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RESEARCH ARTICLE

Visualisation of latent fingerprints on non-porous object immersed in stagnant tap water using safranin-tinted *Candida rugosa* lipase reagent

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Abstract

Waterways have been frequently used for disposing evidence by perpetrators, especially during homicide, rendering difficulties for forensic fingerprint investigators since water may destruct the amino acid components of fingerprints. Although the use of small particle reagent for visualising the lipid components of a fingerprint has been suggested, its use must be reduced due to its toxicity on human as well as environment. Therefore, this present research has developed a new environmentally-friendly safranin-tinted *Candida rugosa* lipase reagent (i.e. Lipase-Glutaraldehyde-Safranin, Lip-GA-Saf) for visualising latent fingerprints on non-porous surface (aluminium foils) immersed for up to 4 weeks in stagnant tap water. Results revealed that the quality of latent fingerprints (Fingermark Quality Scale) developed using Lip-GA-SAF was statistically better in all the four different durations of immersion than that of using SPR ($p<0.05$). Declining quality of fingerprints over longer period of immersion was also observed for both the Lip-GA-SAF reagent and SPR developed fingerprints. Considering the better quality of fingerprints developed using Lip-GA-SAF reagent, its usefulness in forensic practical caseworks appears promising.

Keywords: forensic science, wet non-porous object, latent fingerprints, *Candida rugosa* lipase

INTRODUCTION

The admissibility of fingerprints in the court of law has always been due its uniqueness, persistency, and systematic classifications of general ridge patterns [1]. In general, fingerprints are made up of a mixture of intrinsic (water-soluble and lipid-soluble components) and extrinsic (e.g. bacteria and dust) residues [2]. In view of forensic context, fingerprints can be classified into three, viz. visible, plastic, and latent prints [1]. Because of the hidden nature of latent fingerprints, its visualisation is often a challenging task for the forensic investigators [2]. Among many others, small particle reagent (SPR) has been routinely used for visualising wet latent fingerprints on non-porous objects [3]. However, its formulation mainly contains toxic compounds i.e. titanium dioxide [4] and molybdenum disulphide [5]. While titanium oxide is carcinogenic, molybdenum disulphide poses a great risk to the environment. Therefore, such harmful usage needs to be reduced. Taking into consideration the possible adverse effects of these hazardous compounds, as well as the need to visualise latent fingerprints for forensic purposes, development of an environmentally benign alternative (i.e. Lipase-Glutaraldehyde-Safranin, Lip-GA-Saf reagent) is therefore deemed necessary. The research was aimed at visualising latent fingerprints on aluminium foils immersed in a stagnant tap water for up to 4 weeks. Subsequently, the quality of visualised fingerprints following the use of Lip-GA-SAF reagent was compared to that of the routinely used SPR using the Fingermark Quality Scale (FQS) suggested by the previous study [6].

EXPERIMENTAL

Experimental design

Triplicates of groomed fingerprints from 2 males and 1 female donors were deposited on strips of acetone-cleaned aluminium foils, prior to immersion in stagnant tap water (from the laboratory) for 1, 2, 3, and 4 weeks in a plastic basin. Following the protocol suggested by Peel and Bond [7], the donors were asked to wash their hands with soap and water 15 minutes prior to deposition. Fingerprint was deposited by pressing the right thumb of a donor onto aluminium foils for 1–2 sec with a light pressure, sufficient to ensure contact between the thumb and aluminium foils. After the plastic basin was covered with transparent plastic to prevent interferences from dust and insects, it was placed in the laboratory. To determine the best formulation, a pre-screening analysis using different screening formulations (Table 1) was done to visualised fingerprints immersed in stagnant tap water for 4 days, prior to performing the real analysis. The best formulation was then used to visualise fingerprints on other immersed aluminium foils in the real analysis upon removing them from the water. Next, the quality of the visualised fingerprints was then graded using FQS [6] detailed in Table 2.

Parameters of the water, viz. temperature, pH, and Biochemical Oxygen Demand (BOD) were measured weekly before the development of fingerprint. BOD_5 testing was done according to 5210B APHA Standard Methods [8]. For BOD_5 testing, we used specialized 300 mL BOD bottle with ground-glass stopper which was designed to allow full filling with no air space and provide an airtight seal. A dissolved oxygen meter was used to measure the initial dissolved oxygen concentration (mg/L) in each bottle. Each bottle then placed into a dark incubator at 20 °C for five days. The BOD was determined by calculating the difference between the initial and final concentration of dissolved oxygen in the span of five days.

Table 1 The different screening formulations of the newly developed reagent.

Formulation No.	Solution
1	Lipase (Lip)
2	Glutaraldehyde (GA)
3	Safranin T (Saf)
4	Lip + GA
5	GA + Saf
6	Lip + Saf
7	Lip + GA + Saf

Table 2 The Fingermark Quality Scale suggested by the previous researches [6].

Grade	Detail visualised
0	No visible prints
1	Poor quality, very few visible ridges
2	Poor quality, some ridge-details visible or partial mark with limited characteristics
3	Reasonable quality, ridge-details and some characteristics visible, identification possible
4	Good quality prints, ridge-details and characteristics visible, probable identification
5	Excellent quality, very clear prints, identification assured.

Preparation of the solutions

For Lip solution, the lipase from *C. rugosa* (5 mg/mL) was mixed with phosphate buffer (pH 6.5) by centrifuging them at 6000 rpm for 2 min. The supernatant was then used as the Lip solution. As for GA and Saf solutions, 5 mL of glutaraldehyde in 10 mL of phosphate buffer (pH 6.5) and 500 mg of Saf in 10 mL distilled water were prepared accordingly. The solutions (Lip, GA, and Saf) were kept chilled (about 4 °C) in separate plastic spray bottles until further use.

Visualisation of latent fingerprints

The aluminium foils bearing fingerprints were first sprayed with Lip solution and left for 5 min at room temperature. Next, the excess Lip solution was removed gently using distilled water. Then, the GA solution was sprayed onto the study materials. After 10 min of incubation, the excess GA solution was rinsed using distilled water. Lastly, Saf solution was sprayed onto the study materials and left to incubate for 10 min prior to rinsing it with distilled water. The study materials were left to dry prior to grading analysis.

Statistical analysis

Data analysis in this present research was done using the UTM licensed IBM SPSS version 22.0 and the normality of the data prior to conducting the hypothesis testing was checked using the Kolmogorov-Smirnov and Shapirov-Wilk tests. Considering the small number of samples tested for quality scale assessment ($n < 100$) used in this present research, indication provided by the Shapiro-Wilk test prevailed over the Kolmogorov-Smirnov. Hence, whenever the values of Shapiro-Wilk test were found to be smaller than 0.05 ($p < 0.05$), the data can be construed as not normally distributed. Since it was found that the fingerprint grading scheme data were not normally distributed, the Kruskal-Wallis with the pairwise comparisons using the Mann-Whitney U test was used for comparing the differences between the group medians.

RESULTS AND DISCUSSION

Pre-screening analysis

A pre-screening analysis was executed to determine the best bio-chemical formulation that would give sufficiently identifiable visualised prints on aluminium foils immersed in tap water for 4 days. The pre-screening analysis involved optimising the parameters required for formulating a new visualising reagent using Lip, GA, and Saf solutions. The results for the pre-screening analysis are illustrated in Fig. 1 (a–g).

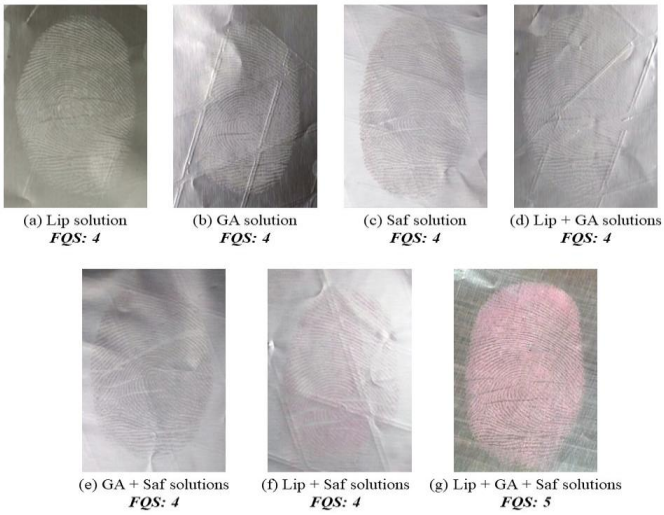

Fig. 1 The results for the pre-screening analysis using the different formulations.

It was found that the best formulation to afford the highest quality (contrast) and identifiable prints was obtained following the use of Formulation 7 that consisted of Lip, followed by GA and Saf solutions (Fig. 1g). It was shown that the use of the cationic Saf solution had markedly enhanced the contrast of the developed fingerprints, and was found indispensable in obtaining identifiable visualised fingerprints (Fig. 1g). It was observed that by merely spraying Lip and Saf solutions (without the presence of GA solution as a cross-linker (Fig. 1f)) as well as spraying GA and Saf solutions (without Lip solution as an lipid-specific agent to react with the lipid constituents of fingerprints (Fig. 1e)), the contrast of the developed fingerprints was not sufficient to provide identifiable characteristics that could be used for identification purposes.

Actual analysis

The feasibility of utilising Lip-GA-Saf reagent in visualising latent fingerprints on aluminium foils immersed in a stagnant tap water for up to 4 weeks was further explored in this present study. Table 3 represents the quality of fingerprint on aluminium foils immersed in stagnant tap water. It is known that the quality of visualised fingerprints naturally deteriorates over time [6], and in this present study, such aspect was observed. It was observed that the range of median quality of visualised fingerprints on aluminium foils immersed in stagnant tap water for up to 4 weeks by Lip-GA-Saf reagent (1.00-4.00) was categorically higher than that of SPR (0.00-4.00). Notwithstanding, the median of fingerprint grading on aluminium foils immersed in stagnant tap water using Lip-GA-Saf reagent were significantly better ($p < 0.05$) when compared to that of SPR. While the fingerprints developed by Lip-GA-Saf reagent (3.00±2) remained forensically identifiable even after two weeks of immersion, the same for SPR (2.00±2) was only observed within the first week of immersion. Hence, the results clearly demonstrated the comparability of the newly-developed Lip-GA-Saf reagent with that of SPR for forensic applications. Fig. 2–5 depict the representative photographs of visualised fingerprints over certain immersion periods using both methods. Because of the differences in temperature (28.0–28.5 °C), BOD_5 (0.0–0.8), and pH (7.05–7.42) of water utilized in this present study were observably marginal (Table 3),

further studies exploring the feasibility of Lip-GA-Saf reagent for visualising latent fingerprints on objects immersed in water with varying temperatures, BOD_5, and pH appear imperative for elucidating the real potential of the reagent for forensic practical casework.

Table 3 Quality of fingerprint on aluminium foils immersed in stagnant tap water.

Immersion period / Surface	Method	Week 1	Week 2	Week 3	Week 4
Aluminium foils	Lip-GA-Saf	4.00±2* (3-5)	3.00±2* (2-4)	1.00±5* (0-5)	1.00±2* (0-3)
	SPR	4.00±1 (2-5)	2.00±2 (1-4)	1.00±1 (0-3)	0.00±2 (0-2)
Temperature (℃)		28.5	28.5	28.0	28.0
BOD_5 (mg/L)		0.8	0.3	0	0
pH		7.42	7.23	7.12	7.05

Kruskal-Wallis with the pairwise comparisons using the Mann-Whitney U test was used for comparing the differences between group medians in tap water using the two methods. The data are presented as median ± interquartile range. The data in parentheses, ( ) indicated the range of the values. The symbols (*) indicate the significant differences in the grading scale of fingerprint between the development of fingerprint on surfaces using Lip-GA-Saf when compared with development of fingerprint on aluminium foils using SPR ($p < 0.05$).

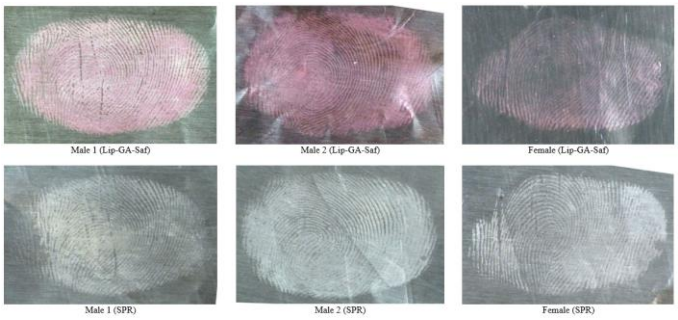

Fig. 2 Development of fingerprints on aluminium foils immersed in stagnant tap water for 1 week using Lip-GA-Saf reagent and SPR.

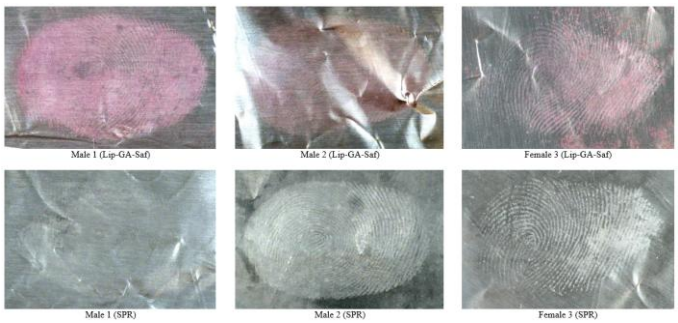

Fig. 3 Development of fingerprints on aluminium foils immersed in stagnant tap water for 2 weeks using Lip-GA-Saf reagent and SPR.

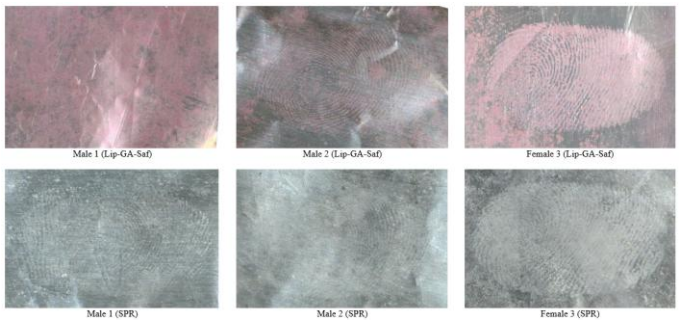

Fig. 4 Development of fingerprints on aluminium foils immersed in stagnant tap water for 3 weeks using Lip-GA-Saf reagent and SPR.

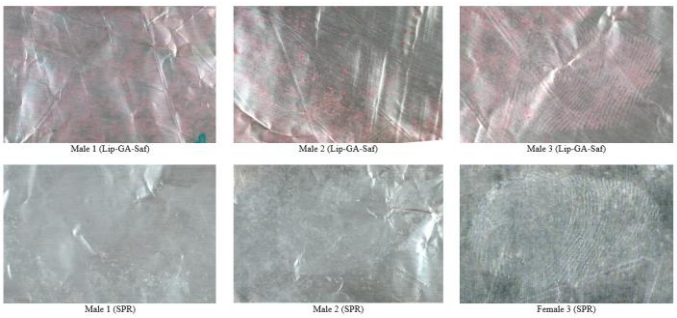

Fig. 5 Development of fingerprints on aluminium foils immersed in stagnant tap water for 4 weeks using Lip-GA-Saf reagent and SPR.

CONCLUSION

In a nutshell, this present study reported the possibility of using greener biotechnological route for developing latent fingerprint immersed in stagnant tap water for providing an environmentally friendly alternative to the commercially used but hazardous SPR reagent for forensic investigation. The results showed that the combination of Lip-GA-Saf formulation provided the best formulation. In addition, the Lip-GA-Saf reagent had successfully facilitated the development of latent fingerprints on aluminium foils immersed for up to 4 weeks in stagnant tap water. Interestingly, following the use of Lip-GA-Saf reagent, the quality of developed fingerprints was statistically better ($p < 0.05$) when compared with that of SPR. Such findings accentuate the possibility of using this newly developed biotechnological method for forensic applications, necessitating further studies covering varying types of water commonplace at crime scenes.

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