Supporting Information to

Electrochemiluminescence Imaging of Latent Fingermarks through the Immunodetection of Secretions in Human Perspiration

*Linru Xu, Zhenyu Zhou, Congzhe Zhang, Yayun He, and Bin Su**

Institute of Microanalytical Systems, Department of Chemistry, Zhejiang University, Hangzhou 310058, China
Fax: (+) 86 571 88273572
E-mail: subin@zju.edu.cn
Homepage: http://mypage.zju.edu.cn/binsu

Table of Contents

S-1. Chemicals and materials

All chemicals were used as received without further treatment. Ultrapure water (>18.2 MΩ cm) purified by a Millipore system was used throughout the experiments. Hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4 \cdot 3H_2O$), sodium borohydride ($NaBH_4$), and tris(hydroxymethy)aminomethane (Tris) were ordered from Alfa Aesar. Luminol, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl), *p*-iodophenol and Tween 20 were purchased from Aladdin Chemicals. Ethylenediamine anhydrous was bought from TCI. Formaldehyde (HCHO), sodium sulfite (Na_2SO_3), sodium chloride (NaCl), hydrochloric acid (HCl, w% = 36.0–38.0), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$), and bovine serum albumin (BSA) were received from Sinopharm Chemical Reagent. Dipotassium hydrogen phosphate trihydrate ($K_2HPO_4 \cdot 3H_2O$) and potassium dihydrogen phosphate (KH_2PO_4) were bought from Huzhou Chemical Reagent. The stock solution of 0.25 M $Na_3Au(SO_3)_2$ was purchased from Changzhou Institute of Chemical Research. Polystyrene (PS) sheets of 1.5 mm thick were bought from Hangzhou Shuangjian Plastic Co.

Human immunoglobulin G (hIgG), goat anti-human IgG, rabbit anti-lysozyme antibody, rabbit anti-human EGF antibody were provided by Shanghai Sangon Biotech Co., Ltd. Rabbit anti-dermcidin antibody was purchased from Abgent Inc. Horseradish peroxidase (HRP)-conjugated rabbit anti-goat IgG (rabbit anti-goat/HRP), HRP-conjugated goat anti-rabbit IgG (goat anti-rabbit/HRP), and biotinylated goat anti-rabbit IgG (goat anti-rabbit/biotin) were received from Jackson ImmunoResearch Laboratories, Inc. HRP-conjugated streptavidin (streptavidin/HRP) was bought from Beijing Biosynthesis Biotechnology Co., Ltd.

Phosphate-buffered saline (PBS, pH 7.2) contained 0.8% NaCl, 0.18% K_2HPO_4, 0.024% KH_2PO_4, and 0.02% KCl. Immunoreagents were dissolved in PBS solution with 2% (w/v) BSA and 0.1% (v/v) Tween 20. PBS with 0.1% (v/v) Tween 20 was used for washing steps. 0.1 M phosphate buffer (PB, NaH_2PO_4-Na_2HPO_4, pH 7.0) was employed during the amination steps of the fabrication of gold film electrodes. Tris-HCl buffer (0.1 M Tris, 0.15 M NaCl, pH 8.5) containing 0.5 mM luminol and 0.23 mM *p*-iodophenol was used for ECL reactions.

S-2. Fabrication of gold working electrodes

The procedure for fabrication of gold film electrodes on PS plates by UV-directed electroless gold plating has been previously reported by Kong et al.[1] Briefly, PS plates of 30 mm × 25 mm × 1.5 mm were exposed

to UV light emitted from a low-pressure mercury lamp (λ = 254 nm, 30 mW) for 2.5 ~ 3 h to generate carboxyl groups on the plate surface. The PS plates were then immersed in a PB solution containing 0.36 M ethylenediamine and 50 mM EDC at RT for 3 h for amination of the exposed area. After amination, the plates were followed by sequential treatments using 1 mM $HAuCl_4$ solution for 2.5 h and 0.1 mM $NaBH_4$ for 10 min to produce a thin layer of gold nanoparticles (AuNPs). These AuNPs functioned as the catalytically active centers during the following electroless plating, which was typically performed by immersing the plates into an electroless gold plating bath containing 8 mM $Na_3Au(SO_3)_2$, 0.125 M Na_2SO_3, and 0.6 M HCHO for about 3 h at RT. After being rinsed with water and dried with warm air, the plated gold electrodes were annealed at 80^{o}C for 3 h. Finally, the electrode was connected to a copper tape at one end for electrical contact and kept for further use.

S-3. Fingermark deposition

The gold film electrodes were used as the substrates for supporting fingermarks. Prior to fingermark deposition, volunteers were asked to wash their hands with soap, rinse thoroughly with deionized water and dry them under an argon stream. To prepare an hIgG groomed fingermark, 50 μL of 1 mg mL^{-1} hIgG was dropped on the fingertip and rubbed dry. Then the fingertip was pressed on a substrate for 1 min. To produce pure eccrine fingermarks, volunteers warmed their hands in a PE glove for "sweating" 2 ~ 10 min and then stamped their fingertips by contact with the substrate for 10 ~ 60 s.

To prepare natural fingermark samples, donors during working hours gently pressed their fingertips on the substrates. No special instructions were given to the donors to ensure the fingermark depositions were completely natural.

S-4. Immunodetection

The area around each fingermark was inked by a Gene Tech pen to form a hydrophobic barrier capable of retaining ~ 100 μL of solution over the fingermark surface. The gold film electrode bearing a fingermark was placed in an immunohistochemical wet box throughout the following treatments.

To detect the hIgG groomed fingermark, 100 μL of 0.05 mg mL^{-1} goat anti-human IgG was distributed onto the surface of the fingermark, and incubated for 30 min at RT. After the incubation period, the fingermark was washed twice with ~ 10 mL washing buffer to remove unbound primary antibodies, and dried with argon. Subsequently, the fingermark was covered with 100 μl of 0.1 mg mL^{-1} solution of rabbit anti-goat/HRP, followed by incubation for 30 min at RT, a thorough wash with buffer, and gas dry with an

argon stream.

To detect epidermal growth factor (EGF), lysozyme or dermcidin in an eccrine fingermark or natural fingermark, 100 μL of 0.1 mg mL^{-1} rabbit anti-human EGF, rabbit anti-lysozyme or 5 μg mL^{-1} rabbit anti-dermcidin antibody was dropped onto the fingermark. After incubation at RT for 30 min, the fingermark was washed with buffer and dried with argon, then covered with 100 μL of 0.01 mg mL^{-1} goat anti-rabbit/biotin solution. Excess antibodies were removed by washing the fingermark twice with washing buffer and a gas dry with argon. This step was followed by adding 100 μL of 5 μg mL^{-1} streptavidin/HRP. The avidin-biotin reaction was allowed to occur at RT for 30 min. Finally the fingermark was washed with buffer and dried with an argon stream. The treated fingermark samples were kept at 4°C before electrochemical characterization or ECL imaging.

S-5. ECL imaging system and ECL measurements

The ECL imaging system was assembled as described previously.[2] The gold film electrode with a fingermark was inserted horizontally into an electrochemical cell made from Teflon, and served as the working electrode. On the side wall of the cell body, a conduit was drilled for accommodating the silver/silver chloride (Ag/AgCl/3 M KCl) reference electrode. The counter electrode was a platinum wire ring mounted closely above the working electrode. The electrochemical cell was positioned in a ChemiScope 2950 fluorescence and chemiluminescence imaging system (Clinx Science Instruments, Shanghai, China) composed of a Model VFA2595H Macro Zoom Iris Megapixel lens (Senko ADL, Japan) and a high sensitive CCD camera (Model Clinx Clx210, Finger Lakes Instrumentation). The imaging system was enclosed in a dark box to avoid interference from ambient light and was integrated with a CHI 832C electrochemical workstation (CH Instruments, Shanghai, China) to provide the needed potential for the ECL triggered reaction.

For ECL imaging, the electrochemical cell was positioned on a three-dimensional translational stage with the working electrode directly facing the lens. The electrolyte solution was 0.1 M Tris-HCl buffer (0.15 M NaCl, pH 8.5) containing 0.5 mM luminol and 0.23 mM *p*-iodophenol. The ECL images were acquired by the CCD camera while applying a constant potential of −0.7 V vs. Ag/AgCl. According to the intensity of the ECL, the CCD exposure time was set to 30 s for detecting hIgG groomed fingermarks, and 1 min for eccrine fingermarks or natural fingermarks, unless otherwise noted.

ECL measurements were performed with a model MPI-E ECL analyzer (Remax Electronic Co. Ltd.,

Xi'an, China) in the ECL reaction solution from −0.2 to −1.0 V vs. Ag/AgCl at a scan rate of 0.05 V s^{-1}. The photomultiplier tube (PMT) was biased at 600 V.

S-6. Supporting figures

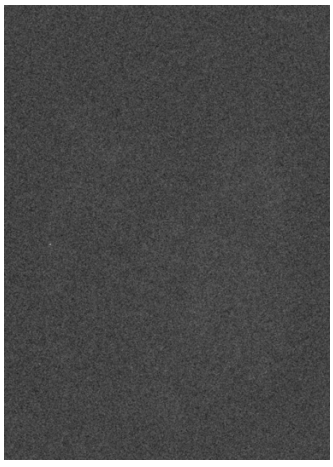

Figure S1. ECL image of a BSA groomed fingermark for the control detection of hIgG. The fingermarks were sequentially incubated with goat anti-human IgG and rabbit anti-goat/HRP.

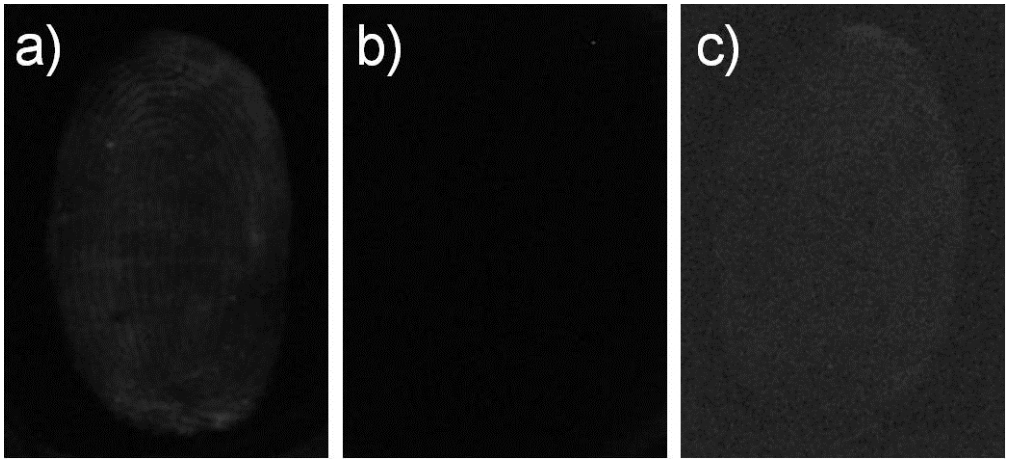

Figure S2. ECL images of the BSA groomed fingermarks treated by the multiple-HRP route for the control detection of a) EGF, b) lysozyme, and c) dermcidin. BSA groomed fingermarks were sequentially incubated with rabbit anti-EGF (anti-lysozyme, or anti-dermcidin) antibody, goat anti-rabbit/biotin, and streptavidin/HRP.

References

1. Y. Kong, H. Chen, Y. Wang and S. A. Soper, *Electrophoresis*, 2006, **27**, 2940.
2. L. Xu, Y. Li, S. Wu, X. Liu and B. Su, *Angew. Chem. Int. Ed.*, 2012, **51**, 8068.