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Curvature-Mediated Surface Accessibility Enables Ultrasensitive Electrochemical Human Methyltransferase Analysis

Guangli Wang,[†,∥] Jagotamoy Das,[†] Sharif Ahmed,[†] Carine R. Nemr,[‡] Libing Zhang,[†] Mahla Poudineh,[§] Edward H. Sargent,[§] and Shana O. Kelley*[,†,‡]

[†]Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON M5S3M2, Canada

[‡]Department of Chemistry, Faculty of Arts and Sciences, University of Toronto, Toronto, ON M5S3M2, Canada

[§]Department of Electrical and Computer Engineering, Faculty of Engineering, University of Toronto, Toronto, ON M5S3M2, Canada

Supporting Information

ABSTRACT: The development of new tools for tracking the activity of human DNA methyltransferases is an important goal given the role of this enzyme as a cancer biomarker and epigenetic modulator. However, analysis of the human DNA (cytosine-5)-methyltransferase 1 (Dnmt1) activity is challenging, especially in crude samples, because of the low activity and large size of the enzyme. Here, we report a new approach to Dnmt analysis that combines nanostructured electrodes with a digest-and-amplify strategy that directly monitors Dnmt1 activity with high sensitivity. Nanostructured electrodes are required for the function of the assay to promote the accessibility of the electrode for human Dnmt1. Moreover, DNA-templated deposition of silver nanoparticles (for signal amplification) is combined with DNA Exonuclease I digestion to yield optimal target-to-control signals. We achieve high sensitivity for the detection of human Dnmt1, and particularly Dnmt1 from crude cell lysates. Specifically, the detection limit of our electrochemical assay is 20 pM, which is 2 orders of magnitude lower than previously reported methods. In crude lysates, we detected Dnmt1 from as few as five colorectal cancer cells (HCT116). With biopsy samples, we were able to distinguish colorectal tumor tissue from healthy adjacent tissue using only 10 μg of sample. The strategy enables analysis of an important marker underlying the epigenetic basis of cancerous transformation.

KEYWORDS: *human methyltransferase, Dnmt1, nanostructured electrode, cancer, electrochemistry*

Deoxyribonucleic acid (DNA) methylation, the predominant epigenetic modification in mammalian cells, plays critical roles in gene expression, chromatin structure modulation, and genomic integrity.[1,2] The methylation of cytosine bases in DNA is catalyzed by a group of enzymes known as DNA methyltransferases (DNMTs). The abnormal expression/activity of DNMTs is linked to epigenetic alteration (hypermethylation or hypomethylation) of the genome that can lead to tumorigenesis.[3−6] Abnormalities in DNMT activity usually occur far before other signs of malignancy and could thus potentially be used as a biomarker of early stage disease.[7−9] A growing body of evidence demonstrates that human DNA (cytosine-5)-methyltransferase 1 (Dnmt1), the most abundant DNMT in somatic cells, plays an important role in cancer initiation, progression and recurrence[5,9−15] and is thus a promising target for discovering the epigenetic basis of cellular transformation and to meet the demand for cancer diagnosis, prevention, and treatment.

Gold standard methods to probe the activity of DNMTs are based on radioactive labeling,[16,17] a method that is not readily implemented for clinical testing. Commercially available enzyme-linked immunosorbent assay (ELISA) kits[18] are proposed as a replacement for radioactive methods; however, these assays can only detect purified DNMTs or the total activity of human DNMTs in real biological samples.

Electrochemical techniques represent promising solutions for bioanalysis in view of their sensitivity, rapidness, cost-effectiveness, and easy miniaturization.[19] Electrochemical assays have been developed to detect purified bacterial DNA methyltransferases;[20−22] however, electrochemical protocols for the detection of human Dnmt1 are at their early stages.[23,24] New electrochemical sensors are needed for label-free detection[24] of Dnmt1 in complex biological matrices that preserve the sensitivity, precision, and speed of detection.

Human Dnmt1 is a large enzyme (∼200 kDa) that exhibits a low level of activity.[25,26] These properties present a challenge

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in the development of electrochemical techniques where the enzyme must bind to and react with DNA on an electrode surface. Assays for human Dnmt1 detection reported previously were performed on planar electrodes and were not able to achieve sensitivity comparable to conventional radioactive methods or commercially available ELISA kits. Evaluating the activity of Dnmt1 using electrochemical methods, especially in complex biological matrices, remains an unsolved problem.

Here, we describe an electrochemical assay for human Dnmt1 activity. Three-dimensional nanostructured microelectrodes (NMEs) were used to facilitiate the display of DNA on the elctrode surface to improve the accessibility of Dnmt1 to recognition sites. NMEs have previously advanced related problems of accessibility and sensitivity in other biosensing assays[27] and especially for nucleic acid detection[28−30] in bacterial lysates and serum. However, the use of these sensors for the detection of DNA-modification related catalytic enzymes remains unexplored. The highly curved features of these structures may provide an ideal environment to promote the interaction of a large enzyme with a DNA substrate: the curved surfaces provide a larger deflection angle between DNA probes[31] and thereby promote higher surface coverage as well as decreased DNA probe aggregation.[32] Here, we coupled NMEs with DNA-templated metallization in order to improve sensitivity of the sensors. The resultant assay exhibits excellent performance with low signal-to-background levels and high levels of sensitivity compared to previously reported methods. We show that the technique leads to sensitive detection of Dnmt1 in complex biological samples, and does not require labeling enzymes or radioactive agents for signal amplification.

EXPERIMENTAL SECTION

Materials. The oligonucleotides employed in this work were purchased from Integrated DNA Technologies (Coralville, USA). The sequences are as follows: probe used for immobilization on the NME: 5′-HS-(CH2)6-*TTT TTT* ACA TGT GCG CGC ATC TCT CCC-3′ (the italicized portion of the sequence does not hybridize with the complementary sequence), partially complementary sequence (of the probe) with hemimethylated base, 5′-GGG AGA GAT GCG mCGC ACA TGT-3′ and partially complementary sequence with unmethylated base 5′-GGG AGA GAT GCG CGC ACA TGT-3′. The recognition site for *BssH*II is underlined. These oligonucleotides were dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) to the desired concentrations and stored at −20 °C. Duplex DNA (as the substrate of Dnmt1) was prepared by heating the mixture of the two oligonucleotides (with equally molar concentrations) and 5 mM TCEP (with the molar ratio of DNA/TCEP of 1:100) in a hybridization buffer (10 mM Tris, 1.0 mM EDTA, 1.0 M NaCl, pH 8.0) to 90 °C for 5 min and then the solution mixture was slowly cooled down to room temperature. TCEP is used to cleave disulfide bonds. This duplex DNA, substrate of Dnmt1, was ready to be immobilized on to the sensor surface of structured electrodes.

Human Dnmt1 was purchased from Active Motif (Carlsbad, CA). The restriction endonucleases (*BssH*II), Exonuclease I (*E. coli*), and *S*-adenosyl-L-methionine (SAM) were purchased from New England Biolabs (Hitchin, England). To avoid the disruption of dithiothreitol (DTT) for the DNA modified electrodes, Dnmt1, *BssH*II, and Exo I were transferred into new buffers using size exclusion column (10 kDa, Amicon) at 4 °C. Dnmt1 was transferred into the methylation buffer containing 50 mM Tris-HCl (pH 7.8 @ 25 °C), 1 mM EDTA, 5% glycerol and 100 μg/mL BSA. *BssH*II was transferred into a 1× CutSmart buffer (50 mM potassium acetate, 20 mM tris-acetate, 10 mM magnesium acetate, 100 μg/mL BSA, pH 7.9 @ 25 °C). Exo I (*E. coli*) was transferred into the Exonuclease I reaction buffer (67 mM glycine-KOH, 6.7 mM $MgCl_2$, pH 9.5 @ 25 °C). Silver nitrate, sodium borohydride (98%), and hydroquinone were purchased from Sigma-Aldrich (St. Louis, MO).

Sensor Chip and NME Fabrication. Fabrication of the chips and NMEs were reported in our published papers. Briefly, Chips were fabricated at Advanced Micro Systems (Ottawa, Canada). Silicon wafers (six-inch) were passivated by a thick layer of thermally grown SiO_2 followed by coating with a 25 nm Ti adhesion layer. A 350 nm gold layer was deposited on the chip using electron-beam-assisted Au evaporation, which was again coated with 5 nm of Ti. The electrodes were patterned employing standard photolithography and a lift-off process. A 500 nm layer of insulating Si_3N_4 was deposited using chemical vapor deposition. The 5 μm apertures were imprinted at the tips of the metal leads through standard photolithography. Contact pads (0.4 mm × 2 mm) were exposed through wet etching.

Chips were oxygen plasma etched for 60 s. Subsequently, they were cleaned by sonication in acetone for 5 min, rinsed with isopropyl alcohol, and DI water, followed by drying with nitrogen. Electrodeposition was performed at room temperature employing the fabricated silicon chips with 5 μm apertures as the working electrode. Contact to the electrodes was achieved through exposed bond pads. First, a gold structure was generated by electroplating in a solution containing 50 mM $HAuCl_4$ (Sigma-Aldrich, MO) and 0.5 M HCl (Sigma-Aldrich, MO) at 0 mV for 100 s using DC potential amperometry. After washing with DI water and drying, the electrodes were replated in a solution of 20 mM $HAuCl_4$ and 0.5 M $HClO_4$ at −400 mV for 3 s to generate nanofeatured at the electrodes.

Dnmt1 Activity Assay. The immobilization of the DNA substrate was performed by applying 20 μL of 50 μM DNA substrate on the NME and incubating for 20 h at room temperature in a dark humidity chamber. The electrode was then washed with the washing buffer (10 mM Tris, pH 8.0) and ultrapure water sequentially to remove the nonspecifically adsorbed DNA. Subsequently, the electrode was treated with 1.0 mM mercapto hexanol (MCH) for 1 h at room temperature to backfill and to obtain a well-aligned monolayer of DNA.

To achieve the methylation at the CpG dinucleotide sites of the immobilized DNA, the DNA-modified sensors were first incubated with 20 μL of methylation buffer containing a specific concentration of Dnmt1 and 160 μM SAM at 37 °C for 1 h. The substrate electrode array was then treated with 5 mg/mL protease solution (in 5 mM phosphate, 50 mM NaCl, pH 7.0) for 0.5 h. After washing with the methylation buffer, the electrode was then incubated with 20 μL of 1,500 units/mL *BssH*II restriction endonuclease (in 1× CutSmart buffer) at 37 °C for 40 min to cleave the unmethylated DNA. After another round of washing with CutSmart buffer and drying, the electrode was incubated in 20 μL of 2 U/μL Exo I solution at 37 °C for 15 min. The surface was then washed by the Exo I reaction buffer and then washed by ultrapure water thoroughly, which was ready for the electrochemical measurements.

For electrochemical detection, the chip was initially immersed in 2.0 mM $AgNO_3$ in ultrapure water for 15 min and followed by washing with water. This allowed the adsorption of silver ions on the DNA skeleton. Subsequently, the chip was dipped into a freshly prepared $NaBH_4$ solution (20 mM in ultrapure water) for 7 min and washed thoroughly with water. After that the chip was emerged in a citrate buffer (pH = 3.5) solution containing 1.0 mM $AgNO_3$ and 2.0 mM hydroquinone under low light condition for 3 min for signal enhancement. The prepared $AgNO_3$ and hydroquinone solutions were filter by a filter membrane to remove any particulate matter. Finally, the chip was placed in a 0.2 M KI solution with 0.1 M $NaNO_3$ as supporting electrolyte for the electrochemical measurements from −0.6 to −0.1 V versus a saturated Ag/AgCl reference electrode.

Dnmt1 Extraction from Cultured Cells and Tissue. Different media were used for culturing different cell lines: parent HCT116 cells were grown in McCoy's 5a media containing 10% FBS; CCD-18Co and HeLa were grown in Eagle's Minimum Essential Medium (EMEM) with 10% FBS; MCF-7 were grown in 0.01 mg/mL human recombinant insulin and 10% FBS. All these cell lines were grown in tissue culture flasks in a 37 °C container under a humidified

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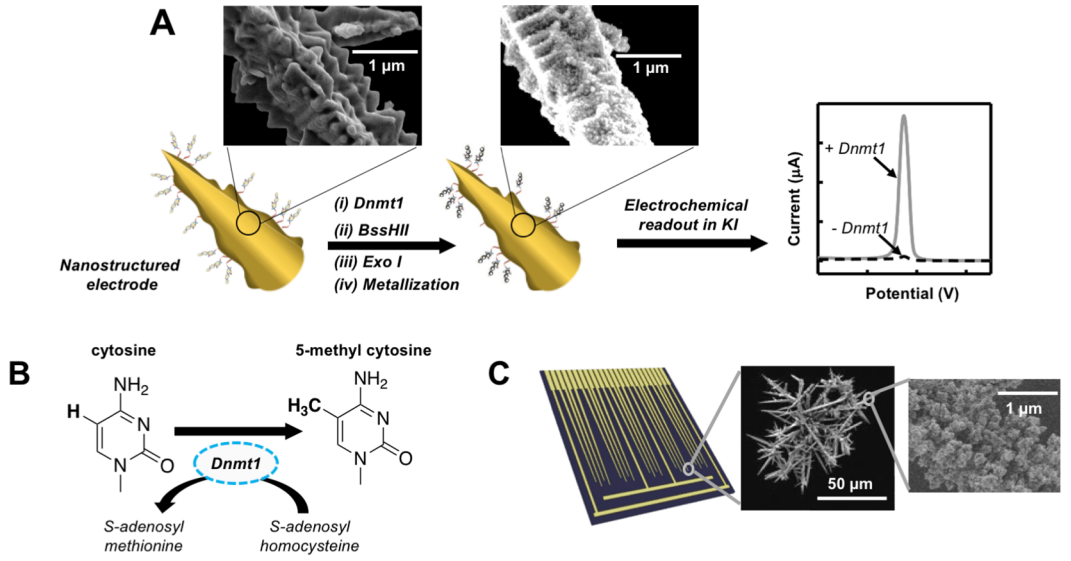

Figure 1. Electrochemical assay for the activity of human methyltransferase. (A) Schematic representing the use of nanostructured electrodes for Dnmt1 detection through DNA-induced metallization. Dnmt1, the restriction enzyme *BssH*II, and Exo I are sequentially applied to the hemimethylated DNA substrate-modified nanostructured microelectrodes (NMEs). Dnmt1 methylates hemimethylated DNA substrates. Only the substrates fully methylated by Dnmt1 are prevented from cleavage by *BssH*II treatment whereas any remaining hemimethylated DNA substrates are digested by *BssH*II. Exo I then digests any single-stranded DNA generated after *BssH*II treatment. Therefore, only fully methylated substrates remain intact to template for the deposition of silver nanoparticles (AgNPs) to generate a sharp electrochemical peak in potassium iodide (KI). SEM images of the gold structures b) before and c) after duplex templated silver deposition. The white features on the Au structured electrode represent AgNPs. See Figure S1 for a more detailed scheme of the detection strategy. (B) Methylation of the cytosine base in the CpG islands catalyzed by the Dnmt1. (C) Layout of the chip. The micropatterns of a microfabricated chip (left), SEM image of an NME (middle) and a magnified SEM of the NME (right) showing nanostructured features.

atmosphere containing 5% CO_2. Approximately one to two million of cells in the exponential phase of growth were harvested from adherent cell culture by trypsinization, followed by washing with cold PBS and pelleting through centrifugation at 500*g* for 5 min. The cells were lysed at 4 °C using a nuclear and cytoplasmic protein extraction kit (Pierce from Thermo Scientific) including a protease inhibitor. The obtained supernatant cell lysate was immediately aliquoted, flash frozen and stored at −80 °C for use. The total amount of protein in the frozen lysate was quantified by a bicinchoninc assay (Pierce), which was found to be 1000−2200 μg/mL.

Snap-frozen tissue samples of colorectal carcinoma as well as its healthy adjacent tissues were obtained from CureLine (Brisbane, CA). Approximately 20 mg of tissue was homogenized with a 2 mL dounce tissue grinder, followed by nuclear extraction using the same nuclear and cytoplasmic protein extraction kit (Pierce from Thermo Scientific) as described above.

The final obtained cell lysates (in buffer) from cultured cells or the biopsied tissue were transferred into Dnmt1 methylation buffer (50 mM Tris·HCl, 1 mM EDTA, 5% glycerol, pH 7.8) through the size exclusion spin column (10 kDa cutoff; Amicon) before the electrochemical assays.

RESULTS AND DISCUSSION

Methylation of DNA by Human Dmnt1 on a High-Curvature Electrode. Human Dmnt1 catalyzes the covalent attachment of a methyl group to the C-5 position of cytosine within double-stranded DNA, using *S*-adenosyl-methionine (SAM) as a methyl donor (Figure 1). We hypothesized that the relatively low senstivity of prior assays for Dmnt1 reflected the crowded surface environment of planar electrodes, which would limit the accessibility of Dnmt1 to immobilized DNA due to steric hindrance. This could lead to limited sensitivity because of the low methylation efficiency even with longer reaction times (ca. 2 h).[23] Though using a sparse DNA monolayer enables the access of Dnmt1 to its recognition sites, the decreased level of signal generated using this approach prevents the improvement of detection sensitivity.[24] To circumvent this problem, we incorporated high-curvature nanostructured features to enhance the accessibility of the Dnmt1 to the recognition sites of the DNA substrate.

To probe the activity of human Dnmt1, a hemimethylated DNA substrate (i.e., double-stranded DNA where one of the two strands is methylated) specific to Dnmt1 was immobilized on the surface of the microelectrodes. In light of the preference of Dnmt1 for hemimethylated substrates over unmethylated ones,[10,16] we designed hemimethylated DNA probes for Dnmt1 detection. The Dnmt1 enzyme present in the sample methylates the 5′-CG-3′ site to form a fully methylated DNA substrate. After methylation, the restriction enzyme B*ssH*II is applied to cleave the hemimethylated duplex, allowing only fully methylated duplex to remain on the electrode surface. The intact DNA that reflects the methylation levels and hence the activity of the Dnmt1 is used to accumulate the electroactive species for signal readout as described below.

DNA-Templated Metallization. To improve the sensitivity, we use a modified DNA-templated metallization strategy[34] for signal readout. DNA binds Ag^+ via the anionic phosphate groups, amino group base functionalities, and the heterocyclic nitrogen atoms within the bases.[35,36] The Ag^+ ions bound to the DNA substrate are reduced to metallic clusters.[37,38] The DNA templated silver clusters can then be used as nucleation sites for further deposition of silver tracers in the presence of Ag^+ and hydroquinone,[34] amplifying the signal through the accumulation of more metal tracers. Scanning electron microscopy (SEM) (Figure 1A) and X-ray photoelectron spectroscopy (XPS) (Figure S2) provided further evidence that DNA-induced metallization forms silver

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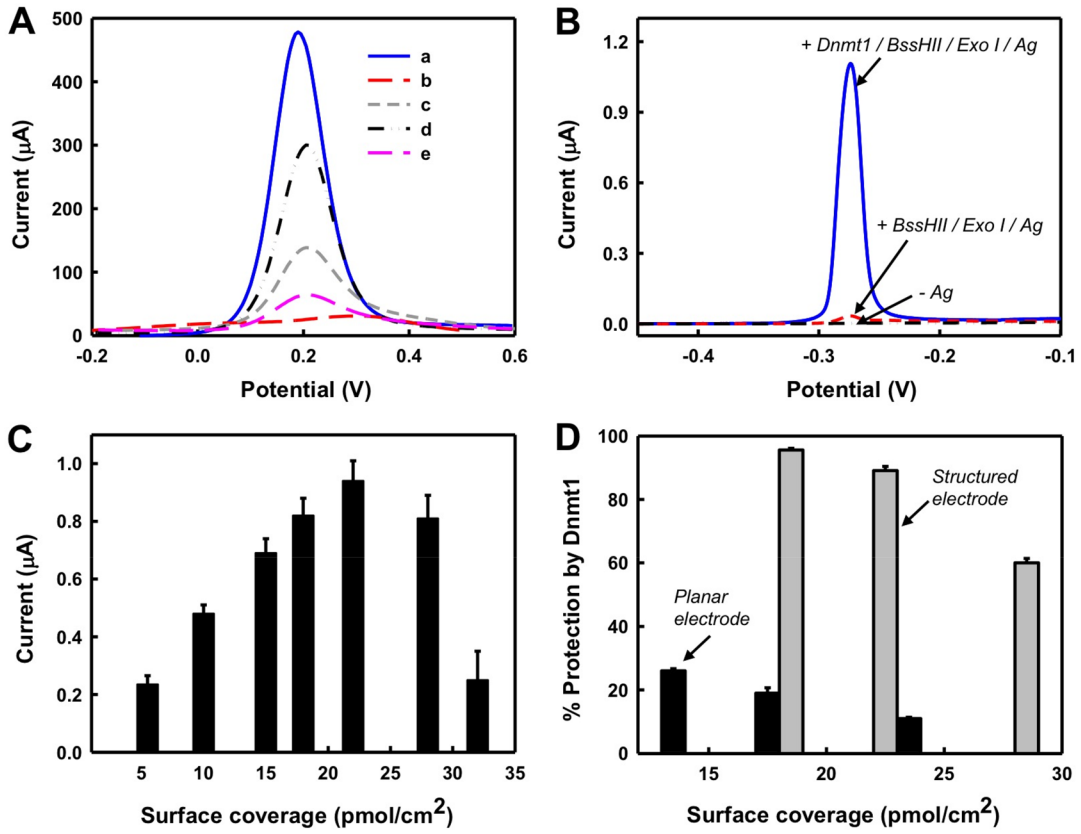

Figure 2. Validation and optimization of the electrochemical assay for Dnmt1 detection. (A) Differential pulse voltammograms (DPVs) of different electrodes in 0.10 M PBS solution containing 5.0 mM $Fe(CN)_6^{3-/4-}$ (1:1 ratio): (a) bare NME; (b) the electrodes after immobilizing the duplex and backfilling with mercaptohexanol (MCH); the duplex/MCH-modified electrodes are treated (c) by the *BssH*II alone, (d) by *BssH*II and Exo I (*BssH*II/Exo I), or (e) sequentially treated by Dnmt1, *BssH*II and Exo I (Dnmt1/*BssH*II/Exo I). (B) Voltammetric responses from differently modified electrodes in NaI solution: DNA modified electrodes without silver deposition (black line); DNA modified electrodes after *BssH*II/Exo I (red line) or Dnmt1/*BssH*II/Exo I (blue line) treatment followed by silver deposition. Scan rate is 50 mV/s. (C) Signal response of the NME sensor when challenged with 20 nM Dnmt1 target at different density of the DNA substrate. (D) Percent protection of the DNA substrate on the sensor surface after challenging with Dnmt1, *BssH*II, and Exo I sequentially at different density of the DNA substrate on the sensor based on planar gold electrodes (PGEs, black bar) and NMEs (gray bar). The % protection represents protection efficiency by Dnmt1. The percent protection was obtained by measuring DNA substrate density on the electrode surface before and after treatment with Dnmt1, *BssH*II, and Exo I.

nanoparticles (AgNPs) on the electrodes that are approximately 20−30 nm in size.

Background Suppression. In order to achieve a high signal-to-background ratio, it is critical to minimize the background signal. The remaining DNA segments cleaved by Bss*H*II would produce a high background signal, deteriorating the sensitivity of detection. Therefore, an oligonucleotide substrate for Dnmt1 was designed to reduce the background signal with the assistance of Exo I, which can digest only single-stranded DNA (ssDNA).[33] After cleavage by B*ssH*II, the DNA substrate with a short segment containing six nonhybridized bases (5′-TTT TTT-3′) and 6 base pairs (5′-ACA TGT-3′ and its complementary sequence) remains at the electrode surface. Given that the melting temperature of this short segment DNA is quite low (∼16 °C), it is expected that this short segment DNA will be dehybridized under the detection conditions leaving a single-stranded DNA (ssDNA) on the electrode surface. The Exo I enzyme suppresses the undesired background signal by digesting ssDNA, while the fully methylated DNA substrate remains intact on the sensor surface. The signals generated at different steps in the detection strategy were monitored by differential pulse voltammetry (DPV) (Figure 2A) based on the electron transfer of the $Fe(CN)_6^{3-/4-}$ redox probe, which reflects the surface states at different stages of the procedure.

Oxidation of AgNPs as a Final Readout of Dmnt1. To avoid interfering signals that could be generated by reducing species present in biological samples, conditions were used that shifted the oxidation potential of AgNPs to more negative potentials. Solid−state voltammetry of AgNPs in chloride solution has proven to be simple and highly characteristic in electrochemical assays.[39,40] The electrochemical signal of the DNA templated AgNPs was tested in 0.2 M solutions containing different halides (chloride, bromide, and iodide). Two well-separated sharp peaks were observed (Figure S3), which are attributed to the electrochemical oxidation of the Ag metal to silver halide and reduction of silver halide back to Ag. This solid state electrochemistry shows that the oxidation potential of the AgNPs shifts to more negative values by changing electrolytes from NaCl to NaBr or NaBr to NaI, which corresponds with the variation trends of the standard electrode potential ($E°$) of the Ag/AgCl, Ag/AgBr, and Ag/AgI redox couples.[41] The low oxidation potential of AgNPs in iodide solution (−275 mV versus saturated Ag/AgCl) eliminates the interference of the reducing species existing in biological matrices and hence improves the precision and sensitivity of the assay.

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In the DNA-mediated metallization process, the silver ion conjugation step is highly selective and is restricted to the DNA template, limiting nonspecific signals. The silver enhancement aims to deposit more silver tracers on the initially formed silver clusters, significantly amplifying the signal while avoiding the spontaneous nucleation onto other parts of the assay. We chose hydroquinone as a selective reducing agent which is unable to reduce Ag^+ itself; however, it can effectively participate in the particle growth in the presence of metal clusters.[42] In order to reduce undesired contributions to the background, the concentration of silver enhancer solution (Ag^+ and hydroquinone aqueous solution) and the enhancement time were adequately optimized. As Figure 2B demonstrates, after the DNA-mediated metallization process, a negligible peak current is obtained from the *BssH*II/Exo I treated hemimethylated DNA-modified electrode, indicating that the background signal is successfully suppressed. A high signal-to-background ratio is achieved after Dnmt1/*BssH*II/Exo I treatment, which offers appealing analytical performance.

Influence of Electrode Nanostructuring and Surface Density. Subsequent to the validation of the electrochemical assay, we investigated the influence of the surface density of the hemimethylated DNA substrate on the analytical performance (Figure 2C). The best signal response was attained at a relatively high substrate density (22 pmol/cm^2). The surface area of the NME was estimated to be $(2.6 \pm 0.4) \times 10^{-4}$ cm^2, as previously described.[43] The densities of the DNA on the electrode surface obtained with different concentrations of DNA probe solutions and different immobilization times were analyzed according to the reported chronocoulometric method.[44] Assessing the surface density and the methylation efficiency of Dnmt1 on its DNA substrate (Figure S4) confirmed that the high-curvature sensors improved the methylation efficiency compared with planar electrodes (Figure 2D). The highly anisotropic nature of NMEs with curved branches protruding out into solution appears to overcome the accessibility barrier of Dnmt1 associated with dense DNA packing, facilitating the efficient methylation of Dnmt1 in a relatively short time (1 h).

After optimizing the detection conditions, we assessed the sensitivity of our method by investigating the dependence of the signal on concentration of Dnmt1 in buffered solution (Figure 3A). In order to determine the detection limit, we examined concentrations of Dnmt1 ranging from 20 pM to 20 nM. As shown in Figure 3B, the current increased with increasing concentration of Dnmt1, and the detectable signal can be observed with concentrations as low as 20 pM. Furthermore, the method is highly reproducible. At least four individual and independent experiments were run for each conditions and the low level of variability is represented by the error bars shown in Figure 3B. These results demonstrate that the detection limit of our method is 2 orders of magnitude lower than previously reported sensors.[23,24] Moreover, our method is the first technique to exhibit better sensitivity than the conventional radioactive[16,17] or ELISA-like kits[18] for detection of large and low active Dnmt1.

Cancer Cell and Tumor Sample Analysis. The ultimate goal of this work was to analyze unpurified samples such as tumor tissue. However, analysis of unpurified samples is always challenging due to their heterogeneity and complexity: in particular, direct analysis of crude cell lysates or tumor tissue by electrochemistry is often impeded by large background signals. We challenged our assay with unpurified, lysed

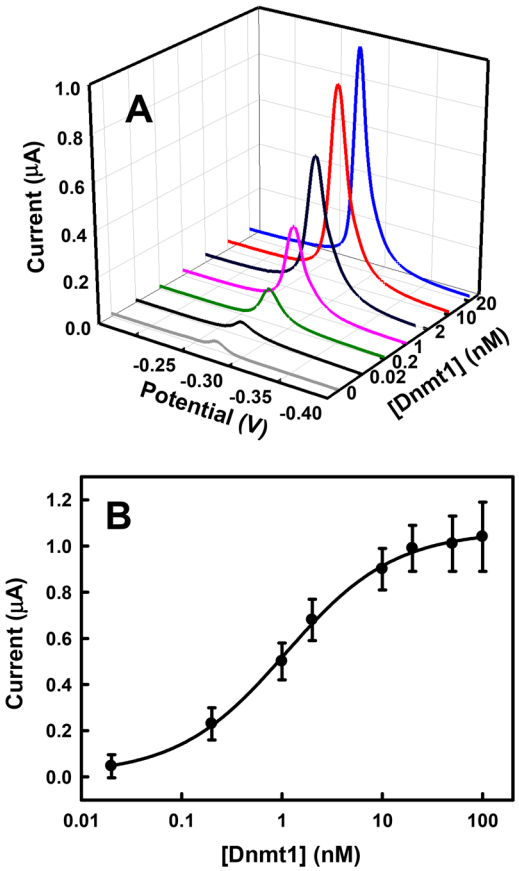

Figure 3. Evaluation of performance of the assay for Dnmt1 in buffered solution. (A) Current–potential plots demonstrating the signal intensity at different concentrations of Dnmt1. (B) Concentration dependence of the signal for Dnmt1 on a logarithmic scale.

colorectal carcinoma cells (HCT116) as a model system. Several studies have demonstrated a link between Dnmt1 and tumorigenesis in this cell line.[5,9,12−15] The detection of as few as five HCT116 cells, present as a crude lysate, indicates that the assay is highly sensitive and robust (Figure 4A). Three-dimensional NMEs coupled with the DNA-induced metallization offers highly sensitive detection of Dnmt1 in crude biological samples.

Further, to demonstrate that our assay is a general and reliable protocol for Dnmt1 detection, we analyzed two more cancer cell lines: MCF-7 (breast adenocarcinoma cells) and HeLa (human cervical cells). The Dnmt1 activity expressed by the cancer cells was measured and the results were normalized to the activity of CCD-18Co cells (a normal colon cell line) (Figure 4B). The measured relative Dnmt1 activity of cancer cells is greater than 1, providing evidence that Dnmt1 is overexpressed in human cancer cells.[3] We further evaluated human biopsy tissue samples purchased from a commercial source. The colorectal carcinoma tissue as well as the adjacent healthy tissue were collected and stored by snap freezing and the cell lysates were extracted (Experimental Section). Typical colon punch biopsies yield 350 mg of tissue; however, the electrochemical assay is able to distinguish the colorectal tumor tissue from healthy adjacent tissue using only ∼10 μg tissue sample per electrode (Figure 4C). This illustrates the effectiveness of the detection platform for demanding clinical applications.[45] The electrochemical assay presented has improved sensitivity for Dnmt1 detection in crude cell lysates

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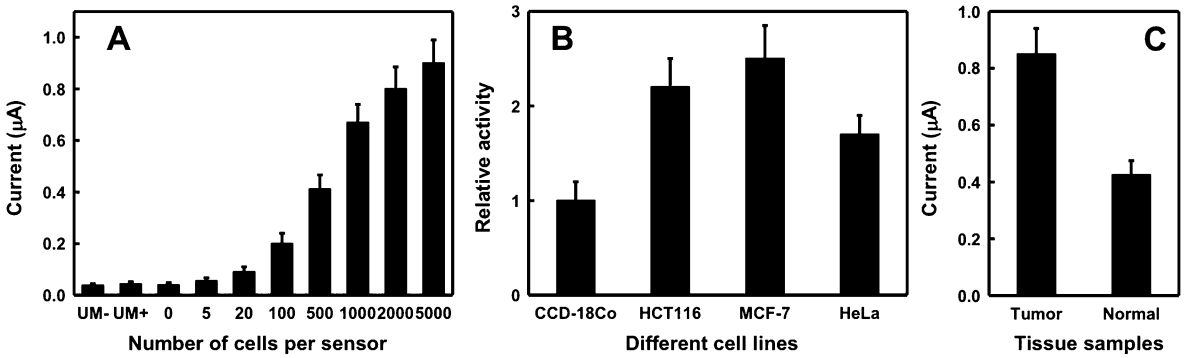

Figure 4. Analysis of different cancer cell lines and tumor tissue. (A) Sensitivity of the NME sensor to analyze crude cell lysates. Current response from lysates of different numbers of cells of HCT116 cancer cell lines. UM− and UM+ represent signal obtained with unmethylated DNA substrate-modified sensors in absence and presence of cells lysates, respectively. (B) Analysis of different cancer cell lines. Normalized activity of colorectal carcinoma HCT116, breast adenocarcinoma MCF-7, and cervical carcinoma HeLa in comparison with the normal colon CCD-18Co cultured cells. The relative activity was measured by the developed electrochemistry using 1000 cells from each and was calculated from the ratio of the Dnmt1 concentrations. The concentration of Dnmt1 was obtained by comparing signal obtained from the cell lysates with the calibration plot. See Figure S5 for comparative titrations. (C) Analysis of tumor tissue. Current response of Dnmt1 for the colorectal tumor tissue in comparison with healthy adjacent tissue.

(five versus hundreds of cancer cells) or biopsied tissues (10 μg versus 500 μg) in comparison with the previously reported electrochemical assay.[24]

CONCLUSIONS

In sum, we developed a new electrochemical strategy for probing the activity of human Dnmt1 via coupling high-curvature nanostructured electrodes with DNA-mediated growth of AgNPs (for signal amplification) assisted by Exo I-mediated background suppression. The anisotropic NMEs enable enhanced accessibility of Dnmt1 to the DNA substrates, promoting a higher turnover rate and methylation efficiency in comparison to the planar electrode. The solid state electrochemistry of the AgNPs accumulated in reponse to methylation of DNA permits detection with a high signal-to-background readout. The merits of our assay include ultrasensitivity, radioactivity-/label-free readout, low cost, and small sample volume requirements (1 μL per electrode) compared to other methods. The chip-based electrochemical readout makes it promisingly portable and adaptable to multiplex. The ability to directly detect Dnmt1 in complex cellular lysates with high sensitivity is an important advance. More generally, this work may easily be extended to sensing other biomolecules (enzymes, proteins, etc.) involving DNA as a substrate, and represent an important step in advanced biosensing technologies.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.8b00494.

Surface coverage, chronocoulogram, XPS, linear-sweep voltammogram of the DNA templated AgNPs in different electrolytes (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: shana.kelley@utoronto.ca.

ORCID

Jagotamoy Das: 0000-0003-2724-1827

Edward H. Sargent: 0000-0003-0396-6495

Shana O. Kelley: 0000-0003-3360-5359

Present Address

‖G.W.: International Joint Research Center for Photo-responsive Molecules and Materials,School of Chemical and Material Engineering, Jiangnan University, Wuxi 214122, PR China.

Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

Dnmt1, DNA (cytosine-5)-methyltransferase 1; DNA, deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; NMEs, nanostructured microelectrodes; SEM, scanning electron microscopy; XPS, X-ray photoelectron spectroscopy; AgNP, silver nanoparticles; HCT116, colorectal carcinoma cells; MCF-7, breast adenocarcinoma cells; HeLa, human cervical cells; CCD-18Co, normal colon cell line

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