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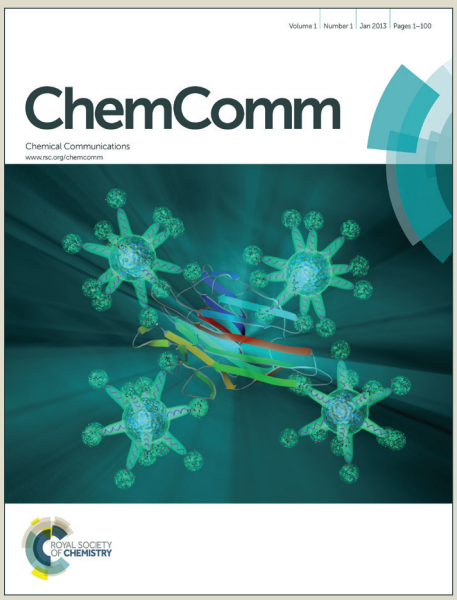

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Electrochemiluminescence Imaging of Latent Fingermarks through the Immunodetection of Secretions in the Human Perspiration

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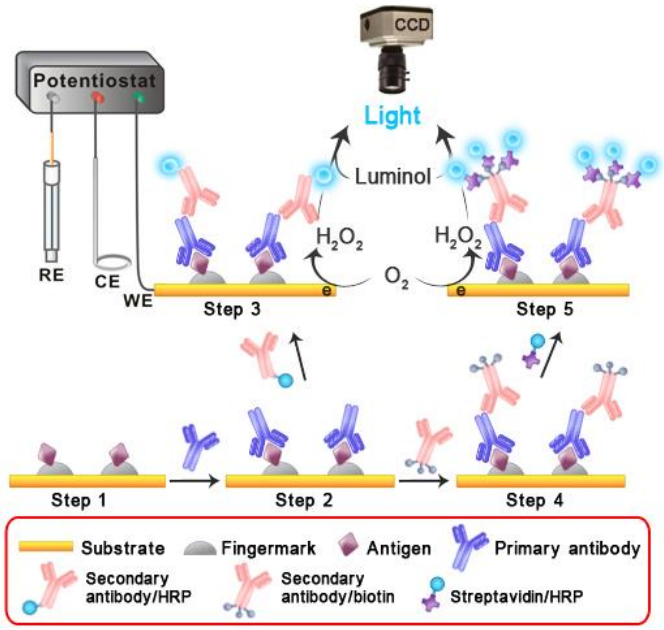
We present the combination of electrochemiluminescence imaging with enzyme immunoassay for the highly sensitive detection of protein/polypeptide residues in latent fingermarks. This technique provides an effective method for fingermark detection that enables both identification of an individual and recognition of the secretions in the human perspiration.

Fingermarks are the contact impressions of the friction ridges of human fingertip. They have long been used as the gold standard for personal identification in forensic investigations since the late 19th century,[1] and continue to be the most reliable tool in crime cases and law enforcement.[2] In addition, the past few years have witnessed a renewal of research interest in the possibility that a fingermark can provide additional information about the donor than just identification,[3] such as gender,[4] diet,[5] the presence of human metabolites with diagnostic values[6] and the evidence of contact with explosives or illicit drugs.[7] To this end, many chemical imaging techniques, including mass spectrometry,[7] electrochemical microscopy[8], Raman spectroscopy,[9] and Fourier transformed infrared spectroscopy,[10] have been employed because of their ability to allow the visualization of a fingermark and the detection of chemicals simultaneously. Recently, methods based on biolabeling have attracted intense interest due to their good sensitivity and selectivity. For example, magnetic particles functionalized by antibodies,[6a, 11] fluorescent tag attached aptamers,[12] aptamer-bound gold nanoparticles,[13] and aptamer functionalized upconversion nanoparticles[14] have been employed to visualize latent fingermarks (LFMs) via the selective detection of the metabolites or contact residues present in fingermarks. However, most of these methods involved complex procedures for the preparation of nanoparticle-antibody (or aptamer) conjugates, or a light source that may cause background interference. In addition, only few works have paid attention to the detection of multiple protein components present in the fingermark residues that originate in the eccrine glands.[4, 15] Herein we present the combination of electrochemiluminescence (ECL) imaging with enzyme immunoassay for the detection of protein/polypeptide components in the human perspiration and simultaneous visualization of LFMs to enable identification of an individual.

ECL, known as a controllable form of chemiluminescence where light emission is initiated by an electrochemical reaction, has been used as a valuable detection method in analytical chemistry.[16] Because the light-emitting species is formed near the electrode surface, ECL provides a superior way for imaging electrochemical and biosensing events on surfaces. In recent years, ECL imaging has received increasing attention in the areas of metabolic toxicity screening,[17] colorimetric device,[18] microfluidic high-throughput analysis,[19] and biosensor arrays.[20] In a previous work, we have demonstrated that ECL imaging can be used as an effective means for visualizing LFMs.[21] For instance, the ECL-active compound, ruthenium bis(2,2′-bipyridine) (2,2′-bipyridine-4,4′-dicarboxylic acid) *N*-hydroxysuccinimide ester, can target amino acids that are omnipresent in the LFM deposits by covalent interaction. Then the immobilized ruthenium luminophore can react with freely diffusing coreactant to generate ECL, eventually producing a distinct image of the LFM. Although this study offered a sensitive method for enhancing the visualization of LFMs, it was unable to selectively detect a particular composition in a LFM, which is potentially extremely useful for forensic work or diagnostic assay.

Among various organic substances present in the LFM residues that are secreted by human eccrine glands, proteins and polypeptides represent the most abundant group of compounds.[22] However, due to the low sample concentration and high background interferences of the chemical imaging techniques, the identification of proteins/peptides is still limited. In this work, we demonstrate that in conjunction with the enzyme-linked immunosensing methodology, ECL can enhance the visualization of LFMs through the recognition of specific protein/polypeptide analytes with high sensitivity and selectivity.



Scheme 1. Illustration of the detection of antigenic residues present in a fingermark using enzyme immunoassay and ECL imaging.

Scheme 1 illustrates the general principle of this approach. Two routes of the immunoreactions were devised according to the abundance of the analytes, namely single- and multiple-HRP routes. Firstly, a fingermark sample was collected from a volunteer to the gold electrode surface (Step 1 in **Scheme 1**). The primary antibodies specific for the target analyte were then incubated over the fingermark (Step 2). In the single-HRP route, the following step was to incubate the above sample with secondary antibodies conjugated with a "single" HRP molecule (Step 3). While in the multiple-HRP route, after Step 2, the sample was sequentially incubated with "multiple" biotinylated secondary antibodies (Step 4) and HRP-labeled streptavidin (Step 5). The ECL detection was carried out by immersing the sample into a buffer containing luminol in an electrochemical cell, which was positioned in an imaging system equipped with a CCD camera and a potentiostat. Applying of a sufficiently negative voltage led to the electrochemical reduction of dissolved oxygen to H_2O_2, with which immobilized HRP can catalyze the chemiluminescent oxidation of luminol.[23] Eventually, sufficiently strong ECL was generated from the developed fingermark ridges. The captured ECL image enables both identification of an individual and recognition of the specific analytes in the LFM.

To exemplify our fingermark visualization approach, we chose human immunoglobulin G (hIgG), the main antibody isotype found in blood, as a test protein. **Figure 1a** shows the ECL image of an hIgG groomed fingermark pre-developed by using the single-HRP method, from which the unique overall pattern of a fingermark, classified as first level detail, can be clearly observed. At a higher magnification, secondary level details such as lake, bifurcation, island, and crossover are observed on the ridge pattern of the fingermark (**Figure 1b**). These details are the significant features that would enable the identity of an individual.

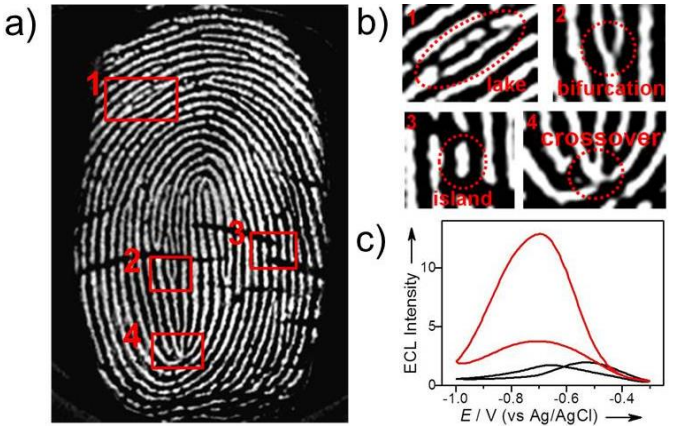


Figure 1. (a) A representative ECL image of an hIgG groomed fingermark treated by the single-HRP route. The image was obtained at an applied potential of −0.7 V in a 0.1 M Tris-HCl buffer solution (pH 8.5, sodium salts) containing 0.5 mM luminol and 0.23 mM *p*-iodophenol. (b) Magnified images showing secondary level details including lake (1), bifurcation (2), island (3), and crossover (4), as indicated by the red rectangles in (a). (c) ECL responses of the gold electrodes carrying an hIgG groomed fingermark without (black line) and with (red line) the single-HRP pre-treatment at a potential scan rate of 0.05 V s^{-1}.

Figure 1c shows the ECL responses recorded for the hIgG groomed fingermarks in a 0.1 M Tris-HCl solution containing 0.5 mM luminol and 0.23 mM *p*-iodophenol without or with the single-HRP pre-treatment during a CV scan. As can be seen, compared with the sample without the pre-treatment (black line), the ECL intensity of the sample with the pre-treatment is efficiently enhanced by the catalytic performance of HRP, confirming the specific binding of the primary antibody and HRP-labeled secondary antibody to the hIgG antigen. Moreover, although the enzyme catalyzes the chemiluminescent reaction between luminol and electrochemically generated H_2O_2, the addition of an enhancer such as *p*-iodophenol could increase the efficiency of the reaction by 3 orders of magnitude.[23c, 24] The ECL emission is shown to reach a maximum at –0.70 V vs. Ag/AgCl corresponding to the electrochemical reduction of O_2 to H_2O_2, thus the best ECL imaging condition was achieved at a constant potential of –0.70 V for 30 s, which also ensures a reasonable detectability with the CCD camera used. Compared with $[Ru(bpy)_3]^{2+}$ labels utilized in most ECL immunoassays, HRP represents a simpler and more cost-effective substitute.

Control experiments were conducted to investigate the specificity of the ECL enzyme immunoassay. The control image (**Figure S1**) shows that no ECL emission is observed for the BSA groomed fingermark that has also been treated following the single-HRP route, confirming that the antibodies specifically target the hIgG antigen in the fingermark residue.

In further experiments, three proteins/polypeptide excreted by eccrine glands[25] were selected as the target analytes for this ECL-immunologic platform technology. Specifically, we have detected epidermal growth factor (EGF), lysozyme, and dermdicin present in eccrine fingermarks. EGF is a single polypeptide chain of 53 amino acid residues that stimulates the biological activities of cell growth, proliferation, and differentiation.[26] Lysozyme is an enzyme present in sweat, tears and many other secretions. It plays a unique role in the defense systems of the skin by damaging and killing the bacteria via breaking down peptidoglycans in bacterial cell walls.[25b] Dermcidin is a

protein specifically and constitutively excreted by sweat glands, which undergoes proteolytic cleavage to produce a 47-amino-acid peptide with antimicrobial activity against a variety of pathogenic microorganisms.[25a, 27] Identification of these proteins/polypeptide from fingermark evidence may be potentially valuable for studying biological activities such as cell growth and antimicrobial behavior.

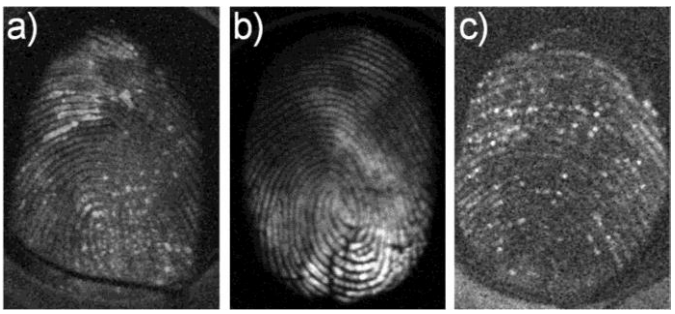


Figure 2. ECL images of the eccrine fingermarks treated by the single-HRP route for the detection of EGF (a), lysozyme (b) and dermcidin (c). The fingermarks were sequentially incubated with rabbit anti-EGF (anti-lysozyme, or anti-dermcidin) antibody and goat anti-rabbit/HRP. The CCD exposure time was set to 4 min.

When using the established single-HRP route for the immunodetection of EGF, lysozyme or dermcidin in the eccrine fingermarks, the obtained images (**Figure 2**) show that only a weak ECL enhancement was displayed. The poor quality of these images can be ascribed to the low sample concentration of the target proteins/polypeptide present in the fingermark residue (the residue is a complex mixture consisting of more than 400 polypeptides and has a very low overall protein content of 384 μg per trace,[28] whilst the content of the test protein of hIgG used above was as much as 25 μg). Therefore, the ECL imaging strategy was further improved by the multiple-HRP route involving the strong interaction between biotin and streptavidin which have been known as a sufficient signal amplification system for bioassays.[29]

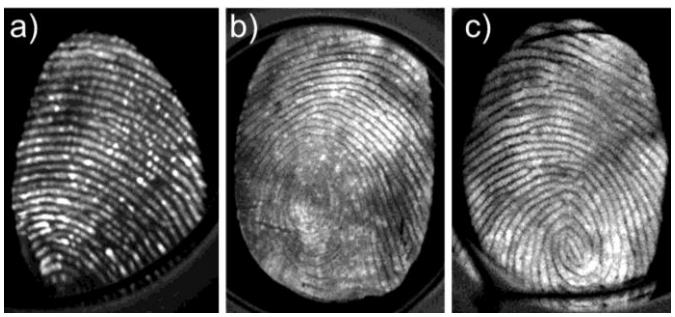


Figure 3. ECL images of the eccrine fingermarks treated by the multiple-HRP route for the detection of EGF (a), lysozyme (b) and dermcidin (c).

In the multiple-HRP route, "multiple" enzyme labels can be achieved since the antibody-biotin conjugates provide multiple binding sites for the HRP-labeled streptavidin, thus resulting in an amplification in the ECL signal than the single-HRP route. The obtained results are shown in **Figure 3**. Compared with the single-HRP route (**Figure 2**), the ECL images (**Figure 3**) after the multiple-HRP treatment show strongly enhanced visualization of the LFMs. These images not only reflect the unique ridge pattern with clearly visible, identifiable features but also provide the chemical evidence of the presence of specific proteins/polypeptide in the eccrine fingermark residue.

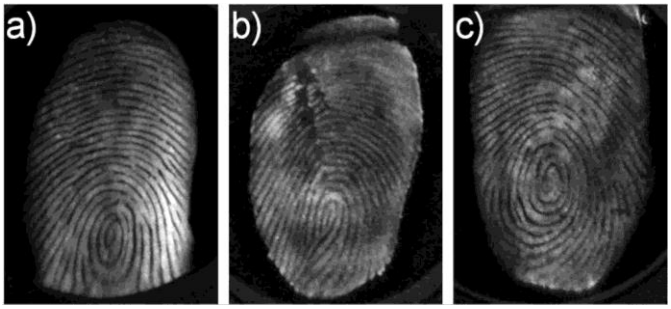


Figure 4. Representative ECL images of the natural fingermarks treated by the multiple-HRP route for the detection of dermcidin.

In the control experiments, BSA groomed fingermarks were again subject to interact with the antibodies specific for EGF, lysozyme, or dermcidin using the multiple-HRP method described above. Since BSA cannot be recognized specifically by these antibodies, no ECL was generated from the fingermarks (**Figure S2b** and **c**) or only very weak background light due to the nonspecific adsorption of the immunological reagents to the underlying substrates (**Figure S2a**) was observed.

Finally, experimental research regarding the visualization of natural fingermarks through the immunodetection of specific secretions, was carried out to verify the practicability of the proposed method. Donors during working hours were asked to press their fingertips on the substrates. No special instructions were given to them so that the fingermark depositions were completely natural. Taking the detection of dermcidin as example, three representative ECL images display sufficient light intensity and clearly resolved fingermark patterns (**Figure 4a** to **c**), which demonstrate the practical utility of this multiple-HRP route to real natural fingermark samples.

Conclusions

In summary, we have demonstrated that proteins/polypeptide deposited in fingermarks, such as EGF, lysozyme and dermcidin, can be identified using ECL enzyme immunoassay with high selectivity and sensitivity. The single-HRP route was firstly established to exemplify the fingermark visualization approach, using hIgG as a test protein. Then a multiple-HRP route was further improved and applied to the highly sensitive detection of the trace residues, *i.e.* EGF, lysozyme and dermcidin that are excreted by human eccrine glands. Since the current method is based on immunodetection, it can provide additional information on specific secretions present in fingermark residues. The ECL imaging approach is complementary to most visualization methods (*e.g.*, spectrometry and fluorescence), in that it does not involve sophisticated or expensive instruments, or a light source hence eliminating the interference of scattered light and luminescent impurities. Taking into account the advantages of electrochemistry methods, for examples low cost and good compatibility with microfabrication technologies, we also believe that this approach can be more easily adapted to fabricate portable hand-held devices and used in the point-of-care test (POCT). Further work is in progress to discover potential, valuable relationships between the fingermark components

and donors (e.g. the abundance and distribution of a particular composition relating to the gender, age of the donor), as well as to detect more other species that are practically and potentially useful for forensic work or diagnostic assay, such as metabolites of illicit drugs, chemical products of explosives, and biomarkers for *in vitro* diagnosis.

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Notes and references

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