Integrated Microfluidic Device with an Electroplated Palladium Decoupler for Electrochemical Detection of the 8-hydroxy-deoxyguanosine (8-OH-dG) DNA Adduct

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Abstract

8-hydroxy-deoxyguanosine DNA adduct (8-OH-dG) is one of the most frequently used biomarkers reporting on the oxidative stress that leads to DNA damage. More sensitive and reliable microfluidic devices are needed for the detection of these biomarkers of interest. We have developed a capillary electrophoresis based microfluidic device with an electroplated palladium decoupler that provides significantly improved detection limit, separation efficiency, and resolving power. The PDMS/glass hybrid device has fully integrated gold microelectrodes covered *in situ* with palladium nanoparticles using an electroplating technique. The quality of the electroplated palladium particles were evaluated electrochemically and via SEM imaging. The performance of the device was tested and evaluated with different buffer systems, pH's and electric field strengths. The results showed that this device has significantly improved resolving power, even at separation electric field strengths as high as 600 V/cm. The detection limit for the 8-OH-dG adduct is about 20 attomoles; the concentration limit is on the order of 100 nM (S/R =3). A linear response is reported for both 8-OH-dG and dG in the range from 100 nM to 150 μM (~100 pA/μM) with separation efficiencies of ~120,000 – 170,000 plates/m.

Keywords: Microfluidic device, DNA-adducts, Biomarkers, Electroplating, Nanoparticles, Electrochemistry.

1. Introduction

Exposure to chemicals has been linked to the oxidative damage to DNA that can cause physiological changes associated with degenerative diseases such as cancer and other age-related diseases [1,2]. The DNA damage results in the formation of urinary adducts that can be used as biomarkers for medical diagnostics [3]. Among the four DNA bases, guanine posses the lowest oxidation potential, that leads to the formation of 8-OH-dG [4]. The analytical methods most often used for the detection of 8-OH-dG DNA adduct include: gas chromatography - mass spectrometry (GC-MS) [5], ELISA [6], and high performance liquid chromatography (HPLC) with electrochemical detection (ED). In particular, this last methodology (i.e. HPLC-ED) is often used since amperometric detection is several times more sensitive than optical detection [7-9]. However, the complexity of HPLC-ED and high operating cost limit the application of this methodology.

Recent studies have also shown the applicability of capillary electrophoresis with amperometric detection (CE-AD) for the separation and detection of the urinary 8-OH-dG adduct [10,11]. Two major difficulties with this approach exist: i) problems with aligning of the working electrode (WE) at the end of the separation channel; and ii) the interference and coupling that occur between the electrophoretic and detection currents. Such problems often lead to an unstable background current that leads to lower sensitivity and poor reproducibility.

Different decoupling techniques have been applied to solve these problems with the conventional CE-AD methodology. In the most frequently used remedy, the electrophoretic ground (EG) is placed before the working electrode.

The introduction of microfluidic chips has revolutionized the landscape of analytical sciences; a wide range of analytes has been studied using microfluidic devices with electrochemical detection. In our previous work, we developed a fully integrated microfluidic device with ED for the separation and detection of different neurotransmitters and dopamine-derived DNA adducts [12]. The working electrode (WE) was placed ~15 μm from the separation channel outlet in this device. This

integrated microfluidic device offered an alternative solution for the aforementioned CE-AD problems, especially in the way that the decoupler is introduced [12].

The introduction of an electrophoretic ground electrode (i.e. cathode) into the separation channel to decouple the separation electric field from the electrochemical detector can be problematic as hydrogen gas bubbles will be generated at the cathode. To overcome this problem electrode materials capable of absorbing hydrogen gas need to be used. Among all metals that can be used for the fabrication of solid-state metal-based decouplers, palladium (Pd) is the most efficient in terms of adsorbing H_2 that can be produced at the EG. Unfortunately, Pd based decouplers are hard to fabricate and process. In contrast, gold (Au) exhibits weak tendency to adsorb H_2, but it is easier to deposit and process. Electroplating also allows for deposition of additional metal on an already deposited metal layer. Although some research groups have used palladium as the decoupler for CE-ED on a chip, problems/ difficulties associated with the fabrication procedure, including optimization of the distance between the decoupler and the working electrode, still exist. The above distance strongly depends upon the separation electric field strength, limiting the universality of such devices.

In this work, we report the fabrication of a PDMS/glass microfluidic device with ED with electroplated Pd decoupler for the separation and detection of 8-OH-dG and dG. The distance between the decoupler and the WE was optimized by utilizing an array of microelectrodes. To the best of our knowledge, this is the first report on the detection of 8-OH-dG adduct on a chip using a microfluidic device with a Pd decoupler.

2. Experiment

Microchip: Details of the fabrication procedure published previously [12]. Briefly, PDMS slabs with channels were casted against SU-8025 photoresist-based mold on a 3″ Si wafer. The electrodes were made of thermally deposited gold (200 nm) and 1 nm of titanium as an adhesion layer. The microdevice was assembled by bonding the PDMS slab to the glass substrate after RF-plasma treatment under a 1-Torr stream of oxygen for 1 min.

Electroplating: 10mM aqueous solutions of K_2PdCl_6, $K_2Pt_2Cl_6$, $NaAuCl_4 \cdot 2H_2O$, were used to electroplate Pd, Pt, and Au respectively. After filling the channels with the solution of the metal to be deposited, a square potential signal between –1800 mV and 0 mV, at a frequency of 2 Hz**,** was applied from a potentiostat.

Electrophoresis: 1mM stock solutions of 8-OH-dG and dG in the running buffer were prepared and kept in the refrigerator at 4 °C. Fresh solutions were prepared daily by diluting the stock solutions with the running buffer. Prior to the separation, the microchannels were flushed for 5 min with NaOH, 10 min with deionized water, and 10 min with the running buffer. 10 mM of three buffers were used, MES (pH = 5.5), phosphate (pH = 7.5), and borate (pH = 9.5) buffers. The sample was injected into the separation via simplified gated-injection mode as previously described [12]. Injection time was 1 second for all runs.

Electrochemistry: All EC measurements were made by a potentiostat from Gamry Instruments, Warminster, PA, model PCl4-FAS2.

3. Results and Discussion

Figure 1 shows a layout of the integrated microfluidic device. This device consists of a PDMS slab with two crossed channels. The separation channel is 3 cm long, and the distance from the crossing points to the other three reservoirs is 0.5 cm. The glass substrate supports an array of ten electrodes placed inside the microchannel (all 50µm wide with a 50 µm spacing between them), two 200 µm wide electrodes that serve as reference and counter (placed inside the waste reservoir), and three other electrodes for injection/separation placed inside the waste, buffer, and sample reservoirs. The microchannel's depth and width are 35 and 75 µm respectively.

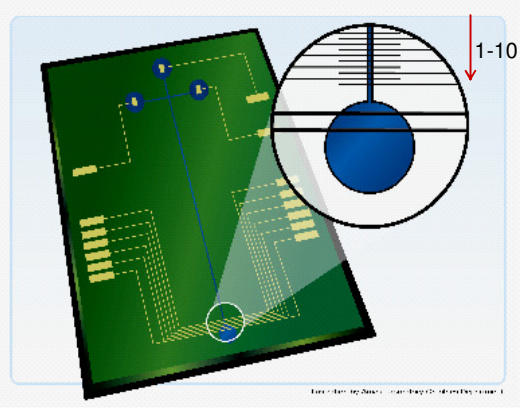

Figure 1: Schematic representation of the fully integrated microfluidic device with electrochemical detection system.

The electroplating process was carried out inside the channel. Figure 2 shows the SEM images for Pd particles, with different magnifications, formed on the surface of gold electrodes after 3.0, 1.5, and 0.5 minutes of deposition time (frames A, B, and C, respectively). As can be seen from the images of low magnification (left frames), there are two distinct regions. The first one, on both sides of the microchannel (center), corresponds to a bare gold surface that was protected by the PDMS. In the middle (channel) region, the surface is covered with Pd particles, which were electrically deposited. As the deposition time was increased (moving from C to A), a significant increase in the size and the density of the particles was observed.

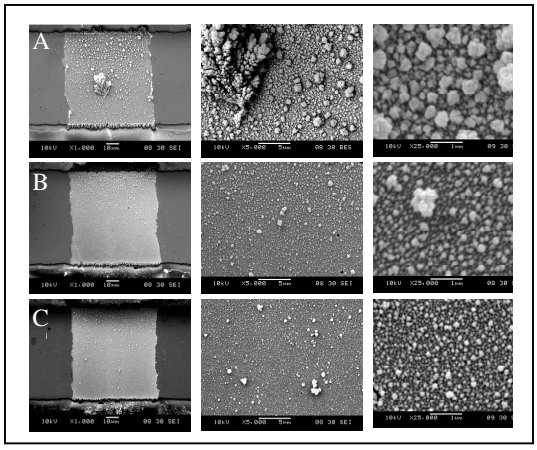

Figure 2: SEM images of channel electroplated with Pd particles. A magnification from left to right is 1000, 5000, and 25000, respectively.

The deposition time was changed in order to increase the surface area of the decoupler without increasing the width of the electrode. In order to estimate the relative increase in the surface area upon increasing the detection time, cyclic voltammograms of 50mM of perchloric acid ($HClO_4$) were generated as a function of deposition time. Figure 3 shows the cyclic voltammograms for three different deposition times. The insert shows the cyclic voltammogram of the gold electrode before the electroplating process for the same solution of $HClO_4$. The H_2 adsorption/desorption peaks are characteristic features of cyclic voltammograms for both Pt and Pd electrodes in aqueous solution, and cannot be observed for gold electrodes. Similar deposition procedures were applied for Au and Pt metals. Significant growth in peaks intensity was observed, which corresponds to an increased area of the decoupler by a factor of ~10. This increased surface area was accomplished by increasing the deposition time without increasing the width of the electrodes.

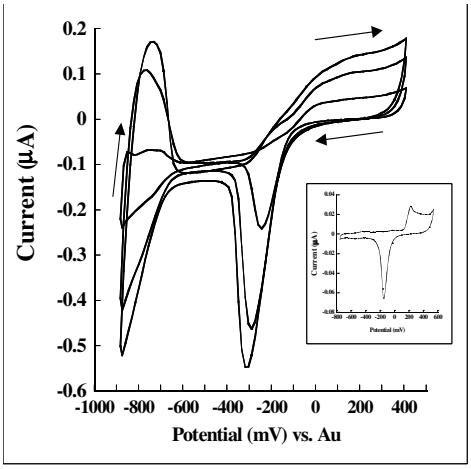

Figure 3: Cyclic voltammograms (CVs) of electroplated decoupler in 50 mM $HClO_4$ as a function of deposition time. The insert shows a CV for the Au electrode in the same buffer.

The performance of the decoupler was evaluated by using different buffer solutions of different pH's, using different separation field strengths. Two important factors were considered: the surface area of the decoupler and the distance between the EG and the WE. The first test was performed without any electrochemical sensing, by just applying a separation electric field through the separation channel to test the stability of the decoupler and to find the maximum separation field that could be applied without the formation of hydrogen gas bubbles. Table 1 summarizes the results for all tested noble metals. As expected, Pd showed the best performance in terms of stability and operation lifetime.

Buffer / ***Electrode***	**Borate pH=9.5**	**Phosphate pH=7.5**	**MES pH=5.5**
Gold	40	>30	70
Platinum	100	30	150
Palladium	600	200	1000

Table 1: Summary of maximum applicable separation field (in V/cm) for different decouplers (Au, Pl, and Pd) in different buffers.

Placing the electrophoretic ground (EG) before the WE would eliminate the interference

between the detection and the electrophoretic current. Since the electroosmotic flow (EOF) vanishes at the EG, thus the sample plug can reach the WE under the influence of the hydrodynamic pressure created by the EOF. The distance between the EG and WE was optimized in order to achieve stable background current. As can be seen from the magnified detection zone in Figure 1, the first electrode is used as the decoupler while the WE could be selected as one of the remaining nine electrodes. This flexibility in the selection of electrodes was essential to optimizing the distance between the decoupler and the WE. Note that too short a distance can result in high interference with the electrophoretic current, whereas too great a distance leads to diffusion problem, thus decreasing the resolving power of the system. A second very important factor in optimizing the performance of the microfluidic device is that the separation distance between the EG and the WE has to be optimized as a function of the separation field strength.

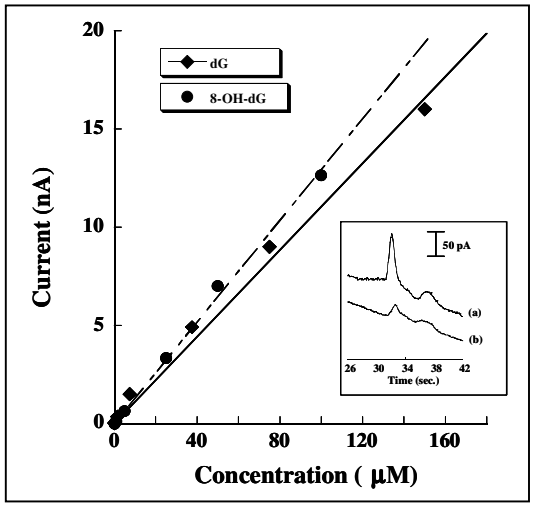

Figure 4: Calibration curves for dG and 8-OH-dG Separation conditions: 10mM boric buffer, pH = 9.5, electric field 300 V/cm, EC potential +0.9 V vs. Au.

We found that the best detection conditions were obtained with a distance of ~600 μm between the EG and WE. The best resolving power was accomplished using an electric field strength of about 300 V/cm. Interestingly, baseline resolved peaks were only obtained in the borate buffer. Figure 4 shows a linear calibration curve for both 4-OH-dG and dG analytes in the range of 100 nM up to 150 μM with separation efficiency of ~120,000 - 170,000 plates/m. The insert shows the separation of these two analytes near the detection limit.

4. Conclusions

We have developed an integrated PDMS/glass device with electrochemical detection with gold microelectrodes covered with *in situ* electroplated Pd decoupler for more efficient separation of 8-OH-dG. The electroplated Pd decoupler provided stable detection background current. Low noise level (~5 pA) has allowed detecting approximately 100 nM of 8-OH-dG. The above detection limit is close to the concentration of 8-OH-dG in urine samples of healthy individuals. Since the level of this adduct in diseased individuals is significantly elevated, we anticipate that our microchip could be used in future clinical applications.

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