Fingerprints

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Visualization of Latent Fingermarks Using an Aptamer-Based Reagent

Michael Wood, Philip Maynard, Xanthe Spindler, Chris Lennard, and Claude Roux*

For centuries it has been known that a mark will be deposited when the ridged skin surface of a finger, palm, or foot comes into contact with a smooth surface.[1] However, not until the late 1800s were these marks, termed friction ridge patterns, used as a means of identification in a criminal context. Since these early beginnings, fingermarks have become one of the most useful and important forensic traces used for identification.[1] Methods for detecting and visualizing fingermarks have evolved greatly over the years as part of the constant search for techniques with improved properties, such as increased sensitivity and selectivity. Despite significant ongoing research within the field of fingermark detection and enhancement, there are still a number of problems regarding current techniques.[2] Almog et al. have highlighted the issue by stating that "there is a considerable number of potentially case-solving latent prints that cannot be visualized by current techniques", referring to the lack of techniques that possess the high levels of sensitivity and selectivity required.[2a] Herein, we report a novel technique that falls in the emerging area of immunogenic fingermark detection[3] and utilizes the highly sensitive and selective recognition power of aptamers as a novel fingermark detection reagent. Aptamers are short single-stranded oligonucleotides that are selected through an in vitro selection technique, referred to as the systematic evolution of ligands by exponential enrichment (SELEX).[4] Through the formation of specific and complex three-dimensional structures, aptamers are able to successfully bind to a wide variety of targets,[5] which results in similar and, in some cases stronger, binding complexes compared to those of their antibody counterparts.[6] By using modified aptamers selected against lysozyme, latent fingermarks were successfully detected and recorded. These developed marks showed strongly fluorescent fingermark ridges with clearly visible, identifiable features.

When a finger comes into contact with a surface, a complex mixture of natural secretions from the pores found along the skin ridges, and external contaminants from the touching of foreign objects, is deposited. These fingermarks are referred to as latent fingermarks, and they are one of the most commonly encountered forms of evidence in forensic examinations.[2c] As these marks are generally invisible to the naked eye, they require the application of one or several appropriate optical, physical, or chemical techniques in order to detect and visualize the marks.[7] Visualization is possible because of the composition and nature of the residues found within the latent mark. Development methods interact with these components in a number of different ways to provide some form of contrast between the surface and the fingermark ridges.

Lysozyme has been clearly identified as one of over 400 polypeptide components found within human sweat,[8] playing a unique role in the defense systems of the skin by hydrolyzing the *N*-acetylmuramic-β-1,4 *N*-acetylglucosamine linkages of bacterial cell-wall peptidoglycans.[9] The lysozyme concentration in skin has been determined[10] however, its concentration in latent fingermarks is not known because of the unique and complex nature of latent fingermark deposits.[11]

DNA aptamers against lysozyme have previously been selected by Cox et al.[12] and Tran et al.[13] (aptamers later referred to as aptamer 1 and aptamer 2, respectively) with low dissociation constants in the nanomolar range. These aptamer sequences were commercially synthesized and modified with CAL Fluor® Orange 560, a fluorescent tag that was chosen to aid visualization after detection. The lysozyme aptamers were diluted to a concentration of 10 μM in the same buffer as that used for their selection against lysozyme.

Fingermark samples were collected from study donors on a polyvinylidene difluoride (PVDF) substrate. Before the fingermarks were collected, donors "charged" their fingers by running them over their forehead and back of the neck. This process provides latent fingermarks that contain both eccrine material from the sweat glands on the hands and sebaceous material (sebum) from the face. Marks were then placed on the PVDF substrate and aged up to 24 hours before being subjected to the aptamer-based reagent. In a Petri dish, 30 mL of tris-buffered saline (TBS) was added together with 100 μL of either of the aptamer solutions. Fingermark samples were placed into the development solution and left at room temperature for between one to four hours while being constantly agitated. Development time differed depending on the nature of the latent marks and the degree of fingermark detail present. Generally, fingermark ridges were visible after one hour, but greater contrast was seen after longer development times. Developed marks were subsequently imaged using a high intensity forensic light source set at a wavelength of 505 nm and a camera fitted with a 555 nm bandpass filter. Figure 1 shows how the detection principle works. For full experimental details see the Supporting Information.

Developed fingermarks exhibited strong fluorescence under the high intensity light source and appropriate filter settings. Figure 2 shows the unique overall pattern type of a fingermark, classified as "level 1" detail. Also clearly visible

[*] M. Wood, Dr. P. Maynard, Dr. X. Spindler, Prof. C. Roux
Centre for Forensic Science
University of Technology, Sydney
Sydney, NSW, 2007 (Australia)
E-mail: Philip.Maynard@uts.edu.au
Homepage: http://www.forensics.uts.edu.au
Prof. C. Lennard
National Centre for Forensic Studies
University of Canberra
Canberra, ACT, 2601 (Australia)

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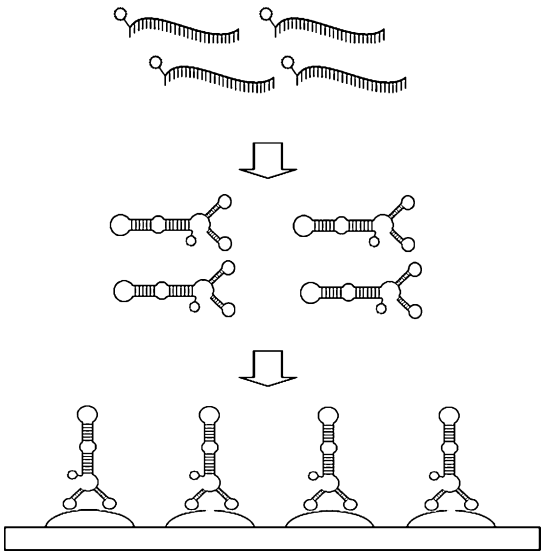

Figure 1. Schematic representation of the detection and visualization of latent fingermarks using the lysozyme-specific aptamer-based reagent. Initially the aptamers remain in their single stranded state with the CAL-Fluor® Orange 560 fluorescent tag attached to the 5′-end. In the presence of lysozyme, the aptamers fold into their specific three-dimensional structures, thus allowing them to bind to the lysozyme within the latent fingermark. Development is illuminated using a 505 nm light source and visualized with a 555 nm bandpass filter.

are the minutiae, or identifying features, of the fingermark, classified as "level 2" detail. These are the features that are necessary for successful fingerprint comparisons. One of the major issues concerning detection techniques is background interaction. If background development occurs, then contrast between the fingermark ridges and the substrate is diminished; thus reducing the potential for visualizing the key minutiae needed for comparison purposes. The magnified section of Figure 2 shows that no significant background interaction has occurred; the ridges are strongly fluorescent, while the valleys of the mark remain dark.

In order to assess the selectivity and sensitivity of the lysozyme aptamers in this context, "spot tests" were carried out on standards for a number of other fingermark components. Squalene, oleic acid, palmitic acid, and glucose were all assessed. Selectivity was also tested against the proteins gelatine and bovine serum albumin (BSA). Small amounts of each standard (10 μg) in solution form were placed onto the PVDF membrane and left to dry. A 1 ng human lysozyme spot was also tested. The samples were subjected to the same development and visualization processes as carried out with the latent fingermarks.

Figure 3 shows a comparison of each of these standards after processing, together with a developed lysozyme standard. Results were identical between the two lysozyme aptamers, with no significant binding visible for any of the fingermark component standards (other than lysozyme) or for the two proteins. The clearly evident detection and visualization of the lysozyme standard at 1 ng scale, with the absence of nonspecific binding (for the other substances tested), highlights the sensitivity and selectivity of the aptamer-based reagents (Figure 3a).

The two aptamer sequences were selected for their slight differences in properties. Aptamer 1 was a 30-mer sequence with a dissociation constant (kD) of 30 nM, while aptamer 2

Figure 2. "Charged" latent fingermark on PVDF, developed using the aptamer-based reagent. Fingermark was aged for 24 h before development with the lysozyme aptamer 1 reagent with CAL Fluor® Orange 560 as the fluorescent tag. A development time of 1 hour was used before image recording in the fluorescence mode. Overall fingermark pattern (left) and a magnified section (right) show completely clear "valleys" of the fingermark, highlighting the lack of background interaction.

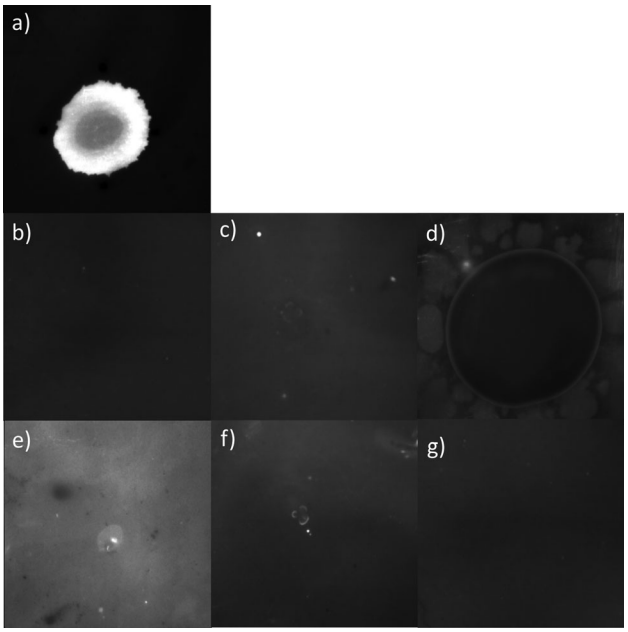

Figure 3. Demonstration of aptamer sensitivity and selectivity. The sensitivity of the lysozyme aptamer reagent is demonstrated with the detection of 1 ng of lysozyme (a). Selectivity is demonstrated through the lack of binding with two proteins (BSA (b) and gelatine (c)) and a number of fingermark components (oleic acid (d), glucose (e)), squalene (f), and palmitic acid (g)), all at 10 μg scale. Images recorded in the fluorescence mode.

was an 80-mer sequence with a kD of 2.8 nM. Comparison between the two sequences showed that there was no significant difference in detection and development for both the lysozyme spots and the latent fingermarks on PVDF. Both of the aptamers were able to develop latent fingermarks of high evidential value (Figure 4).

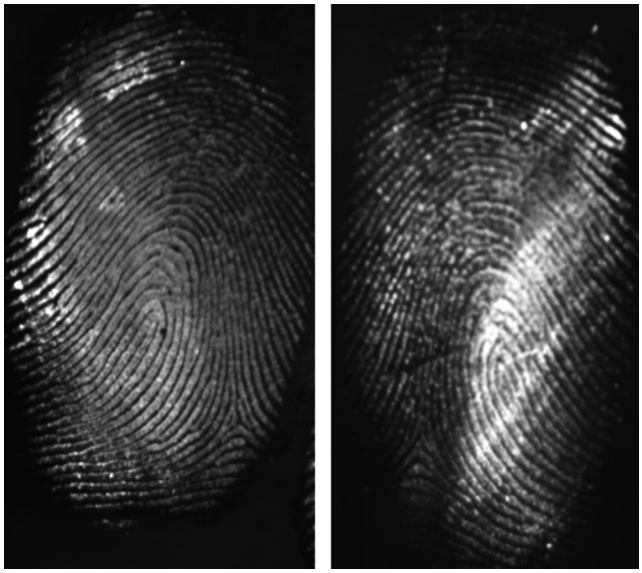

Figure 4. "Charged" latent fingermarks on PVDF, developed using the two aptamer-based reagents. The fingermarks were aged for 24 h before development and imaging under the same conditions, but with different lysozyme aptamers (aptamer 1 (left) and aptamer 2 (right)). Images recorded in the fluorescence mode.

In conclusion, the work shown here has produced the first technique for the detection and development of latent fingermarks using aptamer-based reagents. These reagents are able to develop latent fingermarks on the test substrate (PVDF) which are both strongly fluorescent and easy to visualise. The marks produced were of high evidential value with highly defined level 1 and 2 features clearly visible. The definite potential of an aptamer-based reagent has yet to be fully explored in terms of use on substrates more frequently encountered for fingermark detection, as well as its relative performance compared to traditional methods. However, it has been shown that an aptamer-based reagent is a novel technique capable of developing latent fingermarks with high selectivity and sensitivity. With the ability to create and modify aptamers to almost any target compound, aptamer-based reagents hold enormous potential. By targeting a variety of fingermark components, aptamer-based reagents would be of significant operational use for dealing with difficult fingermark cases. In addition, there is the potential to develop reagents that could provide "extra" information from fingermarks, such as the detection of drug residues or residues from the handling of explosives.

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