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Inkjet-Printed Planar Biochips for Interfacial Detection of Biomoleculars

Abstract

A planar three electrodes system (PTES) chip which is consisted of planar Au, Ag/AgCl, and Pt electrodes on ITO (Indium-Tin Oxide) glass is fabricated using state of the art inkjet printing technology to serve as biosensor for electrochemical detection of biomolecules. The electrochemical detection of melamine and dopamine is successfully performed with this PTES chip and established higher sensitivity in comparison with traditional commercial three electrode system, indicating this PTES chip has great potential in practical applications as electrochemical sensor for biomolecules.

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Title

Inkjet Printed Bio-chip: Planar Three Electrodes System for Electrochemical Biosensing

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Keywords:

Inkjet printing, PTES, melamine, dopamine, electrochemical sensor

A Planar Three Electrodes System (PTES) Chip which is consisted of planar Au, Ag/AgCl and Pt electrodes on ITO glass was fabricated using state of the art inkjet printing technology to serve as biosensor for electrochemical detection of biomolecules. The electrochemical detection of Melamine and Dopamine was successfully performed with this PTES chip and established higher sensitivity in comparison with traditional commercial three electrode system, indicating this PTES chip has great potential in practical applications as electrochemical sensor for biomolecules.

1. Introduction

It has been reported that excessive melamine additive in milk caused serious health problems, such as renal failure and kidney stone on thousands of children. Since then, the importance of food safety has been paid more attention. Therefore, there is an increasing need for the fast, simple and convenient method to detect harmful food additives, pesticide residue and biomolecules. Currently, a series of methods, such as enzyme linked immunosorbent assay,^[1,2] chromatographic techniques (GC, HPLC),^[3] mass spectrometric (MS), spectrometric (FTIR, UV–VIS)^[4] and electrochemical method have been employed in laboratory to detect these molecules.^[5] As compared to other methods,^[6–8] electrochemical method demonstrated significant advantages including rapid detection, ease of operation, high sensitivity and low limit of detection (LOD). Besides, electrochemical method do not need complicated maintenance and calibration compared to other detection techniques. In a typical electrochemical characterization, sensor signals are obtained directly and real-time response are provided. Thus electrochemical method showed great potentials in the utilisations of everyday life due to the advantages discussed above. For example, most of the commercial domestic glucometers in the market for daily blood sugar monitoring are based on electrochemical method.^[9,10]

A series of electrochemical biosensors have been developed for the detection of harmful additives and biomolecule. However, most of these applications have been limited to laboratory utilisation as traditional three electrodes methods were too complicated to be employed in real time detection for field work. Therefore, portal planar integrated chips which can fully perform complete functions of traditional three electrodes and be used in practical field work are highly desired to transfer electrochemical biosensor from laboratory to commercial utilisations (as glucometers).

Inkjet printing is a novel drop on demand technique to fabricate electronics and biochips in large scale. The advantages of inkjet printing include digital and additive patterning, reduction

in materials wastage, non-contact and compatibility with a wide range of substrates. Therefore, inkjet printing has been employed in this work to fabricate a planar three electrodes bio-chip (**Figure 1**) for as electrochemical sensor platform for practical applications.^[11,12]

In this study, we designed a planar three electrodes system (PTES) chip, which is consisted of an inkjet printed Au working electrode (WE), an inkjet printed Ag/AgCl reference electrode (RE) and a sputter coated Pt counter electrode (CE) as shown in **Figure 1**. The Au electrode can be used directly to electrochemically detect biomoleculars and it can also be further modified with other electrode materials which widely broaden its application area. This PTES Chip showed improved sensitivity in electrochemical detection of melamine^[13,14] and dopamine,^[15-20] indicating the potential of this planar chip to be utilise as a portable device with supporting device similar to glucometer for practical electrochemical sensing under a variety of circumstances and conditions.^[21]

2. Results and Discussion

2.1 Fabrication of Inkjet Printed PTES Chips

Figure 1 shows the fabrication process of this inkjet printed PTES Chips. Briefly, Au and Ag electrodes were inkjet printed on the substrates (glass slide or PET etc.) and then annealed at 150 °C for 30 minutes with designed pattern as shown in **Figure S2** Pt electrode was fabricated by sputter coating with mask. Then a 5 V potential was applied on Ag electrode in 0.1 M NaCl solution to chloridize the outer layer of Ag electrode to fabricate Ag/AgCl reference electrode. Herein, PTES Chips was prepared successfully.

The physical properties of Au and Ag nanodispersions have been characterised before printing. Particle size distribution measured by dynamic light scattering (DLS) (**Figure S1**), illustrating that nearly all of AuNPs were smaller than 50 nm while the size of AgNPs located between 70 and 300 nm. The viscosities of these two ink was characterised as 17.4 mPa·s and 32.1 mPa·s while the surface tension values were read as 24.1 and 32.7 mN·m⁻¹ from giometre respectively.

The morphology of each electrodes and the conductivity of all three electrodes were characterised to identify the printing quality of the electrodes. SEM (**Figure 2**) and AFM images (**Figure 3**) demonstrated that the continuous films of Au, Ag and Pt were obtained with densely packed NPs. AuNPs accumulated as clusters after printing with a higher roughness value (**Table S2**) than that of relatively smoother Ag film. This may increase the effective surface area of Au electrode when is employed as a working electrode for sensing. After the transformation from Ag to AgCl, small AgNPs (**Figure 2 (B)**) grew into larger AgCl particles **Figure 2 (C)** with obvious increase in roughness as well.

The conductivities of Au, Ag and Pt electrodes were tested and compared with bulk metal (**Table S1**). Results showed that inkjet printed Au and Ag electrodes and sputter coated Pt electrode demonstrated comparable conductivity to bulk metal,^[22] indicating that these three electrodes had good printing quality and were qualified to serve as bio-chips in electrochemical sensing applications.

2.2 Electrochemical characterization of individual electrodes

The electrochemical performances of individual inkjet printed Au, Ag/AgCl and sputter coated Pt electrodes have been characterised respectively before assembled into integrated biochips.

The function of inkjet printed gold electrode (IPGE) was characterised by CV performance in 5 mM $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_4^{2-}$ and 0.01 M PBS solution and compared with commercial gold electrode (CGE) (**Figure 4A** and **B**). The results demonstrated that IPGE performed pretty well in both $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_4^{2-}$ and PBS solution. The CV of IPGE in $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_4^{2-}$ system, which is often utilised to characterise the quality of working electrode, indicated that IPGE demonstrated much higher current response compared to CGE although ΔE_p of IPGE (108 mV) is slightly higher than that of CGE (94 mV), where ΔE_p is defined as the potential difference between oxidation and reduction peaks ($\Delta E_p = E_{\text{ox}} - E_{\text{red}}$). **Figure 4B** showed that IPGE demonstrated a wider horizontal window (-0.2 – 1 V) than CGE (0 – 1 V) in 0.01 M

PBS solution. These results illustrated that IPGE can serve as work electrode platform in a wide potential window in the traditional electrolyte with high electrochemical activities.

The performance of planar Ag/AgCl and Pt electrodes were tested by replacing RE and CE in a traditional three electrodes (commercial GCE, Ag/AgCl, Pt electrodes) system with prepared electrodes respectively. CVs were characterization in 5 mM $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_4^{2-}$ solution at $50 \text{ mV}\cdot\text{s}^{-1}$ (**Figure 4C and D**) and compared to identify whether prepared planar electrodes can perform as well as commercial electrodes. Results illustrated that CV curves had nearly the same shape and redox peak potentials, indicating that both planar Ag/AgCl and Pt electrode can be utilised as replacements for commercial electrodes in electrochemistry characterization.

2.3 Electrochemical detection of Melamine and Dopamine (DA)

Whether this planar three electrode bio-chip can be utilized in practical application for biosensing was verified by using this bio-chip to electrochemically detect Melamine and Dopamine (DA). The prepared planar electrodes were examined with both traditional triangular arrangement and integrated chip mode (**Figure 1**) and compared with control group (commercial Au-Ag/AgCl-Pt and Glass Carbon-Ag/AgCl-Pt system).

The electrochemical detection of Melamine was carried out using a previously reported method with the assistance of DOPAC.^[16] DOPAC is electrochemically active and recognizable thus can be utilised as electrochemical probe. Melamine has a good H-bonding ability and can spontaneously bind to the carboxyl group and two phenolic-OH groups of DOPAC to form a non-electroactive complex.^[17] Meanwhile, the hydrogen-bonding interaction restricts the electroactivity of the phenolic-OH group of DOPAC, which means melamine bonded DOPAC cannot be detected electrochemically. Therefore, with the addition of melamine, the detectable amount of DOPAC will decrease and lead to the decrease of current signal of DOPAC, which could be used for electrochemical sensing of melamine.

It is clear that prepared planar electrodes with traditional triangular arrangement showed the highest current response in both CV and DPV characterization (**Figure 5(A)** and **(B)**), which is thousands of times higher than commercial Au-Ag/AgCl-Pt and Glass Carbon-Ag/AgCl-Pt system. When planar electrodes were assembled as a chip on ITO-glass, it showed similar shape in CV test and slightly lower current response than triangularly arranged, but still much higher than traditional commercial systems as control. This is probably due to the high electrochemical activity and surface area of inkjet printed Au electrode from nanogold dispersion. The assembly of planar electrodes as a chip on ITO-glass may affect the transfer of ions and decrease the peak current but this planar biochip still showed better function when used as electrochemical sensor. That indicated that PTFE chip can be utilised as an electrochemical biosensor to detect melamine and can be expected to demonstrate high sensitivity.

Figure 5(C) showed CV of PTES chip detecting Melamine in the presence of 10 nM Melamine with 0.3 mM DOPAC in 0.01 M PBS solution at various scan rates from 20 $\text{mV}\cdot\text{s}^{-1}$ to 100 $\text{mV}\cdot\text{s}^{-1}$. As seen from the inset in **Figure 5(C)**, the cathodic peak currents increased linearly with the square root of the scan rate. The linear equations are as follows: $I_{pa} = 3.4v^{1/2} - 443.4$ ($R^2 = 0.9987$). The result demonstrated that the electrode reaction of detecting Melamine is controlled by the diffusion of DOPAC.

Assembled PTES chip was then utilised as biosensor to electrochemically detect melamine in 10 mL solution containing 0.3 mM DOPAC in 0.01 M PBS solution using DPV characterisation. Inkjet printed Au electrode performed as working electrode without any further modification. Melamine was added in to the solution gradually for detection. DPV curves of PTES chip in the presence of various concentrations of melamine from 10 nM to 5 μM were demonstrated in **Figure 5(D)**. It can be observed that peak current of DPV curve decreased with the increase of melamine concentration. Inset figure of **Figure 5(D)** illustrated

that there is a linear relationship between DPV peak current (I_{pa}) and $\lg C_M$, where C_M is the concentration of melamine.

The linear relationship between peak current and melamine concentration can be described using the equation $I_{pa} = -4.5 \lg C_M + 9.5$ ($R^2 = 0.9987$). The sensitivity of this PTES biochip can reach as high as $1.82 \times 10^4 \mu A \cdot mM^{-1} \cdot cm^{-2}$. The limit of detection was calculated as $2.4 \times 10^{-11} M$ ($S/N = 3$), which is two orders of magnitude higher than previously reported values ($3.0 \times 10^{-9} M$).^[14]

The function of this planar biochip was also verified in the electrochemical detection of DA. Inkjet printed Au electrode served as a platform and modified with Nafion/MWCNTs dispersion following the protocol utilised in our previous publication.^[20] Similar to what was observed in Melamine detection, prepared planar electrodes with traditional triangular arrangement showed the highest current response in both CV and DPV characterization (**Figure 6(A)** and **(B)**). Although the difference of peak current between this system and commercial Au-Ag/AgCl-Pt and Glass Carbon-Ag/AgCl-Pt system is not as significant as in Melamine detection, the peak current in DPV was still 5 times higher than traditional commercial system (**Figure 6(B)**), which can be attributed to the high surface area that Au platform provided. DPV peak current of PTES chip is lower than prepared planar electrodes with traditional triangular arrangement but doubled in comparison with traditional commercial system, which agreed well with previous result in Melamine detection. This illustrated that PTES chip can be modified with other electrode materials to detect various biomolecules. In addition, PTES chip showed better electrochemical properties compared to traditional electrode systems and can be expected to exhibit high sensitivity in electrochemical biomolecule detections.

CV of the PTES Chips under various scan rates were demonstrated in **Figure 6(C)**. As seen from the inset figure of **Figure 6(C)**, the cathodic peak currents increased linearly with the square root of the scan rate ($I_{pa} = 4.2 v^{1/2} - 17.84$ ($R^2 = 0.998$)). This result illustrated that the

electrode reaction of detecting DA is controlled by the diffusion of DA from solution to Nafion/MWCNTs modified planar Au electrode.

Figure 6 (D) showed DPV of the PTES when the concentration of DA increased from 0.4 μM to 10.0 μM . As seen from the inset figure in **Figure 6(D)**, the cathodic peak currents increased linearly with the C_{DA} . This linear relationship can be represented by $I_{pa} = 1.6 C_{\text{DA}} + 9.58$ ($R^2 = 0.991$). Sensitivity calculated from detection result can reach as high as $6.4 \times 10^3 \mu\text{A} \cdot \text{mM}^{-1} \cdot \text{cm}^{-2}$. The limit of detection of this PTES Chip for DA detection can achieve $7.46 \times 10^{-9} \text{M}$ ($\text{S/N} = 3$).

3. Conclusion

In conclusion, a Planar Three Electrodes System Chip consisted of an inkjet printed Au as working electrode, an ink printed Ag/AgCl reference electrode and a sputter coated Pt as counter electrode was successfully made. This PTES chip can not only serve directly as biosensor for electrochemical detection of melamine, but it can also be modified with various electrode materials for the detection of different biomolecules. Sensitivity obtained from this PTES chip is higher than traditional commercial three electrodes system. The successful fabrication of this integrated biochip enable the possibility of transferring complicated electrochemical detection of biomolecules to everyday life utilisation.

4. Experimental Section

Chemicals:

Gold (III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), tetraoctylammoniumbromide (TOAB), toluene, ethanol, 6-mercaptohexanoic acid, melamine, dopamine (DA), hydrochloride, 3-mercaptopropanol, sodium borohydride, potassium chloride (KCl), multiwalled carbon nanotubes (MWCNTS), nafion, 3,4-dihydroxyphenylacetic acid (DOPAC), tetrahydrofuran (THF), and phosphate buffered saline (PBS, $\text{pH} = 7.00$) were purchased from Sigma-Aldrich. Nanosilver ink (Jet-600C) was purchased from Kunshan Hisense Electronic co.,Ltd. All the solutions were prepared with doubly distilled water (DI). Commercial Pt electrode (CPE, L/W/thickness = 10/10/1 mm), commercial Ag/AgCl

reference electrode (CRE), gold electrode (CGE) and glassy carbon electrode (GCE) were purchased from Shanghai Chenhua.

Fabrication of the AuNPs:

Inkjet printable Au nanoparticles (AuNPs) were prepared via Bibin T. Anto's method.^[22] The obtained AuNPs has been collected at the concentration of 50 mg·mL⁻¹ in DI water.

Fabrication of PTES chip:s

Inkjet printed Au electrode and Ag electrode were fabricated by Dimatix 2800(FUJIFILM Dimatix, Inc. USA), equipped with a 10 pL cartridge (DMCLCP-11610) at room temperature. The formulation was typically printed at 25.0 V, a frequency of 5.0 kHz, and a customized wave form.

Fabrication of the Au and Ag electrodes:

As shown in the **Figure 1**, Au electrode was printed for 20 layers with as prepared Au ink (drop space = 20 μm). Printing of Ag electrode was performed under the same condition with as purchased Jet-600C Nanosilive ink for 3 layers with 20 μm drop space. As printed electrodes were hearted at 150 °C for 15 minutes for annealing.

Fabrication of the Ag/AgCl reference electrode:

Ag/AgCl reference electrode was obtained by applying a 5 V potential on annealed Ag electrode for 60 s in 0.1 M NaCl solution via constant voltage method. Then Ag/AgCl reference electrode was soaked in saturated KCl solution for at least 24 hours before use.

Fabrication of the Pt electrode:

Pt electrode was coated via sputter coating device (JEC-3000FC, JEOL, Tokyo, Japan) as designed with pre-designed mask. After coating, mask was removed to obtained patterned Pt electrode.

Physical characterization:

Scanning electron microscopy images (SEM) of Au, Ag, Ag/AgCl and Pt electrodes were taken by JSM-7800F (JEOL, Tokyo, Japan). Atomic force microscope (AFM) images of Au, Ag, Ag/Agcl and Pt electrodes were taken by Bruker Dimension FastScan (USA). The Optical

microscope (OM) images of AuNPs, AgNPs and PtNPs etc. were characterised by Nikon ECLIPSE Ti-S (Japan). The thickness of inkjet printing electrodes were measured by KOSAKA ET200 (Japan) profilometry. The particle sizes of printable Au and Ag nanodispersion were measured by Malvern Zetasizer Nano-ZS90 (United Kingdom). Viscosity values of nanoformulations were characterised by Brookfield DV-II+ Pro viscometer. Conductivity measurements were performed on printed films using the four-point probe method.

Electrochemical characterization:

Cyclic voltammetry (CV) and differential pulse voltammetric (DPV) were characterised using an CHI 660 system (Shanghai Chenhua, China) under various conditions.

Electrochemical detection of Melamine:

CV and DPV of various electrode system for comparison were carried out in the presence of 10 nM Melamine in 0.01 M PBS solution with 0.3 mM DOPAC, CV of PTES under various scan speed was carried in the presence of 10 nM Melamine with 0.3 mM DOPAC in 0.01 M PBS solution at various scan rates from $20 \text{ mV}\cdot\text{s}^{-1}$ to $100 \text{ mV}\cdot\text{s}^{-1}$, DPV of PTES under various Melamine concentration was measured in 0.01 M PBS solution with 0.3 mM DOPAC, Melamine was added gradually from 10 nM to 5 μM .

Electrochemical detection of DA:

Nafion/MWCNTs modified electrodes was prepared as previously reported.^[20] MWCNTs (4 mg, Nanocyl) were added to Nafion water solution (10 mL, 0.05 wt%, Sigma) followed by ultrasonication (Branson Digital Sonifier Model 102C) for 30 min. Then the dispersion was stirred overnight until well dispersed MWCNTs were achieved. Then planar Au electrode, commercial glass carbon electrode and commercial Au electrode were coated with 20 μL Nafion/MWCNT and dried in an oven at 60 °C. CV and DPV of various electrode system for comparison were carried out in the 0.01 M PBS solution containing 0.4 μM DA. CV of PTES

under various scan speed was carried in the 0.01 M PBS solution containing 0.4 μM DA at various scan rates from 40 $\text{mV}\cdot\text{s}^{-1}$ to 200 $\text{mV}\cdot\text{s}^{-1}$; DPV of PTES under various DA concentration was measured in 0.01 M PBS solution, Melamine was added gradually from 0.4 to 10 μM .

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Figure 1. The schematic diagram of the fabrication of inkjet printed PTES chips on glass side

Figure 2. SEM images of (A)Au electrode, (B)Ag electrode, (C)AgCl electrode and (D) Pt electrode

Figure 3. Tapping mode AFM surface morphologies images of various electrodes (A) Au electrode, (B) Ag electrode, (C) AgCl electrode and (D) Pt electrode; E, F, G, H, images corresponding to the 3D morphologies

Figure 4. CV of (A) Au electrode in 5 mM $\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_4^{2-}$ and (B) in 0.01 M PBS solution (a: IPGE, b:CGE) at $50 \text{ mV} \cdot \text{s}^{-1}$ with CPE as counter electrode and CRE as reference electrode; (C) CV of CGE with various reference electrodes (a: IPRE, b: CRE) in 5 mM $\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_4^{2-}$ solution at $50 \text{ mV} \cdot \text{s}^{-1}$ with CPE as counter electrode; (D) CV of CGE with various counter electrodes (a: CPE, b: SCPE) with CRE at in 5 mM $\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_4^{2-}$ solution at $50 \text{ mV} \cdot \text{s}^{-1}$.

Figure 5. (A) CV and (B) DPV of various detection systems in the presence of 10nM Melamine with 0.3 mM DOPAC in 0.01 M PBS solution ((a) commercial Au-Ag/AgCl-Pt system (b) commercial Glass Carbon-Ag/AgCl-Pt system; (c) PTES chip mode and (d) planar electrodes with traditional triangular arrangement, scan rate: $50 \text{ mV} \cdot \text{s}^{-1}$) (C) CV of PTES chip in the presence of 10 nM Melamine with 0.3 mM DOPAC in 0.01 M PBS solution at different scan rates; (D) DPV of PTES Chip under various Melamine concentrations (10 nM $\rightarrow$ 5 μM : 10 nM, 50 nM, 100 nM, 500 nM, 1 μM , 5 μM)

Figure 6 (A) CV and (B) DPV of various detection systems in the 0.01 M PBS solution containing 0.4 μM DA ((a) commercial Au-Ag/AgCl-Pt system (b) commercial Glass Carbon-Ag/AgCl-Pt system; (c) PTES chip mode and (d) planar electrodes with traditional triangular arrangement, scan rate :50 mV·s⁻¹) (C) CV of PTES chip in the 0.01 M PBS solution containing 0.4 μM DA at different scan rates; (D) DPV of PTES Chip under various DA concentrations (0.4 μM→10.0 μM: 0.4 μM, 1.0 μM, 2.0 μM, 4.0 μM, 6.0 μM, 8.0 μM, 10.0 μM)

Keyword

inkjet printing, PTES, Melamine, Dopamine, electrochemical sensor

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Supporting Information

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Figure S1 The particle size distribution of (a) gold nanoparticles and (b) silver nanoparticles

Figure S2 The images of Inkjet printed electrodes and the FESEM images of silver and silver/silver chloride; (A) the design image of inkjet printed electrode, (B) platinum electrode, inset: 2, platinum (C) silver/silver chloride electrode , inset: 2, silver; 3, silver chloride (D) gold electrode, inset: 2, gold . a, b, c) silver; d, e, f) silver chloride

Figure S3 The thickness of (a) silver electrode, (b) gold electrode and (c) Pt electrode

Table S1 The conductivity of various electrodes

Electrodes	Ag	Au	Pt
Resistivity (Ω/square)	0.2335 ± 0.024	0.1083 ± 0.036	9.4066 ± 0.048
Film thickness (nm)	1275	250	140
Conductivity measured (S/m)	$5.46 \pm 0.6 \times 10^6$	$3.69 \pm 0.4 \times 10^7$	$7.6 \pm 0.3 \times 10^6$
Reference conductivity (S/m)	5×10^7	2×10^7	9.1×10^7

Table S2 The roughness of various electrodes

Electrodes	Ag	AgCl	Au	Pt
R_a (nm)	11.2	38.8	35.4	21.2
R_q (nm)	14.0	48.3	44.2	25.3
R_{max} (nm)	102	297	285	177