Fingerprinting

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"Intelligent" Fingerprinting: Simultaneous Identification of Drug Metabolites and Individuals by Using Antibody-Functionalized Nanoparticles**

*Richard Leggett, Emma E. Lee-Smith, Sue M. Jickells, and David A. Russell**

The identification of individuals from indentations made by their fingers has been known for almost 4000 years with the discovery of finger impressions pressed into the clay surface of Babylonian legal contracts.[1] The use of fingerprints for forensic analysis was first suggested in the 19th century with reports that both the study of "bloody finger-marks or impressions on clay or glass may lead to the scientific identification of criminals"[2] and that a "signature by means of finger-marks" prevented impersonation or repudiation of a handwritten signature.[3] Fingerprint identification is today still the cornerstone of forensic evidence. However, currently the fingerprint is useful solely when police or other security agencies are able to obtain a positive match with those prints present on databases. Herein, we show that it is possible to obtain direct chemical information from drugs or drug metabolites present in minute quantities of sweat deposited with a latent fingerprint to provide "lifestyle intelligence" regarding an individual. Such information could prove vital to focus police investigations. We have devised an approach with antibody–nanoparticle conjugates that provides information on the presence of drug metabolites through binding to an antigen in the sweat. The high-definition fluorescence images obtained as the sweat diffuses along the unique ridge pattern additionally enables the direct visualization of the fingerprint to allow identification of the individual. The ability to detect the crime while simultaneously determining the perpetrator has enormous potential for forensic science. However, it is anticipated that this technique could equally be applied to the screening of athletes and for the detection of biomarkers for medical diagnostics.

When visualized under a microscope, the skin on the palms and fingers appears as ridges and grooves. It is the pattern of these friction skin ridges that produces the unique fingerprint.[4] Each skin ridge has a single row of pores through which sweat is excreted and deposited on the surface of the skin. When a finger touches a surface, sweat is transferred leaving an impression of the finger's ridge pattern, referred to as a latent fingerprint. Such fingerprints are considered "invisible prints" as they require physical or chemical treatments to enable visualization.[5] Sweat, the ultrafiltrate of blood plasma, contains inorganic ions, lactate, urea, and amino acids, and these species are therefore present within a freshly deposited fingerprint. It is also known that orally ingested and metabolized drugs are excreted in sweat.[6] These drugs have been measured in sweat through the use of collection devices, such as patches of adsorbent cotton, followed by extraction and subsequent analysis.[6] However, the methods used are laborious, require a large amount of sweat collected over a period of time and are therefore not suitable for rapid analysis, for example, roadside testing of persons suspected of driving under the influence of drugs.

While detection of a drug in a fingerprint would show that an individual had come into contact with a drug, such detection would not necessarily prove use of the drug. Detection of a specific metabolite of a drug would provide evidence of use. To exemplify our fingerprint visualization approach, we have chosen to detect cotinine, a metabolite of nicotine, from individuals who smoke tobacco products. It is known that after metabolism, cotinine is present in saliva, serum, urine, and sweat.[6,7] A mean combined concentration of nicotine plus its metabolites in eccrine sweat has been previously determined, using a radioimmunoassay, as 780.8 $ng\,mL^{-1}$ for active smokers.[8]

In nature, there are numerous instances where weak interactions between individual ligands and receptors are enhanced through multivalent or polyvalent interactions; the binding of viruses to cell surfaces is one such example.[9] Based on such interactions, we have fabricated gold nanoparticles functionalized with multiple anti-cotinine antibodies to enhance the specific interaction between the antibody and the cotinine antigen within a latent fingerprint. The simple, yet robust, construction of the antibody-functionalized nanoparticles is shown in Figure 1.

Gold nanoparticles 16 nm in diameter were formulated by using the Turkevich method that uses citrate as both a reductant and a stabilizing agent.[10,11] Protein A, a cell-wall component of *Staphylococcus aureus*, was tagged with *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) to facilitate the binding of the protein on the surface of gold nanoparticles.[12] Upon addition of the SPDP-modified protein A to a solution of the nanoparticles, the thiolated ligand

[*] R. Leggett, E. E. Lee-Smith, Prof. D. A. Russell
School of Chemical Sciences and Pharmacy
University of East Anglia
Norwich, Norfolk, NR4 7TJ (UK)
Fax: (+44) 1603-593-012
E-mail: d.russell@uea.ac.uk
Dr. S. M. Jickells
Department of Forensic Science and Drug Monitoring
King's College London
Franklin-Wilkin's Building
150 Stamford Street, London, SE1 9NH (UK)

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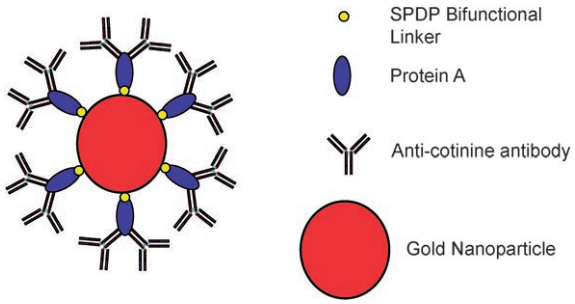

Figure 1. Representation of the antibody–nanoparticle conjugates. The conjugates are formulated by depositing protein A, which acts as a biological linker to orientate the anti-cotinine antibody on the gold-particle surface. SPDP = *N*-succinimidyl 3-(2-pyridyldithio)propionate.

displaced the citrate layer on the gold-particle surface. Protein A was chosen as the linker as the protein specifically binds to the Fc (fragment crystallizable) portion of two antibody molecules simultaneously.[13] Importantly, by binding the Fc component, the protein A orientates the antibody such that the recognition component, the $F(ab')_2$ binding region (Fab = fragment antigen binding), is optimally presented for direct antigen binding. The anti-cotinine antibody was readily assembled onto the protein A monolayer surrounding the gold nanoparticles.

The anti-cotinine–nanoparticle conjugates were applied to the detection of cotinine in the fingerprints of people that smoke. In a typical experiment, the volunteer would provide a fingerprint on a glass microscope slide (such fingerprints are described "as presented") and then wash their hands, after which sweating was induced by placing the volunteer's hand in a sealed glass beaker. Fingerprints, deposited onto glass slides, were then taken at regular intervals. The anti-cotinine–nanoparticle conjugates were pipetted onto the fingerprints, incubated, and then the unbound nanoparticles were removed by washing. A fluorescently tagged secondary antibody fragment ($F(ab')_2$ region) was then pipetted onto the fingerprint and again incubated. Excess reagent was removed by washing with water. Fluorescence images were then taken of the fingerprints. Figure 2 shows the fingerprint images obtained from a smoker (reported to smoke between 5–7 cigarettes per day) by using the antibody–nanoparticle conjugates.

The images obtained from the volunteer "as presented" (Figure 2, A and F) clearly show the typical fingerprint ridge pattern including whorls and loops that would enable identification of an individual. The evolution of the fingerprint as sweating is induced in the volunteer over 10–40 min is seen in Figure 2 (B–E) and (G–J). At the 10-min time point, the images (Figure 2, B and G) show the ridge structure of the fingerprint with sufficient detail to enable identification. However, between 20–40 min, the clarity of the images intensifies, culminating in the image obtained at 40 min, which highlights the quality of the latent fingerprint images obtained with the nanoparticle conjugates.

To determine whether the nanoparticles were necessary, the experiment to image a smoker's fingerprint was repeated by using anti-cotinine antibodies not bound to the gold nanoparticles. The typical images obtained (see the Supporting Information) show that a partial ridge pattern of a fingerprint appears to be discernable when the fingerprints are fluorescently imaged by using the anti-cotinine antibody. Such images do indicate the presence of cotinine, confirming that the individual is a smoker. However, the poor quality of these images would not enable identification of the person who deposited the fingerprints. It is thought that the enhanced valency provided by the antibody–nanoparticle conjugates, with approximately 50–60 antibodies present on each nanoparticle,[14] increases the avidity of the antibody–antigen complex. Additionally, an enhancement in the fluorescence signal would be obtained as the antibody–nanoparticle conjugates provide multiple binding sites for the fluorescently tagged secondary antibody fragment. Enhanced fluorescence signals have been obtained previously by using dyes encapsulated within nanoparticles for microarray detection.[15] The multivalency and enhanced fluorescence signal together contribute to the high-quality

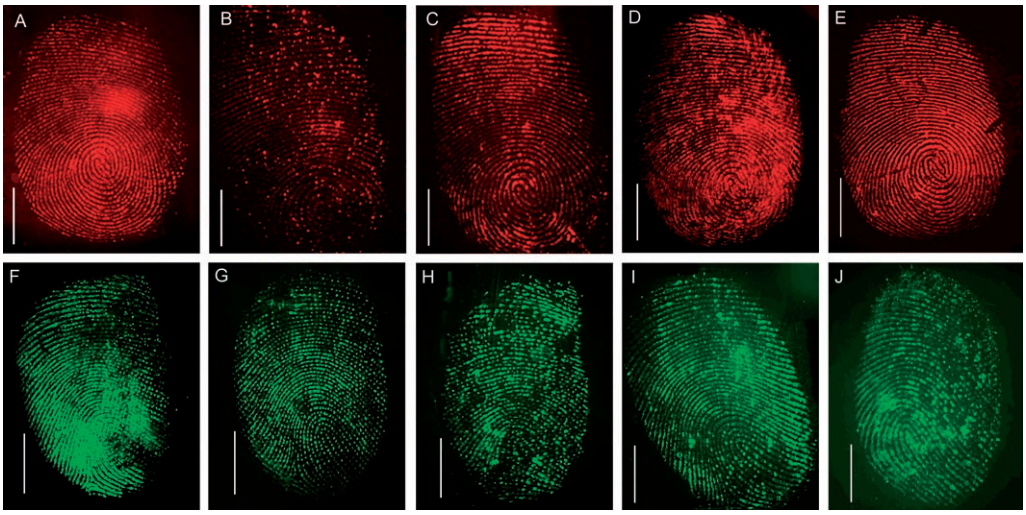

Figure 2. Evolution of fingerprint development by using anti-cotinine–nanoparticle conjugates. The fingerprints are from a male smoker; the fluorescence images are derived from the binding of a secondary antibody fragment tagged with either Alexa Fluor 546 (A–E) or Alexa Fluor 488 (F–J) to anti-cotinine–nanoparticle conjugates. Images A and F are fingerprints taken as presented; images B–E and G–J were taken after the volunteer washed his hands and then gave a fingerprint after a predetermined "sweating" time: B) and G) 10 min; C) and H) 20 min; D) and I) 30 min; and E) and J) 40 min. Images A–E are taken from the thumb; images F, G, and I are of the middle finger; image H is of the index finger; and image J is of the little finger. The scale bars represent 5 mm.

fingerprint image obtained with the antibody–nanoparticle conjugates as compared with the antibody alone.

In a further important control experiment, no fluorescence images of fingerprints were obtained from nonsmokers by using the nanoparticle conjugates (see the Supporting Information), confirming that the anti-cotinine-functionalized nanoparticles specifically target the cotinine antigen deposited within the sweat of the fingerprint.

When the fingerprints are visualized by using the antibody–nanoparticle conjugates and imaged at a higher magnification (Figure 3), the high evidential quality of the print is

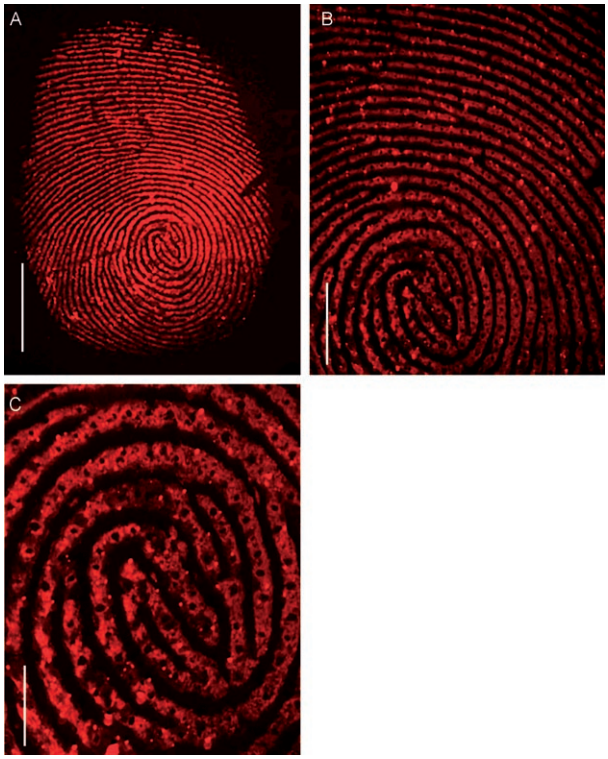

Figure 3. Fluorescence images, at varying magnifications, showing detailed fingerprint information by using the antibody-functionalized nanoparticles. The images are taken from the thumb of a male smoker after 40 min sweating and illuminated by using an Alexa Fluor 546-tagged secondary antibody fragment. Scale bar: 5 mm (A), 2 mm (B), and 1 mm (C).

apparent. Figure 3B shows the typical features of the ridge pattern of the fingerprint, namely, bifurcations and ridge endings. Such features, referred to as second-level detail, are used by fingerprint experts to make an identification through database matching. At higher magnification (Figure 3C), the clarity of the images is such that it is possible to visualize the pores running along the fingerprint ridge pattern (seen as regular dark spots along the length of the ridge pattern) together with the shape of the ridges. This "third-level" detail is also used for matching purposes. Importantly, such second- and third-level detail is not visible when imaging with the anti-cotinine antibodies alone.

The pores, from which the sweat containing the cotinine is deposited along the friction ridge pattern, are further highlighted in Figure 4, with fingerprint images from both a male and female smoker. With the male smoker, the image (Figure 4A) was obtained 10 min after the volunteer had

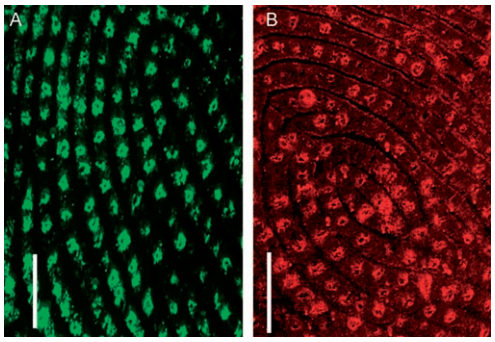

Figure 4. High concentrations of cotinine detected immediately surrounding the sweat pores. A) Middle finger of a male smoker following 10 min sweating. B) Ring finger of a female smoker "as presented". The scale bars represent 1 mm.

washed his hands. It can be seen that the cotinine in the sweat has been excreted from the pores but has yet to diffuse fully along the ridge pattern of the fingerprint. Similar "hot-spots" of excreted cotinine surrounding the individual pores can be seen in the image taken from the female volunteer (Figure 4B), although in this instance, the sweat and hence the cotinine has diffused further along the friction ridges.

The clarity of the fluorescence images achieved by using the antibody–nanoparticle conjugates provides a method of fingerprinting that enables both identification of an individual and simultaneous determination of the chemical makeup of the sweat deposited in the fingerprint. The ultimate sensitivity of this chemical detection capability has yet to be established, but the potential applications of this methodology are enormous as the functionalization of nanoparticles with other antibodies enabling the specific detection of numerous antigens within a fingerprint is possible. Such applications would include illicit-drug detection (driving under the influence, workplace screening, screening of athletes) and medical diagnostics as examples in which a simple but rapid screening method in conjunction with confirmatory identification of an individual are essential.

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[14] By using the fabrication procedure, it is estimated, based on flocculation assays, that 25–30 protein A molecules are assembled onto each nanoparticle. Although there are four antibody binding sites on each protein A, only two binding sites are accessible at anytime.[13] Therefore, the maximum number of antibodies per gold nanoparticle would be 50–60.
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