Fingermarks, more than just a ridge pattern
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CHAPTER 4

THE COMPATIBILITY OF FINGERPRINT VISUALIZATION TECHNIQUES WITH IMMUNOLABELING

ABSTRACT
The chemical composition of a fingerprint potentially holds a wealth of information about the fingerprint donor, which can be extracted by immunolabeling. Immunolabeling can be used to detect specific components in fingerprints, however in order to be applicable in the forensic field it should be compatible with commonly used fingerprint visualization techniques. In this study the compatibility of immunolabeling with two different fingerprint visualization techniques, magnetic powder and ninhydrin, was investigated on fingerprints deposited on glass and on nitrocellulose membranes. With dermcidin as antigen of interest, immunolabeling was performed successfully on all developed fingerprints. We can conclude that immunolabeling is compatible with magnetic powdering and ninhydrin staining, which can be of great forensic value.
1. INTRODUCTION
Fingermarks can be divided into three groups, visible, impression and latent fingermarks [1]. All three types of fingermarks can be encountered during criminal investigations. Latent fingermarks are invisible and need development in order to visualize them. There are several techniques that can be used, such as powders, ninhydrin, cyanoacrylate and 1, 8-diazafluoren-9-one (D.F.O.) [1-3]. The technique of choice is selected based on the specific characteristics of the carrier material on which the fingermarks are left [1, 4].

A method to extract more information from fingermarks, such as the presence of drug metabolites, is immunolabeling [5-8]. One of the first to describe the use of antibodies in fingermarks was Checka et al. In their study immunolabeling was used to type the bloodgroup of the donor of the fingermark [9]. Immunolabeling can also be used to improve the quality of poorly developed fingermarks and obtain more detailed information about the skin ridge pattern, like third level details [10-11]. The compatibility of immunolabeling with commonly used visualization techniques has not yet been investigated. The use of two successively applied techniques will facilitate both pattern recognition based on the skin ridge pattern and secondly retrieval of important knowledge based on the chemical composition of the fingermark.

In this study we explored the effect of fingerprint visualization techniques on the immunolabeling of fingermarks. Latent fingermarks left on a non-porous and porous carrier material were visualized using two common fingerprint visualization techniques, magnetic fingerprint powder and ninhydrin. After visualization immunolabeling was performed on treated fingermarks. Dermcidin, a naturally occuring antimicrobial peptide released by sweat glands, was detected using anti-dermcidin [10, 12]. Preliminary experiments performed by our group showed that dermcidin is present in most fingermarks and is thus a good choice to demonstrate the compatibility of immunolabeling with other fingerprint visualization techniques. In addition the pores visualized by dermcidin add to the elucidation of the ridge pattern.

2. MATERIALS AND METHODS
All experiments were performed under room temperature unless mentioned otherwise.

2.1 Fingermark collection.
Fingermarks were collected during working hours. Donors were asked to deposit a fingerprint on glass slides (Superfrost plus, Gerhard Menzel GmbH, Germany) or on nitrocellulose membranes (Millipore, Merck KGaA, Germany). No special instructions were given to the donors, which mean that the fingerprint depositions were completely natural. Every experiment included at least six different donors, both female and male donors. Samples were left for one day before treatment.
2.2 Powdering fingermarks.
Magnetic fingerprint powder was obtained from BVDA (Magnetic Black, BVDA International bv, The Netherlands). The powder was applied to the latent fingermark on the glass slide with a magnetic applicator, (Magnetic brush, BVDA International bv, The Netherlands) by moving the applicator above the mark. Fingermarks were visualized and after imaging, fingermarks were prepared for immunolabeling.

2.3 Ninhydrin treatment.
Ten fingermarks left on nitrocellulose membrane were sprayed with Ninhydrin NIN-Print Aerosol Spray (BVDA International bv., The Netherlands). Sprayed fingermarks were left to develop for one day. Fingermarks were visualized and after imaging, fingermarks were prepared for immunolabeling.

2.4 Single labeling of fingermarks developed with magnetic fingerprint powder on glass slides.
Fourteen fingermarks left on glass slides developed with magnetic powder were fixated in methanol (Sigma Aldrich, Germany) for twenty minutes at -20°C. Next, the slides were washed in MilliQ water (Millipore, Merck KGaA, Germany) for two minutes and were taken out, this step was repeated another two times. Samples were air-dried for about ten to fifteen minutes. A layer of fixogum (Marabu, Germany) was applied around the fingermark and dried for thirty minutes. The fixogum serves as a water-repellent barrier that keeps staining reagents localized on the fingermark sections. MilliQ was added to cover the section for five minutes. Next, phosphate buffered saline (PBS) (PBS obtained from Biowhittaker, Lonza Cologne GmbH, Germany) was applied to the samples. After five minutes, samples and surface were incubated with blocking buffer 1 (PBS + 3% bovine serum albumin (BSA) (BSA was obtained from Sigma Aldrich, Germany) for one hour. Mouse monoclonal IgM anti-dermcidin (Santa Cruz Biotechnology, INC, USA) was diluted in blocking buffer 1 1:20 (v/v) and 100 µl was applied to the fingermark and left overnight in a wet chamber. Next day, samples were washed in PBS for two minutes and this step was repeated another two times. The fingermark was incubated with the secondary antibody, goat anti-mouse FITC 1:200 (v/v) (Anti Mouse IgM FITC, Jackson Lab, Brunschwig, Switzerland) for one hour in a dark room. Samples were washed in PBS for two minutes and this step was repeated another two times. Fixogum was removed and a drop of DAKO fluorescent mounting media (Dako, Corp. Denmark) was applied to every glass slide, after which the cover slips were put on. Negative control experiments included the exclusion of the primary antibody and the exclusion of both antibodies.

2.5 Single labeling of fingermarks developed with ninhydrin left on nitrocellulose membranes
Ten ninhydrin developed fingermarks left on nitrocellulose membranes were incubated for one hour in blocking buffer 2 (PBS + 0.1% Tween-20 + 5% skim milk powder (SMP)) (Tween-20 obtained from Merck KGaA, Germany, SMP was obtained from Sigma Aldrich, Germany). Next the membrane was incubated with anti-dermcidin diluted in bloc-
king buffer 2 1:20 (v/v) for one hour. Upon incubation the membrane was washed three times with washing buffer (PBS + 0.1% Tween-20) for two minutes. Next the membrane was incubated with the secondary antibody, goat anti-mouse FITC 1:200 (v/v) diluted in blocking buffer 2 for thirty minutes. Then the membrane was washed for two minutes with washing buffer. This washing step was repeated another two times. Finally, the membrane was washed once with MilliQ for five minutes. Negative control experiments included the exclusion of the primary antibody and the exclusion of both antibodies.

2.6 Imaging
After the samples were dried, bright field and fluorescence imaging was performed using a Nikon Eclipse E600 microscope using a FITC filter (FITC: Excitation filter 465-495 nm, dichroic mirror 505 nm, barrier filter 515-555 nm) and a Nikon Coolpix 990 digital camera. Overall images were taken using a Nikon D40X digital camera (Nikon, Japan). Fluorescent images of immunolabeled fingermarks were obtained using a blue Crime-lite® 2 torch (445 nm, 10% band width 420 – 470 nm, Foster and Freeman, UK) and yellow filter (GG495, Foster and Freeman, UK).

3. RESULTS AND DISCUSSION
All latent fingermarks left on glass slides were successfully visualized using magnetic fingerprint powder. Ridges and furrows could be easily visualized, as shown in figure 1-A. No detectable (auto)-fluorescence could be observed in developed fingermarks prior to immunolabeling (fig 1-B and C). Figure 1-D shows the bright field images of fingermarks visualized using magnetic powder after immunolabeling. Washing steps performed during the immunolabeling caused loss of powder from the fingermarks. The overall pattern of the fingermarks, including ridge endings and other fingermarks features were still visible after these washing steps, except for one fingermark. Fluorescence images were obtained from the immunolabeled fingermarks, which indicated that specific detection of dermcidin was performed successfully on all fingermarks treated with magnetic powder. As depicted in figure 1-E, the green fluorescence corresponds to the presence of dermcidin. No non-specific binding of the primary and secondary antibody to the magnetic powder was observed. Negative controls (fig. 1-C and F) which included the exclusion of the primary antibody yielded a negative result, which supports the finding of specific detection of dermcidin. High detail levels can be observed when detecting dermcidin. Also, dermcidin is located only at the pore-sites, which is in agreement with results shown in previous studies [10, 12]. In all fingermarks the detection of dermcidin was performed successfully, albeit that the amount of fluorescence and thus the amount of dermcidin present in the fingermarks varied.

Fingermarks left on the porous nitrocellulose membrane, were visualized successfully using ninhydrin. Ninhydrin reacts with amines, amino acids, peptides and proteins in the fingerprint residue and the reaction product gives a red/purple coloration, called Ruhemann’s purple [13-14]. Bright field and fluorescence images were obtained from
the developed fingermarks. In fingermarks left on nitrocellulose membrane and which were developed with ninhydrin, Ruhemann’s purple can be observed as depicted in figure 2-A. Figure 2-B shows the result of detection of dermcidin in the same ninhydrin treated fingermark. Third level details can be observed and the fluorescence image is of high quality.

![Figure 1. Immunolabeling of fingermarks left on glass visualized with magnetic fingerprint powder. A-F: 4 × magnification. A and D: Bright field images. B-C and E-F: Fluorescence images. A-C: Fingermarks developed with magnetic powder prior to immunolabeling. D: Bright field image of fingermark after immunolabeling with anti-dermcidin. E: Fluorescence image of a fingermark after immunolabeling with anti-dermcidin. F: Fluorescence images of a negative control, which included the exclusion of the primary antibody.](image)

In figure 3 magnified images of a ninhydrin treated fingermark before and after immunolabeling and their negative controls are shown. A red/purple coloration could be observed in all ninhydrin treated fingermarks as illustrated in figure 3-A. However, in fingermarks of some individuals instead of ridges or furrows only one single large purple spot could be observed. We attribute this observation either to a high amount of excretion products, as free amino acids, peptides and/or proteins, which will diffuse into one single purple spot when applied with a lot of moisture or to the use of a high pressure while depositing the fingermark and thereby inducing a lack of ridge details. In the FITC-channel Ruhemann’s purple absorbs light, which results in black spots as can be seen in figure 3-B and were hardly visible in the negative control (fig. 3-C). Immunolabeling following the imaging of ninhydrin treated fingermarks resulted in the successful detection of dermcidin in all ninhydrin treated fingermarks as illustrated in figures 3-D and E. Bright field images showed that Ruhemann’s purple was washed away during the immunolabeling (fig. 3-D). Ninhydrin treatment and the washing steps did not influence
the immunolabeling of dermcidin in fingermarks. The fluorescence images demonstrate that detection of dermcidin was successful (fig 3-E). Dermcidin extended along the pore-sites of the fingermark, which is consistent to findings in previous work [10, 12]. Negative controls yielded a negative result (fig. 3-C and F).

![Image](image_url)

**Figure 2.** Overall image of a ninhydrin developed fingermark left on nitrocellulose membrane prior to (A) and after immunolabeling (B) with anti-dermcidin. A: Brightfield image of a ninhydrin developed fingermark. B: Fluorescence image of a ninhydrin developed fingermark after immunolabeling with anti-dermcidin.

Both fingerprint visualization techniques, magnetic powder and ninhydrin reagents, do not influence the immunolabeling of fingermarks. Dermcidin was present in all investigated fingermarks. The amount of dermcidin left in fingermarks varied between individuals. However, the detection of dermcidin provides relevant information about the location of the pore-sites in fingermarks; therefore this antigen can be helpful in obtaining third level details, which can be useful for identification purposes. Also, in cases whereby ninhydrin development resulted in a lack of ridge details, the labeling of dermcidin can give more information about the overall ridge pattern, as shown in figure 2.

To apply immunolabeling in the forensic field this technique should be applicable on fingerprints found and lifted from crime scenes. Therefore, future research may include the investigation of immunolabeling of lifted fingermarks. Also, investigating the effect of other fingerprint visualization techniques, like cyanoacrylate and D.F.O., on the immunolabeling of fingermarks is a next step to make this technique applicable in the forensic field.
4. CONCLUSION

Successful immunolabeling was performed on fingermarks developed with two commonly used fingerprint visualization techniques. Developing latent fingermarks with magnetic powder or ninhydrin prior to immunolabeling did not inhibit the specific detection of our antigen, dermcidin. Immunolabeling can thus be used on developed fingermarks to detect specific components or to enhance the quality of fingermark ridge patterns. In addition, the use of anti-dermcidin provides information about the location of the pore-sites and will therefore help in obtaining high level features of the fingermark.

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