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VIP Drug Detection

Imaging of Latent Fingerprints through the Detection of Drugs and Metabolites**

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No two individuals possess identical fingerprints.[1] Thus, since the realization of its importance in the 19th century, fingerprint identification is still an indispensable part of any forensic investigation today.[2] When a finger touches a surface, sweat and components within the sweat are transferred to the surface, thus leaving a latent fingerprint. In order to visualize a latent print, chemical or physical treatments are required.[3] Magnetic powder[4–6] is one such treatment which has been used in forensic investigations since its development in the 1960s. In forensic practice, the magnetic powder is applied to the latent print with a magnetic brush. The brush distributes the powder onto the latent print and removes nonadherent powder, which reveals the enhanced characteristic pattern of ridges and grooves of the fingerprint. Herein, we report the combination of the properties of magnetic powders with that of antibody recognition through the formation of antibody–magnetic-particle conjugates in order to detect drugs and drug metabolites within latent fingerprints. These conjugates are attractive as not only can they provide evidence of drug use but they also enable identification of an individual with the ease of application and removal of magnetic fingerprint powder. The antibody–magnetic-particle conjugate platform is exceptionally versatile. Variation of the antibody bound to the magnetic particles enables binding to specific drugs or drug metabolites within the fingerprint to provide, for the first time, high-definition fluorescence images of the fingerprint pattern whilst providing chemical information regarding drug usage.

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While the detection of drugs in sweat is known,[7,8] reports of the detection of drugs in latent fingerprints are typically based on fingerprints that have been artificially doped with drugs of abuse.[9–11] Detection of a drug residue in a fingerprint would not necessarily prove usage as the residue may have come from accidental contact. The detection of a metabolite of the parent drug is therefore advantageous as evidence of drug use can be inferred. Recently, nanoparticles have attracted much attention in forensic science research. For example, we have shown that antibody–gold-nanoparticle conjugates can be used to detect cotinine, a metabolite of nicotine, in a latent fingerprint whilst simultaneously providing fluorescence images of the print to enable identification of an individual.[12] Additionally, Au[13–15] and CdSe/ZnS[14] nanoparticles have been used for the enhancement of latent fingerprints. The use of nanoparticles for fingerprint detection has recently been reviewed.[16] In this work, we have functionalized magnetic particles with a variety of antibodies for the detection of a range of drugs and drug metabolites deposited within latent fingerprints. Specifically, we have detected: Δ^9-tetrahydrocannabinol (THC), the main psychoactive component of marijuana;[17,18] methadone, a synthetic opioid, generally prescribed as a substitute pharmacotherapy to heroin-dependent patients;[19,20] 2-ethylidene-1,5-dimethyl-3,3,-diphenylpyrrolidine (EDDP), the major metabolite of methadone;[21,22] and benzoylecgonine, the major metabolite of cocaine.[23]

A schematic representation of the method used for the detection of drugs and drug metabolites in fingerprints using the antibody–magnetic-particle conjugates is shown in Figure 1. Primary antibodies were conjugated to Protein A/G coated magnetic particles. Protein A/G is a recombinant fusion protein that combines IgG binding domains of both Protein A and Protein G.[24] Such binding optimally orientates the $F(ab')_2$ binding region of the antibody for antigen recognition. Fingerprints from volunteers were collected on glass microscope slides. The primary-antibody-functionalized magnetic particles were then incubated over the fingerprint. After a 30 minute incubation period, excess nonbound particles were removed using a magnetic brush. A secondary antibody fragment tagged with an Alexa Fluor dye was used to fluorescently label the bound magnetic-particle conjugates. After incubation for 30 minutes, excess secondary antibody fragments were removed by washing with phosphate buffer. Finally, the fingerprint was imaged using a stereomicroscope (Figure 1).

To assess the performance of the antibody magnetic particles, the conjugates were initially used to detect THC. Figure 2a shows the brightfield image obtained immediately after collection of the fingerprint of a drug user, prior to

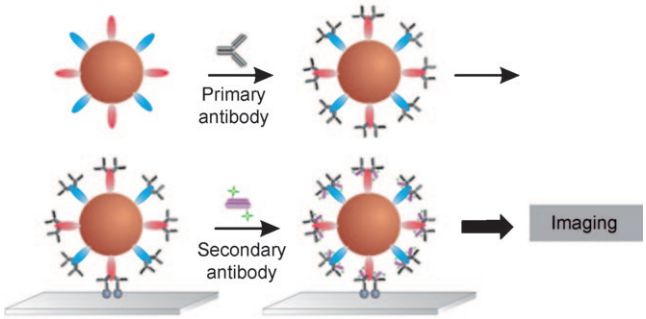

Figure 1. Schematic representation of the detection of drugs and metabolites in latent fingerprints using antibody–magnetic-particle conjugates. In the first step, Protein A/G coated magnetic particles were combined with a primary antibody to prepare the antibody-functionalized magnetic particle conjugates. In the second step, the conjugates were incubated over a fingerprint that had been collected on a glass microscope slide. Excess particles were removed using a magnet. Subsequently, a secondary antibody fragment tagged with an Alexa Fluor dye was incubated over the fingerprint. Finally, the fingerprint was imaged using a stereomicroscope.

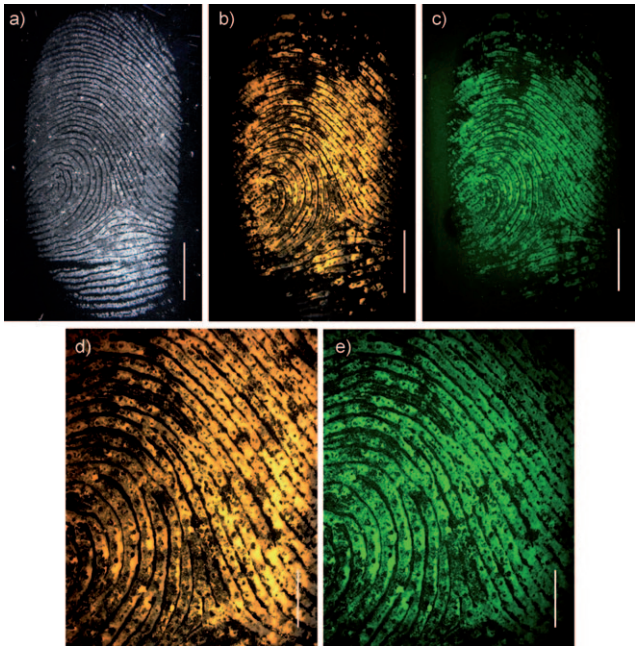

Figure 2. Detection of THC in a fingerprint. Brightfield images of a fingerprint a) before and b) after incubation of antiTHC–magnetic-particle conjugates and a secondary antibody. c) Fluorescence image of the fingerprint following incubation of the reagents used in (b). d) Brightfield and e) fluorescence images of a section of the same fingerprint at higher magnifications. Scale bars in (a), (b), and (c) are 5 mm; those in (d) and (e) are 2 mm.

incubation of any reagents, to confirm the presence of the latent fingerprint on the glass slide. Figure 2b also shows a brightfield image, obtained after the subsequent incubation of the slide with antiTHC antibody–magnetic-particle conjugates and Alexa Fluor 488 dye-tagged secondary antibody fragment. A change in color of the fingerprint to yellow-brown is observed after the incubation steps. The yellow-brown coloration is due to the antiTHC–magnetic-particle conjugates that have bound to the THC antigens present in the sweat deposited along the unique ridge pattern of the fingerprint. Figure 2c shows the fluorescence image of the fingerprint obtained with the antiTHC–magnetic particles and fluorescently tagged secondary antibody, again providing the chemical evidence of the presence of the THC antigen in the sweat of the fingerprint. At higher magnifications (Figure 2d,e), significant details of the ridge pattern of the fingerprint are clearly evident. Such details include bifurcations and termination points (known as second-level detail), which are used by fingerprint investigators to establish the identity of an individual. The sweat pores, from which the sweat that contains the THC is excreted prior to deposition along the ridges of the fingerprint, are also visible as dark spots along the ridge length. The position of these pores (known as third-level detail) is also used by fingerprint examiners to establish identification. It is clear therefore that both the brightfield and the fluorescence images show the ridge pattern of the fingerprint with sufficient detail to enable identification of the individual.

Control experiments were performed to ensure that the antiTHC–antibody-functionalized magnetic particles were specific for the THC antigen detection. These control images (Figure S1 in the Supporting Information) show that the THC is not detected in either the brightfield or fluorescence images of a volunteer not taking cannabis.

To establish the applicability of this platform technology for the detection of other drugs and metabolites in fingerprints, ten volunteers who are prescribed methadone were recruited from a drug treatment clinic, with the aim of detecting both methadone and its major metabolite EDDP in fingerprints. Figure 3a,b show the brightfield and fluorescence images, respectively, of a fingerprint, obtained from a volunteer, after incubation of the antimethadone-antibody-functionalized magnetic particles and Alexa Fluor 488 tagged secondary antibody fragment. It was again observed that after incubation with the antibody–magnetic-particle conjugates, the fingerprint color changed to yellow-brown because of binding of the magnetic particle conjugates to the methadone antigen (Figure 3a). Both brightfield and fluorescence images (Figure 3a,b) confirm the detection of methadone in the fingerprint. The typical fingerprint ridge pattern that is required to identify an individual is also clearly evident. Similar results were obtained from the other nine volunteers. Figure 3c,d are brightfield and fluorescence images, respectively, of a fingerprint obtained using antiEDDP-antibody-functionalized magnetic particles for the detection of EDDP, the major metabolite of methadone. Again, the change in color of the fingerprint to yellow-brown after incubation of the antiEDDP-antibody-functionalized magnetic particles was observed (Figure 3c), while the fluorescence image of the fingerprint ridge pattern was also clearly visible (Figure 3d). Thus, both methadone and its metabolite EDDP can be readily detected in fingerprints of methadone users. The detection process is specific, as controls carried out by incubating both antimethadone- and antiEDDP-antibody-functionalized magnetic particles with fingerprints of volunteers who are not prescribed methadone neither showed any change in color in the brightfield image nor resulted in any

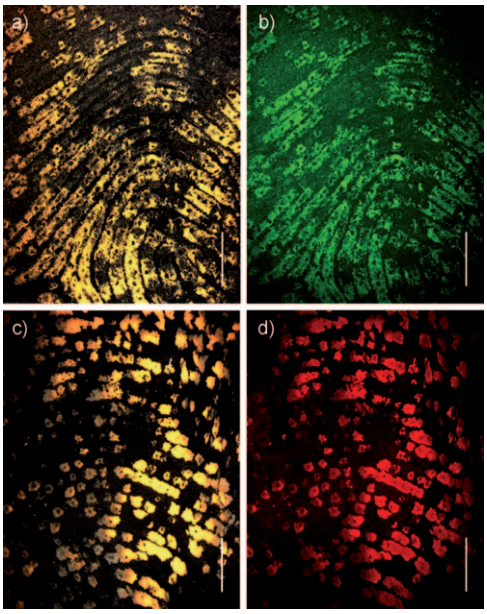

Figure 3. Detection of methadone and EDDP in a fingerprint. a) Brightfield and b) fluorescence images of a section of a fingerprint, obtained after incubation of antimethadone-antibody-functionalized magnetic particles and Alexa Fluor 488 dye-tagged secondary antibody. c) Brightfield, and d) fluorescence images of a section of a fingerprint, obtained after incubation of antiEDDP-antibody-functionalized magnetic particles and Alexa Fluor 546 dye-tagged secondary antibody. The scale bars are 2 mm.

detectable fluorescence signal from the fingerprint (see Figure S2 and S3 in the Supporting Information).

Finally, an experiment was performed in order to detect benzoylecgonine, the major metabolite of cocaine, in fingerprints. It was anticipated that, because of its short elimination half-life of about 7.5 hours, this drug metabolite would be difficult to detect.[25] Figure 4 shows the brightfield and fluorescence images of a section of fingerprint after incubation of antibenzoylecgonine-antibody-functionalized magnetic particles and Alexa Fluor 546 dye-tagged secondary antibody. The images in Figure 4 show the successful detection of benzoylecgonine in a fingerprint, as confirmed by both brightfield (Figure 4a) and fluorescence (Figure 4b) images.

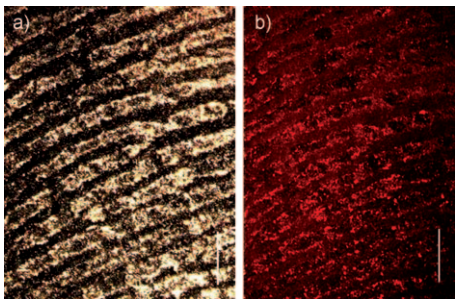

Figure 4. Detection of benzoylecgonine in a fingerprint. a) Brightfield and b) fluorescence images of a section of a fingerprint after incubation of antibenzoylecgonine-antibody-functionalized magnetic particles and Alexa Fluor 546 dye-labeled secondary antibody. The scale bars are 1 mm.

The fingerprint images are not as intense as the images obtained for the other drugs and drug metabolites described. This could be because of a lower concentration of the benzoylecgonine metabolite in the sweat deposited in the fingerprint with its short half-life. However, detection of this drug metabolite has clearly been achieved. Control experiments for benzoylecgonine detection again confirmed the specificity of the detection method (Figure S4 in the Supporting Information).

In conclusion, it has been shown for the first time that various drugs and drug metabolites, such as THC, methadone and its metabolite EDDP, and benzoylecgonine can be readily detected in latent fingerprints of drug users through the use of antibody–magnetic-particle conjugates imaged by using either brightfield and/or fluorescence microscopy. Importantly, the ability to see a change in color of a fingerprint by using brightfield microscopy following binding of the antibody–magnetic-particle conjugates provides the option of detecting latent fingerprints using a simple white light source, thus eliminating the need for expensive instrumentation, and thereby providing a simple, portable method for scene of crime investigations. The fluorescence imaging capability of latent fingerprints provides a method that is potentially more sensitive for trace residue detection. A key advantage of the magnetic-particle conjugates is that excess reagents can be removed using a magnet in a process similar to that currently used by fingerprint examiners at the scene of a crime. However, the versatility of the antibody–magnetic-particle conjugate platform is a particularly exciting feature as this enables the direct chemical imaging of latent fingerprints through the detection of both drugs and drug metabolites producing images of high evidential quality.

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