0.44 nm for PtNPs using ImageJ analysis (Figure S-6). Particle size differences can impact the electroactive area; however, we did not observe significant differences in oxidative current improvements between these two metals.\textsuperscript{38,45}
Scan rates tested ranged from 50 to 800 V s\(^{-1}\). The log of the current vs the log of scan rate was plotted, and the slope of the linear regression provides insight into the limiting reactions at the surface (Figure 6). A slope of 0.5 describes a diffusion-controlled interaction, whereas a slope of 1.0 indicates an adsorption-controlled process.\(^4\) We observed increases in the slope from 0.634 at bare carbon-fiber electrodes to 0.855 (AuNP-modified carbon) and 0.815 (PtNP-modified carbon), indicating changes in the surface interaction to more adsorption-limited processes (n = 6–8).

The surface coverage explains the monolayer surface saturation coverage of the adsorbate on the electrode. The average surface coverage for ATP increased from 7.16 pmol/cm\(^2\) to 42.6 pmol/cm\(^2\) after introducing AuNPs on the carbon-fiber surface, equating to an approximate 495% increase in surface coverage (Figure 7, Table 1). At PtNP-modified electrodes, the surface coverage increased to 74.3 pmol/cm\(^2\) resulting in a average increase of 938% (Figure 7 and Table 1). The thermodynamic equilibrium constant (\(\beta\)) for adsorption helps describe the rate of desorption at the electrode surface. The oxidative current for ATP was recorded as a function of scan rate. Scan rates tested ranged from 50 to 800 V/s. The log of the peak oxidative current (\(i_p\)) is plotted vs the log of scan rate. A slope closer to 1.0 indicates an adsorption-controlled process, whereas a slope closer to 0.5 indicates diffusion-controlled processes. The slope of the line changes from 0.634 at bare carbon (CF, black), AuNP-modified (orange), and PtNP-modified (blue) electrodes (n = 6–8).

**Table 1. Metal Nanoparticle Modified Carbon-Fiber Increase Sensitivity and Adsorption Strength of ATP on the Surface (n = 6–8)**

| electrode            | \(\Gamma_s\) (pmol/cm\(^2\)) | \(\beta\) (x\(^{10}\) pmol\(^{-1}\)) | sensitivity of ATP (µM) | adsorption strength (b, cm) |
|----------------------|-------------------------------|-------------------------------------|------------------------|---------------------------|
| bare CFME            | 7.2 ± 1.1                     | 0.48                                | 0.9 ± 0.1              | 0.30 ± 0.06               |
| PtNP-modified        | 74.3 ± 7.8                    | 0.04                                | 4.2 ± 0.2              | 2.07 ± 0.01               |
| AuNP-modified        | 43.0 ± 4.7                    | 0.06                                | 3.8 ± 0.2              | 1.51 ± 0.02               |

\[\Gamma = \Gamma_s \beta \text{[ATP]} \left(1 + \beta \text{[ATP]}\right)\]  
(1)  
\[i_p = \frac{n^2 \theta F^2}{2.718RT} \nu A \Gamma\]  
(2)  
\[\Gamma_A = b[A]\]  
(3)  
\(\Gamma_A\) is surface concentration, \(\Gamma_s\) is the saturation surface coverage, and \(\beta\) is the thermodynamic equilibrium constant for adsorption. The surface coverage for purines was determined using eq 2 since ATP’s oxidation involves both reversible and irreversible steps.\(^3\) For eq 2, \(i_p\) is the peak oxidative current, \(R\) is the ideal gas constant, \(T\) is the temperature (25 °C), \(F\) is Faraday’s constant, \(\nu\) is the scan rate, \(n^2\) is the number of electrons transferred prior to the chemically irreversible steps, \(\theta\) is the total number of electrons transferred in the redox reaction divided by \(n^2\), and \(A\) is the area of the electrode surface (1.68 \(\times\) 10\(^{-5}\) cm\(^2\) for the 75 µm length cylinder carbon fiber microelectrode and for ATP \(n^2 = 2\) and \(\theta = 3\)).\(^3\) The linear region of the isotherm can be used to calculate \(b\) (eq 3), the equilibrium coefficient which dictates adsorption.

**Figure 6.** Metal nanoparticles change the interaction of ATP at the electrode surface. The oxidative current for ATP was recorded as a function of scan rate. Scan rates tested ranged from 50 to 800 V/s. The log of the peak oxidative current (\(i_p\)) is plotted vs the log of scan rate. A slope of 0.5 describes a diffusion-controlled process, whereas a slope of 1.0 indicates an adsorption-controlled process.\(^4\) A slope closer to 1.0 indicates adsorption-controlled processes, and a slope closer to 0.5 indicates diffusion-controlled processes. Figure 6, n = 6–8. At the bare carbon-fiber, ATP interactions are governed primarily by diffusion; however, at metal nanoparticle-modified carbon, ATP’s interactions become more adsorption-limited.

**Figure 7.** Higher sensitivity, surface coverage, and stronger adsorption strength are observed for ATP at metal-nanoparticle modified carbon-fiber microelectrodes. (A) The oxidative current for ATP was measured for concentrations ranging from 1 µM to 100 µM (B). The linear region of the curve spanned from 1 µM to 10 µM ATP and was used to compare the sensitivity of each electrode type for ATP (\(r^2\) Bare CF = 0.9384, \(r^2\) AuNP = 0.9588, \(r^2\) PtNP = 0.9864). (C) Langmuir isotherms for ATP at the bare carbon-fiber (CF, black), AuNP-modified (orange), and PtNP-modified (blue) electrodes (n = 6–8).
electrode surface. β decreased by 12-fold at PtNP-modified electrodes and by 8-fold at AuNP-modified, which suggests that the rate of desorption of ATP is faster at metal nanoparticle modified electrodes. This result may help elucidate why less ATP fouling is observed at metal nanoparticle-modified carbon electrodes. For the adsorption strength (β), the number increased 5-fold and 6.9-fold for AuNP and PtNP, respectively, compared to bare carbon fiber. This result shows that ATP adsorbs strongly to metal nanoparticle-modified surfaces, but the oxidation product desorbs quickly. The sensitivity (Figure 7B, Table 1) improved 4.7-fold at PtNP-modified electrodes and 4.3-fold at AuNP-modified electrodes compared to the traditional bare carbon-fiber. Higher sensitivity measurements of ATP are crucial for applications in vivo and demonstrates that metal-nanoparticle-modified surfaces could be a critical advance in real-time ATP sensing.

3.8. Detection of Exogenous ATP in Lymph Node Slices Using Metal Nanoparticle Electrodes

The AuNP and PtNP-modified electrodes were used to detect exogenous ATP in slices of live murine mesenteric lymph node. ATP is known to be the “currency” of biological processes and has been monitored within the immune system to assess sensitivity to allergens and characterize T-cell motility.51,52 Likewise, ATP is copackaged with norepinephrine in sympathetic neurons which innervate lymphoid organs and can be rapidly released to modulate immune signaling. Despite this knowledge, subsecond detection of ATP release in the lymph node has not been explored, introducing a fundamental gap in our understanding of rapid ATP regulated immune processes. To validate detection within biological tissue and demonstrate the relative power of the nanoparticle modifications,12,13,53 both AuNP and PtNP-modified electrodes were implanted in mesenteric lymph node slices approximately 100 μm away from a picospritzing pipet. The pipet was backfilled with 150 μM ATP; large concentrations are common for this procedure due to diffusional loss of analyte in tissue, with a few micromolar actually reaching the electrode surface.30 A short bolus was administered to the tissue to mimic a rapid endogenous event. Detection was successful using both the AuNP and PtNP-modified electrodes; ATP was detected in four slices using seven AuNP-modified electrodes and in five slices using seven PtNP-modified electrodes (Figure 8). Example CVs and color plots for ATP using the AuNP (Figure 8A) and PtNP-modified electrodes (Figure 8B) are comparable to in vitro data. The primary (1°) and secondary (2°) oxidation peaks are observed; small shifts in peak potential are a common phenomenon when detecting adenine-based purines within a biological matrix.54,55 Likewise, differences in the current magnitude between the two electrodes are likely due to the inability to precisely place the picospritzing pipet at the same location in the X- and Z-directions from the implanted electrode across all trials. Electrodes which are closer to the picospritzing pipet will measure larger concentrations than ones further away. Because of this, the oxidative current for ATP detected at each electrode was variable ranging from 5 to 30 nA. Rapid decay of the signal over time indicates native metabolism and uptake of ATP within the lymph node slice.

3.9. Multiplexed Detection and Biofouling at Metal-Modified Carbon-Fiber Microelectrodes

ATP is copackaged with norepinephrine (NE) in vesicles in the lymph node, which can be coreleased to modulate immunity. To demonstrate that our metal-modified electrodes are capable of codetecting both NE and ATP and this co-detection is improved compared to bare CFMEs, we tested a mixture of 5 μM ATP with 1 μM NE at each electrode type (Figure S-8). Minimal oxidative peak for ATP is observed when in a mixture with NE at bare carbon-fibers; however, both oxidation peaks are clearly observed at AuNP-modified (Figure S-8) and PtNP-modified electrodes (Figure S-8). This demonstrates the utility of these electrodes for multiplexed detection in the lymph node in the future.

Nonspecific adsorption of proteins and bioproducts on the electrode surface can lead to biofouling. To test the stability of metal-modified and bare carbon-fiber electrodes in tissue, we measured the change in oxidative current for both DA and ATP after soaking in homogenized tissue. Electrodes were pretested with 5 μM ATP and 1 μM DA and then soaked in homogenized lymph node tissue for 1 h. Following soaking, the electrodes were post-tested, and the ratio of the post-test current to pretest current was plotted (inset: pre) as a function of electrode type (bare carbon fiber, AuNP-, and PtNP-modified). Overall, we observed reductions in measured current for all electrodes indicating that all electrodes experience biofouling in tissue (Figure S-9). The ratio of post-test current to pretested current for 5 μM ATP and 1 μM DA is 0.37 ± 0.11 and 0.63 ± 0.03 on bare carbon-fibers. On AuNP, the ratio is 0.38 ± 0.16 for ATP and 0.62 ± 0.03 for DA. For the PtNP treated electrodes, the ratio is 0.80 ± 0.10 for DA and 0.46 ± 0.13 for ATP. The decreases in current for metal-modified electrodes were not significantly different from bare carbon-fibers for both ATP (one-way ANOVA, F(2,18) = 0.138, p = 0.873, n = 6) and DA (one-way ANOVA, F(2,18) = 1.1817, p = 0.204, n = 6). Overall, these results indicate that metal-modified surfaces do not provide an improvement for the impacts of biofouling compared to traditional carbon-fibers; however, future work focusing on nanostructuring the

![Figure 8. Exogenous detection of ATP within mesenteric lymph node slices at both AuNP and PtNP-modified electrodes. (A) Example color plot, current vs time (top) and CV (bottom) for exogenous ATP at a AuNP-modified electrode. The blue arrow indicates when ATP was pressure-ejected into the tissue. Both the primary (1°, 1.2 V) and secondary (2°, 1.0 V) oxidation peaks are on the CV for ATP. (B) Example color plot, current vs time, and CV for exogenous ATP at a PtNP-modified electrode. The primary (1°, 1.2 V) and secondary (2°, 1.0 V) peaks are visible on the CV for ATP.](https://doi.org/10.1021/acsmeasuresciu.1c00026)
metal nanoparticle surfaces could provide better biofouling resistance, according to previous reports.  

4. CONCLUSION

Electrodeposition is an easy method to introduce metal nanoparticles on carbon−fiber microelectrode surfaces to facilitate studies of analyte−metal nanoparticle interfaces and to advance fundamental understanding of nanoscale electrochemical reactions and kinetics. Here, we show that the surface structure and catalytic properties of metal nanoparticles enhance ATP detection with FSCV. Exogenous detection within living lymph node tissue was shown to be feasible using both nanoparticle types. This work provides critical insight into the mechanisms of ATP interaction at electrode surfaces, which will enable strategic design of ultrasensitive electrodes for real-time ATP sensing in the future.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/10.1021/acsmeasuresciu.1c00026.

Optimization of metal electrodeposition procedures, control experiments, assessment of changes in temporal resolution after modification, quantification of ATP oxidative conversion at the electrode, analysis of other purines at modified surfaces, quantification of approximate metal nanoparticle size, EDS spectra, codetection of ATP with norepinephrine, and biofouling data (PDF)

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Notes

The authors declare no competing financial interest.

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