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Evaluation of multi-target immunogenic reagents for the detection of latent and body fluid-contaminated fingerprints

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ABSTRACT

Fingerprint enhancement reagents capable of molecular recognition offer a highly selective and sensitive method of detection. Antibodies and aptamers provide a high degree of adaptability for visualisation, allowing for the selection of the most appropriate visualisation wavelength for a particular substrate without the need for specialist equipment or image processing. However, the major hurdle to overcome is the balance between sensitivity and selectivity. Single-target molecular recognition is highly specific, purported to have better detection limits than chemical reactions or stains, and can provide information about the donor or activity, but often results in incomplete ridge pattern development.

Consequently, the development and evaluation of multi-target biomolecular reagents for fingerprint enhancement was investigated, with the focus on endogenous eccrine secretions. To assess the suitability of the immunogenic reagents for potential operational use, a variety of parameters (i.e., processing time, fixing and working solution conditions) were optimised on a wide range of non-porous and semi-porous substrates. The relative performance of immunogenic reagents was compared to that of routine techniques applied to latent marks and marks in blood, semen and saliva. The incorporation of these novel reagents into routine technique sequences was also investigated. The experimental results indicated that the multi-target immunogenic reagents were not a suitable alternative to routine detection methods or sequences, but may have promise as a “last resort” method for difficult substrates or cases.

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1. Introduction

Fingerprints are one of the most common traces used for identification purposes. Unfortunately, routine detection methods currently employed by practitioners are limited by factors such as insufficient sensitivity or selectivity [1–3]. According to Champod et al. [4], an ideal detection method should have the following desirable features: increased sensitivity; portability to crime scenes; compatibility with other fingerprint detection methods and forensic analyses (e.g., DNA profiling); process simplicity; and reduction in cost and use of hazardous chemicals. Furthermore, the ability to develop fingerprints that remain undetected, or partially detected, by current methods is a highly desirable goal [4].

To address some of the issues raised with current fingerprint detection methods, investigation began into the use of molecular recognition, due to the high sensitivity and selectivity achieved by antibodies, lectins, and enzymes [3,5–14]. Research into the application of aptamers – chemically synthesised short single-stranded nucleic acids that bind to specific targets [15] – to detect fingerprints has also commenced [16]. Most of the published work to date has focused on capture reagents for a single target in the secretion. Due to the inherent intra- and inter-donor variability of fingerprint residues, however, there is a need to create a more “universal” multi-target immunogenic reagent that is compatible with current fingerprint detection methods. This reagent should target endogenous secretions in latent fingerprints, rather than drug metabolites or exogenous residues. Van Dam et al. [17] were able to develop fingerprints using their immunolabelling method targeting dermcidin, albumin, and keratin. Immunolabelling was also used following ninhydrin detection; results were inconsistent as some fingerprints showed improved contrast, while others did

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not. However, the immunogenic reagents were not evaluated as the first step in the sequence.

With the continuing interest into immunogenic reagents, the aim of the research presented in this paper was to optimise relevant parameters for the enhancement of latent and body fluid-contaminated fingerprints deposited on a wider range of substrates commonly encountered in real casework. Also, in order to evaluate these reagents for potential operational use, the relative performances of the antibody- and aptamer-based reagents were compared to those of established latent and blood detection methods. The prospect of their application as viable alternatives or in conjunction with routine detection methods is discussed.

2. Materials and methods

In accordance with the International Fingerprint Research Group (IFRG) guidelines, optimisation and validation studies were performed on locally sourced substrates [18]. Additional details on the initial optimisation experiments can be found in [electronic supplementary information \(ESI\)](#). For the initial proof-of-concept experiments to ensure the selected antibodies and aptamers were capable of binding to fingerprint residue, charged and natural fingerprints were collected on aluminium kitchen foil (Glad[®], Australia). Charged marks were prepared by asking the donors (2 male; 2 female) to lightly wipe their fingers across the back of the neck before touching the substrate.

2.1. Materials

2.1.1. Antibodies and aptamers

Anti-L-amino acid [19] and anti-L- α -hydroxy acid [20] antibodies (raised in rabbit) were produced by published methods and dialysed prior to use. Polyclonal anti-cAMP, anti-D-glucosamine, anti-human red blood cell (RBC), anti-semenogelin I, and anti-ODF3 antibodies were supplied by Abcam[®]. Polyclonal anti-carnosine and monoclonal anti-histatin 3 (clone 4G9) antibodies were supplied by Abnova. Polyclonal anti-acid phosphatase antibody was supplied by Aviva Systems Biology. Monoclonal anti-pan cytokeratin [AE1/AE3], anti-A HE195 clone and anti-B-HEB29 clone, polyclonal anti- α -amylase, anti-mucin 5B, and anti-SPA17 [N1C3] antibodies were supplied by GeneTex Inc. Polyclonal anti-SPAG11A (aa17–46) antibody was supplied by LifeSpan BioSciences Inc. Polyclonal anti-cathepsin D antibody was supplied by Molecular Innovations. Polyclonal (whole antiserum) anti-cortisol, anti-serotonin, anti-haemoglobin, and anti-spectrin antibodies were supplied by Sigma–Aldrich. All antibodies purchased were raised in rabbit, with the exceptions of anti-pan cytokeratin, anti-A HE195 clone, anti-B-HEB29 clone and anti-histatin 3 (clone 4G9) (raised in mouse), and anti-cAMP (raised in sheep).

Helix Aspersa and Ulex Europaeus (lyophilised powders) lectins were supplied by Sigma–Aldrich. Aptamers selected against catalase [21], “cathepsin D” (DGI, GEL, KAI tripeptide sequences) [22], cortisol [23], sperm [24], vitamin B12 [25], and vitamin D [26] were prepared and purified to order by Sigma–Aldrich’s Castle Hill oligonucleotide laboratory.

AttoTec Atto 550 N-hydroxysuccinimide (NHS) ester, Atto 590 NHS ester, Atto 610 NHS ester, and Atto 647N NHS ester were supplied by Sigma–Aldrich and used according to manufacturer’s instructions. Fluorescent Orange 550 reactive and Fluorescent Red 630 reactive were supplied by Fluka. Isoindole 1 was synthesised in-house (refer to [ESI](#) for detailed procedure).

Sodium citrate (ACS grade, Sigma–Aldrich), tetrachloroauric acid (Proscitech, Australia), sodium borohydride (99.99%, Sigma–Aldrich), O-(2-carboxyethyl)-O’-(2-mercaptoethyl) heptaethylene

glycol ($\geq 95\%$, Sigma–Aldrich), NHS and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide ($\geq 97\%$, Fluka) were used as supplied for preparation of antibody-functionalised gold nanoparticles (AuNPs) (refer to [ESI](#) for detailed procedure).

2.1.2. Current fingerprint detection techniques

Indanedione (SHIRAN, Israel), ethyl acetate (99.8%, Sigma–Aldrich), glacial acetic acid (RCI Labscan Ltd.), zinc chloride (reagent grade; Scharlau), ethanol (100%, Chem-Supply, Australia) and HFE7100 (3M Novac) were used as supplied in the preparation of 1,2-indanedione-zinc chloride (IND-Zn) reagent. Maleic acid (99%, Sigma–Aldrich), n-dodecylamine acetate (Optimum Technologies, Australia), Tween 20 (Sigma–Aldrich), silver nitrate (99.5%, Chem-Supply), ferric nitrate (98%, Chem-Supply) and ammonium ferrous sulphate (Analytical Reagent (AR) grade, Chem-Supply) were used as supplied in the preparation of physical developer (PD). Cyanobloom low-density cyanoacrylate (CA) (Foster + Freeman), rhodamine 6G (R6G, Sigma–Aldrich), isopropanol (99.9%, VWR) and methyl ethyl ketone (99.5%, Chem-Supply) were used for CA development and luminescent post-staining of latent fingerprints. 5-sulfosalicylic acid (5-SSA, 99.5%, BDH Chemicals Ltd., England), ethanol (95%, Chem-Supply), glacial acetic acid (RCI Labscan Ltd.), acid yellow 7 (AY7, Optimum Technologies), amido black (AB, Hopkin & Williams, England) and methanol (AR grade; Chem-Supply) were used as supplied in the preparation of blood reagents AY7 and AB.

2.2. Sample preparation

Six non-porous and two semi-porous substrates were chosen because they are often found at crime scenes or submitted as exhibits ([Table 1](#)). All of the substrates were used as is, except for the empty beverage cans and bottles. These were washed with warm soapy water, rinsed, dried, and then an acetone wipe was used to remove any residual traces from the outer surface prior to fingerprint deposition. Samples were prepared by drawing grid lines on the substrate surfaces to ensure that one fingerprint was present per cell and was bisected evenly, so equivalent areas of fingerprint residue were developed and assessed for each technique.

Four donors from the research project team (two male, two female) were asked to deposit both natural latent and blood-contaminated fingerprints. Donors were asked not to wash their hands for at least an hour prior to collecting natural fingerprints (total = 600). Blood-contaminated fingerprints were collected (total = 480) by pricking the finger with a single-use blood sampling lancet and rubbing the blood over the finger pad prior to deposition. All fingerprints were deposited in a series of five depletion fingerprints (i.e., successive impressions from the same digit, providing a sequential reduction in fingerprint residue) and then stored in the dark under ambient laboratory conditions for up to 4.5 months.

Table 1

List of substrates used and their suppliers.

Substrate type	Substrate	Supplier
Non-porous	Plastic ziplock bag	Coles, Woolworths
	Light grey plastic shopping bag	Woolworths
	Black garbage bag	Woolworths
	Cling film	Glad [®] , Woolworths
	Beverage can	Coca-Cola, various
	Plastic water bottle	Cool Ridge
Semi-porous	Glossy magazine (GI Mag)	UTS Playground, Aldi catalogue
	Glossy cardboard (GI Cdbd)	Nabisco

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