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DNA recovery from 3D printed firearms

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ABSTRACT

Over the last few years, additive manufacturing, by means of fused deposition modelling 3D printing, has become increasingly popular and accessible due to the relative low costs, flexibility, and continually lowering barriers for entry. Unfortunately, while this technology is not only used by hobbyists for creative or practical creation of custom objects using a variety of thermoplastic materials, they can also be used in the manufacture of firearms. From fully printed to hybrid designs, AM/3D printing has been used to produce parts for fully functional firearms capable of discharging conventional ammunition. The designs for such firearms can be easily found, downloaded and shared from the internet with 'enthusiasts' developing ever more sophisticated designs.

In environments where lawful possession of firearms is strongly controlled, the ability to manufacture and assemble firearms through 3D printing technologies presents an increasing challenge for law enforcement, especially for forensic units tasked with collection, examination and recovery of evidence from these items. Forensic examination of 3D printed firearms can require a different approach compared to conventional firearms if maximum forensic intelligence is desired. Due to the decentralised and often individual nature of manufacture, biometric identification of both the handler/possessor and the manufacturer/assembler of the firearm can have investigative value. However, this can present a dilemma, as collection of biometric evidence from internal surfaces of the firearm typically requires dismantling of components which may affect functionality in subsequent test firing processes.

This study aims to investigate DNA transfer and recovery on 3D printed firearms (Harlot pistols and FGC-9) in casework-inspired handling scenarios, to determine the most probative regions for the recovery of DNA originating from the assembler and handler of such firearms as well as determining the effect of firing (or test-firing) the firearm. The findings suggest that assembler DNA can be recovered from internal surfaces of the firearms, with significantly more DNA recovered from internal grip pieces than internal triggers. Further, the proportions of assembler DNA recovered on internal surfaces were significantly more than the proportions on external surfaces, based on contributor proportion percentages. In this study, test firing of the firearm, even up to 25 times, did not result in detectable DNA relocation onto internal surfaces from the individual conducting the test fires.

1. Introduction

3D printing, or 'additive manufacturing,' involves creating a three-dimensional object by adding layers of various materials. Using digital print files, 3D printers allow individuals to create complex objects, confined only by the physical limits of the material used and the printers used to construct them. As such, 3D printers have seen a rapid increase in use worldwide due to their flexibility and convenience in many fields, such as medical devices, mechanical engineering, and robotics. They have also been deployed for 3D printed firearms (3DPFs) or "ghost guns" ([1]. Firearm designs can be found, downloaded and shared from the internet, and the plastic components printed at home. These individually

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printed components are commonly assembled with other easily accessible pieces such as springs and screws [2], to form fully functional firearms, capable of firing commercially available ammunition. 3DPF files for the 'Liberator' pistol were released in May 2013 by Defence Distributed and downloaded over 100,000 times before the United States government demanded the files be retracted [1]. The Liberator pistol is an unreliable handgun that is prone to exploding on discharge [3]. However, since the Liberator's release, 3DPFs have experienced extensive development. The FGC-9, released in 2020 and undergoing constant improvements, is one example of the many modern printable firearms, that have been tested and designed to reliably function within material constraints. These firearms can be accurate and lethal. FGC-9 print files and fully assembled firearms have been recovered across the globe and are particularly popular amongst extremist groups and militias [4].

The forensic science community has conducted research into some of the traces that can be recovered from a 3DPF. Most of these revolve around physical traces such as striations on bullets and impressions made by firing pins, or gunshot residue (GSR) and polymer fragments that are ejected following discharge [3,5]. At the time of writing, the authors are not aware of any research into the recovery of DNA from 3DPFs. In a 2024 report on the state of 3DPFs, the South-Eastern and Eastern Europe Clearinghouse for the Control of Small Arms and Light Weapons [6] discussed the need for more research on the forensic examination of 3DPFs, including DNA sampling on internal surfaces which is not necessary for conventional firearms. In order to expose the internal surfaces for traditional swabbing techniques, disassembly of a firearm is required, however, disassembling the firearm before test firing can create challenges for the firearm evidence team as the reassembled firearm is no longer in the condition it was collected. As such, the effects of test firing a 3DPF on DNA recovery are worth examining.

There are many different areas on a firearm that can be targeted for trace DNA collection. The optimal surfaces are likely those that are regions that have the most contact with the handler These include the handle/grip, slide and stock (when present), and the trigger/trigger guard [7–9]. However, DNA recovered from firearms is typically of poor quality [9–11]. Trace DNA refers to DNA-bearing cellular material that is not sourced from a known bodily fluid such as blood or saliva, and is often found in small quantities [12]. The amount of DNA transferred to an object depends on the nature of the contact between the source and the surface. It has been demonstrated that considerable trace DNA transfer occurs in the initial contact between an individual and surface [13,14]. The pressure used by an individual when coming into contact with a surface can affect trace DNA transfer, with higher pressures depositing more DNA [15]. Prolonged contact over an extended period generally results in greater DNA transfer compared to brief contact durations [16].

An individual's shedding propensity is an indication of the amount trace DNA that individual tends to deposit on touched surfaces and is an underlying factor influencing transfer of DNA to the surfaces of the firearms. Shedder tests are used to determine an individual's shedding propensity and typically classify individuals as poor, intermediate or good shedders. It has been observed that there is a significantly higher probability of detecting DNA transferred from a high shedder than from low shedders to surfaces [17]. However, there are a range of shedder tests and when studied or practised, there are a multitude of other variables that can affect their results. As such, shedder classifications are highly variable and strict shedder classification are sometimes considered arbitrary[18].

Consideration of DNA evidence in investigations and court rooms has shifted from contesting the presence of DNA to contesting the events that led to the presence of DNA, where the propositions focus on how the DNA was deposited rather than the source of the DNA profile [19]. Secondary or indirect transfer is when the DNA of an individual is transferred to a surface not through direct contact, but by an intermediate vector such as another individual or object [20]. Secondary transfer is complex, unpredictable and influenced by many interacting factors [16]. One potential outcome of secondary transfer is mixture inversion. This is when an individual contacts a surface yet doesn't appear as the major contributor to the profile. Instead, DNA that is indirectly transferred to the surface, via the individual, has a greater abundance. Mixture inversion is relatively rare, but it has been observed in some secondary transfer studies, for example, one of fifty collected in a study by Goray et al [21].

The transfer, persistence, prevalence and recovery of DNA are complex phenomena and may be impacted by a variety of factors. Multiple reviews and recommendations have been published that summarise these factors including Taylor et al 2018 [19], Gosch and Courts 2019 [22], Kokshoorn et al. 2018 [23] and Gill et al. 2025 [24]. This study focuses specifically on recovery of DNA from 3DPFs post-primary handling and the effects of firing on subsequent profiles, by examining the effects of test firing on the recovery of DNA from internal and external surfaces. This study also examines optimal procedures for the forensic examination of a fired 3DPF. Specifically, the objectives were to:

- determine the most effective method of DNA recovery from 3D printed surfaces;
- determine the most probative regions for the recovery of DNA originating from the assembler and handler of a 3DPF; and
- examine the effect on DNA recovery of increasing the number of times a 3D printed firearm is fired.
- determine if firing the 3DPF is likely to relocate DNA to internal surfaces

2. Methods

2.1. Ethics Approval and participants

Informed consent was obtained from each participant before their inclusion in the study. This research was approved by the [redacted for review]. The participants were adults between the ages of 20–45 and included two assemblers (who assembled the firearms from 3D printed components) and two handlers (who handled the assembled firearms before and during firing) for each firearm (Table 1). There were no instructions or requirements for participant behaviours prior to participation. Participants were allowed to go about their usual daily tasks and routines. The participants share a workplace and, as such, it is possible that the participants could have come into contact with each other prior to their roles as assemblers or handlers.

2.2. 3D printed tiles

Fifteen 4.4 cm by 4.4 cm black plastic tiles were printed on an Ender-3 v2 Neo 3D printer (Creality 3D) with a 0.4 mm brass nozzle using modified polylactic acid (PLA +) 3D printing filament (eSUN). The tiles consisted of a top side and bottom side, with differing textures, the same textures as some exterior and interior surfaces of the 3DPFs, respectively. A DNA source was prepared by diluting neat saliva in sterile water. A volume of 50 μL of the saliva was diluted with 4950 μL of water to make a 5 mL diluted saliva solution. The tiles were cleaned in a bath of 10% bleach solution followed by 70% ethanol and left to dry. An aliquot of 200 μL of diluted saliva was added to the top side of fourteen tiles and

Table 1
Participant roles.

Participant	Role for Harlot Scenarios	Role for FGC-9 Scenarios	Gender
1	Assembler 1	Assembler 1	Male
2	Assembler 2	Nil	Male
3	Handler (both)	Nil	Female
4	Nil	Handler 1	Male

left to dry overnight.

The DNA recovery methods of double swabbing and tape lifting were compared in this experiment, in isolation from the other variables introduced by firearms. Saliva from seven of the tiles was recovered using wet and dry push off swabs (PathTech). The double swabbing involved first swabbing the surface with a swab moistened with sterile/DNA free water ampules, immediately followed by then swabbing the same surface with a dry swab, with both swab heads combined in the same 1.5 mL safelock tube (Sarstedt). The saliva from the other seven tiles was recovered using DNA-free tapelifts (Lovell Surgical Solutions International) and placed in 1.5 mL plastic tubes (Sarstedt). Swabs of both sides of one tile with no saliva deposit were also added to 1.5 mL plastic tubes (Sarstedt) to be used as a manufacturing and cleaning control. The tiles were cleaned and left to dry. The same method was then repeated with the diluted saliva solution being deposited on the bottom side of the tiles.

2.3. Firearms

Two different 3DPFs were employed (Fig. 1). The less complex firearm examined was the Harlot pistol, consisting of all plastic components aside from a metal barrel, springs and fasteners. The Harlot pistol features a basic, manually operated single-shot firing mechanism with no automation and is commonly designed to fire 0.22 Long Rifle (22LR) calibre ammunition. Manual cartridge extraction and reloading is required before the pistol can be subsequently fired again. The FGC-9 MKII (FGC-9) is a substantially updated version of the original FGC-9 and represents a more advanced 3DPF, it is capable of semi-automatic fire and is commonly designed to fire 9x19mm calibre ammunition. The FGC-9 uses a magazine-fed system and features a more complex firing mechanism, as such the firearm requires additional non-plastic components, including large springs and a metal bolt. This increased complexity does not require specialist skills [25], but demands a higher level of technical skill and knowledge for its printing and assembly compared to the Harlots.

2.4. Initial firearm Construction

Both firearms were printed and assembled in accordance with the instructions included in the print file package downloaded from open-

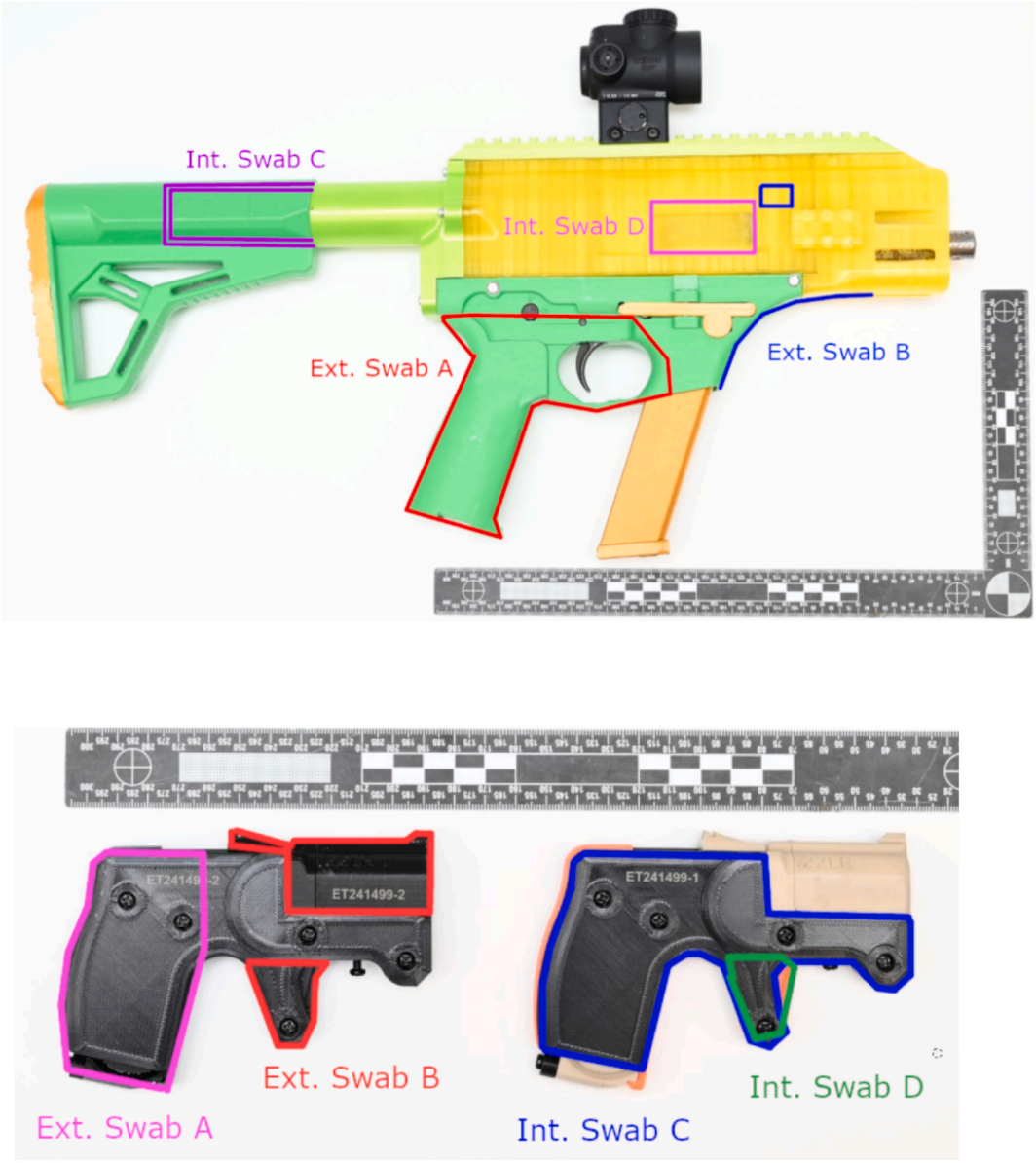

Fig. 1. FGC-9 rifles (A) and Harlot pistols (B) and the areas that were sampled for DNA from the exterior and interior surfaces.

source internet. Printing of the Harlot pistols was performed on an Ender-3 v2 Neo 3D printer (Creality 3D) with a 0.4 mm brass nozzle using eSUN PLA + 3D printing filament (eSUN). After assembly, each Harlot was loaded with a 22LR calibre inert round (Pachmayr) to test the firing mechanism, after which a primed cartridge case was fired by hand. These tests confirmed that the Harlots would be able to discharge a live cartridge. The FGC-9 was originally printed on a Creality Ender 3 v2 printer using a range of PLA based materials, including eSUN Glass PLA and eSUN eSilk PLA. As the FGC-9 had been used prior to this experiment, no test firing was necessary.

2.5. Cleaning and reassembly

Before firing, and after DNA recovery (Section 2.6), the components of the firearms were cleaned. The Harlot pistols were fully disassembled, the components soaked in 10% bleach for 5 min and then 70% ethanol for 5 min. The barrel was plugged at both ends with Forensic Sil (Loci Forensic Products) before soaking to protect the metal lining. The significantly larger FGC-9, having more exposed internals, required only partial disassembly; the stock and bolt were removed to access swabbed surfaces. It was then wiped with 10% bleach-soaked RediWipe sheets, followed by 70% ethanol-soaked sheets. Each firearm was reassembled by a single, ungloved individual, assigned as the 'assembler'. The assembly process involved holding the pieces in place and screwing them together with multiple Allen keys. The firearms were then placed and sealed in an evidence recovery bag, in which it was transported to a firing range.

2.6. Firing

All firing occurred within an indoor firing range at the [redacted for review]. All cartridges were discharged into a granulated rubber bullet trap (Meggitt). The FGC-9 was fired by a designated handler, in a semi-automatic (or self-loading) mode. A set number of 9x19mm cartridges (124 grain, full metal jacket, American Eagle branded) were discharged. The firearm was then placed back into the evidence recovery bag.

The structural integrity of the Harlot pistols was unknown. A remote firing process was implemented to ensure the safety of the participants. Prior to firearm handling, a table and pistol rest underwent sterilization using a combination of bleach then ethanol wiping. Each Harlot was removed from its protective paper packaging and underwent dry firing by hand, three times, by the assigned handler (different to the handler of the FGC-9). A Plastic Safety Snap Cap (Pachmayr) was placed in the chamber during dry firing to minimize potential damage to internal mechanisms. This method produces no recoil, but is otherwise a suitable substitute for live-firing. Subsequently, the firer wrapped the handle of the Harlot with a sterile blanket (Grycol International) that had been cut to size in a sterile environment and securely positioned the firearm within a Master Series Pistol Rest (Ransom International) fixed to the table (Fig. 2). The Harlot remained secured in the rest throughout the remainder of the firing procedure. The firearm was loaded with a single 22 Long Rifle cartridge (40 grain, lead round nose, Winchester Xpert branded) and remotely fired using the integrated lever mechanism of the rest to compress the trigger (Fig. 3). The Harlot pistols underwent a set number of loading and firing cycles before being removed from the rest and returned to their original packaging. The same sequence of procedures was conducted for both Harlot 1 and Harlot 2. The firearms were then placed back into their evidence recovery bags.

2.7. DNA recovery

The firearms were transported in their paper bag packaging to a DNA recovery laboratory. The laboratory bench was cleaned with bleach and ethanol and DNA-free paper was placed on the table. DNA recovery was performed by an individual who was not involved in the manufacturing or firing of the firearms. The firearms were removed from their packaging and placed on the examination paper on the table. Four sets of swabs were collected from each firearm according to Fig. 1 and Table 2. Two sets of double swabs were collected from the external surfaces. After disassembly using the appropriate sterilised tools, two sets of double swabs were collected from the internal surfaces. The swab heads were removed and placed in 1.5 mL plastic tubes (Sarstedt). All the firearm components were placed in the exhibit packaging and returned to the assemblers for cleaning and reassembly. The tubes containing the swabs were kept in a freezer at −20 degrees Celsius before DNA analysis and subsequent profiling.

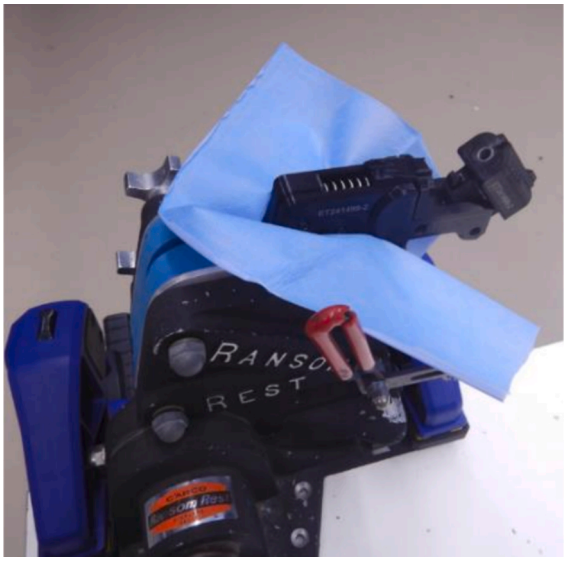

Fig. 2. Unloaded Harlot 2 pistol in pistol rest.

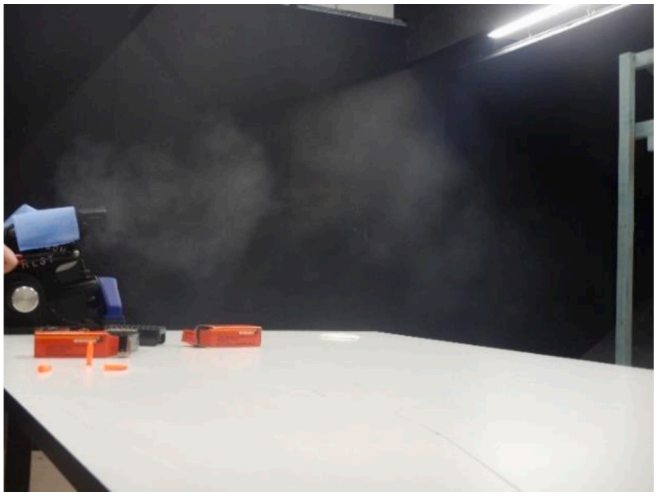

Fig. 3. Harlot 2 being remotely fired.

Table 2
Swabbed surfaces of firearms.

	External Swab A	External Swab B	Internal Swab C	Internal Swab D
FGC-9 surfaces	Grip, trigger	Cocking handle (not visible in Fig. 1A), underbarrel	Internal surface of stock head, stock	Plastic surfaces of bolt
Harlot pistol surfaces	Grip	Trigger, hammer and barrel. (THB)	Grip spacer, internal grip surfaces	Internal trigger surfaces

2.8. Repetitions

The experiment was repeated from the cleaning and reassembly stage of the method to the firing stage. Each repetition differed in the number of times the firearms were fired, resulting in four rounds of DNA recoveries for all firearms. Over 3 rounds, both Harlot pistols were dry fired then discharged 3, 6 and 9 times. In the fourth round the harlots were dry fired 9 times then discharged 25 times. The FGC-9 was discharged 3, 6, 9 and 8 times. The FGC-9 was deemed unsafe to continue firing after 8 discharges in the fourth round due to a malfunction of an internal component.

2.9. Shedder test

Following the results from the Harlot pistols, an [redacted for review] standard shedder test was performed, with an internal methodology modified from Kanokwongnuwut, Martin, Kirkbride and Linacre [26] to determine the individual shedding propensity of the Harlot assemblers and handler. Glass slides were used for the collection of thumbprints. The glass slides were initially cleaned by wiping with 10% bleach for 60 s (minimum 60 s exposure) and then with 70% ethanol. They were subsequently left to dry with the collection surface facing up. The slides were stored in a clean, dry environment until use. The assembler and handler of the Harlots washed their hands using water, then continued their normal administrative activities (not laboratory activities) for approximately 60 min, without further washing their hands. The participants then deposited thumbprints on the slides. Contact was made on the surface of the DNA free side, for 60 s using medium pressure. This was performed with the participants' left and right thumbs.

The slides were heat fixed over a flame and then cooled, after which they were stained using the Christmas tree stain: Nuclear Fast Red for 15 min; rinse with deionised water; picroindigocarmine for 15 s; rinse with 70% ethanol. The slides were then airdried and stored at room temperature before microscopic examination at 100x magnification under brightfield microscopy conditions. For each thumbprint, five random frames were selected for imaging. Cells with characteristics typical of shed epithelial cells were counted. The average count per image for both thumbs was calculated and converted to cells per mm^2.

Individual shedding propensity was determined with cell per mm^2 boundaries used for [redacted for review] in-house research and validation studies (Table 3).

2.10. DNA analysis and profiling

All DNA analysis and profiling procedures were performed according to the manufacturers' recommended protocols unless otherwise stated. DNA extraction was performed using the QIAamp DNA Investigator Kit (QIAGEN) on a QIAsymphony liquid handler (QIAGEN). DNA quantification was performed using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific). The individual real time quantitative polymerase chain reactions (qPCRs) were prepared on a QIAgility liquid handler (QIAGEN), then amplified on a QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific). DNA was normalized to 1 ng of template in 15 μL added to each polymerase chain reaction (PCR) for a total 25 μL reaction volume and then amplified using the GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific) on a VeritiPro™ Thermal Cycler (Thermo Fisher Scientific). The amplified DNA was electrophoresed on a 3500xL Genetic Analyzer (Thermo Fisher Scientific). Genotyping was performed with GeneMapper ID-X Software (Thermo Fisher Scientific) using an analytical threshold of 50 relative fluorescence units (RFU).

Table 3
Individual shedding propensity boundaries.

Classification	Cell per mm^2
Light	< 2.14
Intermediate	2.14 – 9.98
Heavy	> 9.98

STRmix version 2.7 was used to analyse the DNA profiles. For STRMix parameters, the Australian Caucasian Sub-Population database and a theta value of 2% was used as recommended in Taylor, D., et al, (2017) [27]. The number of contributors to a profile was determined by the highest number of alleles at any locus. The most probable number of contributors to each profile was determined after visual inspection. If all loci in the profile contained no more than two alleles (after accounting for stutter), it was deemed to be a single source profile (one contributor). If any locus in the profile contained more than two and no more than four alleles (after accounting for stutter), it was deemed to be a two-person mixture. If any locus in the profile contained more than four and no more than six alleles (after accounting for stutter), it was deemed to be a three-person mixture.

Likelihood ratios (LRs) were assigned for a given profile, G, according to:

$$\mathrm{LR} = \frac{\mathrm{P}(G|H_1)}{\mathrm{P}(G|H_2)}$$

where $\mathrm{P}(G|H_1)$ is the conditional probability of the profile, G, given proposition H_1 and $\mathrm{P}(G|H_2)$ is the conditional probability of G given proposition H_2. In the case of a single contributor, the propositions were:

H1: one known contributor (handler OR manufacturer).

H2: one unknown random member of the population

For two contributors, the propositions were:

H1: one known contributor (handler OR manufacturer) and one unknown random member of the population

H2: two random unknown members of the population.

For three contributors, the propositions were:

H1: one known contributor (handler OR assembler) and two unknown random members of the population

H2: three unknown random members of the population

STRmix returned likelihood ratios for the relevant propositions and deconvoluted contributor proportions for each presumed contributor to the sample DNA profiles.

2.11. Statistical tests

Experimental data were grouped into independent variable classes and then tested for normality using Shapiro-Wilk tests. If all groups or all but one were normally distributed then a multi-factorial analysis of variance (ANOVA) was applied to find variables with a significant influence on the dependent variable. The R Stats Package was used to apply all statistical tests [28]. Cohen's Eta Squared (η^2) Interpretation Scale is used throughout to describe and compare effect sizes on a verbal scale [29].

3. Results

3.1. DNA recovery from 3D printed tiles

A total of fourteen swabs and fourteen tape lifts were taken from the 3D printed plastic tiles, with seven swabs and seven tape lifts collected from both the top and bottom surfaces (Fig. 4). The DNA concentrations recovered from the squares were grouped by surface (top, bottom) and DNA collection method (swab, tapelift) and were found to be normally distributed after Shapiro-Wilk tests ($p > 0.05$).

Analysis of variance (ANOVA) revealed significant differences between surfaces ($p = 0.024$) and DNA recovery method ($p = 7.8\text{x}10^{-8}$). There is a large effect size (>0.16) [29] for both surface and DNA collection method, with the surface type having a lesser effect on the quantity of DNA recovered ($\eta^2 = 0.19$) compared to the DNA collection

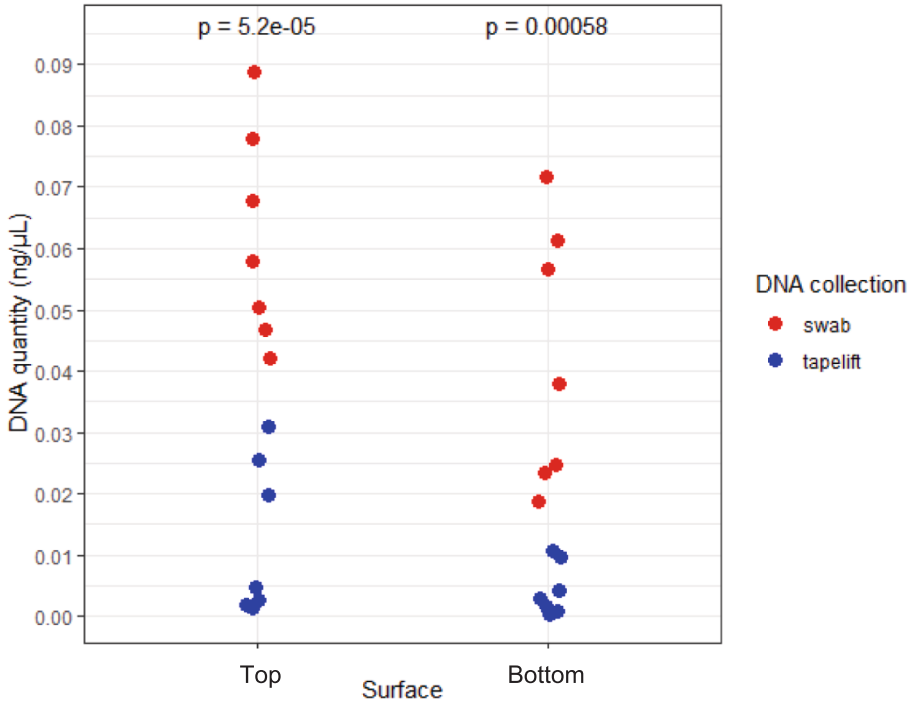

Fig. 4. Quantity (ng/µL) of DNA recovered using swabs and tapelifts from top and bottom surfaces of seven 3D printed tiles.

method ($\eta^2 = 0.71$). Post tests indicated swabs recovered significantly more DNA than tapelifts on both top ($p = 5.2x10^{-5}$) and bottom ($p = 0.0006$) surfaces (Fig. 4). DNA collection method had a similarly large effect on the quantity of DNA recovered on both the top and bottom surface with $\eta^2 = 0.64$ and $\eta^2 = 0.76$ respectively.

3.2. DNA recovery from firearms

A total of 16 swabs were taken from each Harlot pistol and the FGC-9. After each test firing, four sets of swabs were collected from each firearm. Two sets of double swabs were collected from the exterior surfaces and, following disassembly, two sets of double swabs were collected from the interior surfaces.

The DNA concentrations recovered from the FGC9 rifle were grouped by surface (interior/exterior), sample area, and times fired. These groups were found to be normally distributed after Shapiro-Wilk tests ($p > 0.05$). ANOVA revealed no significant differences between surfaces, sample area and number of fires (Figs. 5 and 6). The DNA concentrations recovered from the Harlot pistols were also grouped by surface (interior/exterior), sample area, and times fired. These were found to be normally distributed after Shapiro-Wilk tests ($p > 0.05$) excepts for swabs of the interior trigger ($p = 0.001$). This lone significant result is consistent with a false positive as a result of multiple comparisons and so an ANOVA was applied.

The ANOVA tests applied showed no significant differences between the quantity of DNA recovered on internal vs external surfaces, however a post test indicated that significantly more DNA was recovered from the swabs of the interior grip than from the interior trigger ($p = 0.007$) (Fig. 7), likely due to the larger surface area of the grip pieces compared to the trigger pieces. As surface area was not a variable that was able to

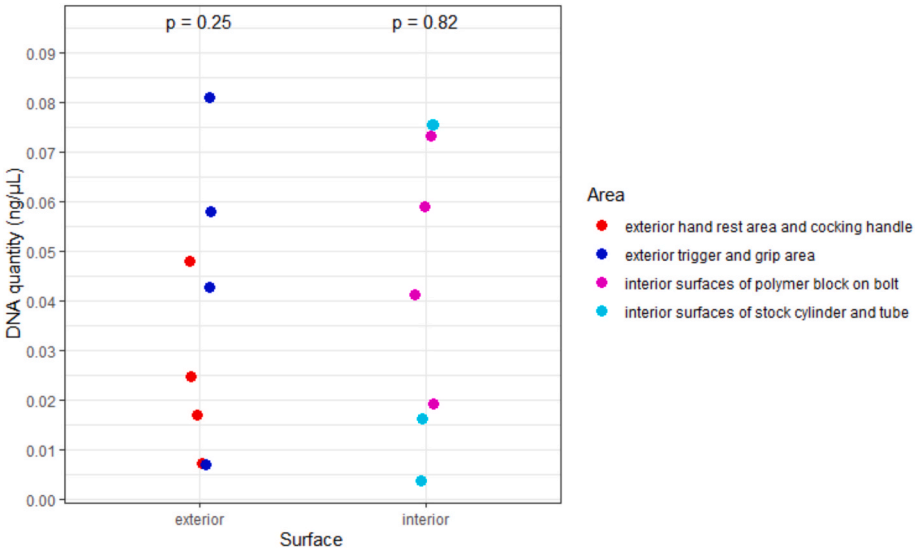

Fig. 5. Quantity (ng/µL) of DNA recovered from the surfaces of the FGC-9 after firing and grouped by sample area.

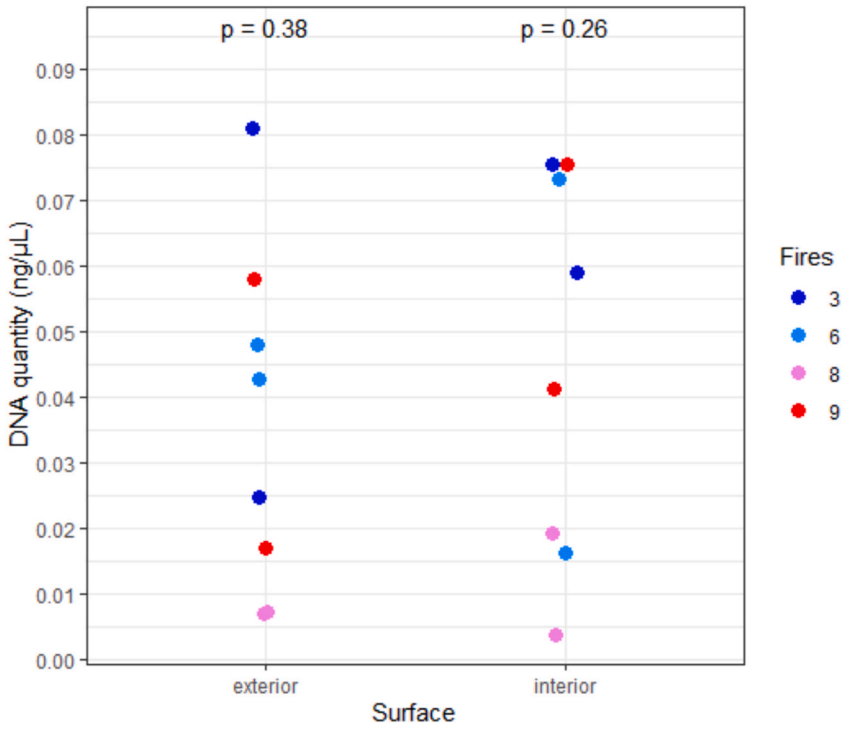

Fig. 6. Quantity (ng/µL) of DNA recovered from the surfaces of the FGC-9 after firing and grouped by number of times fired.

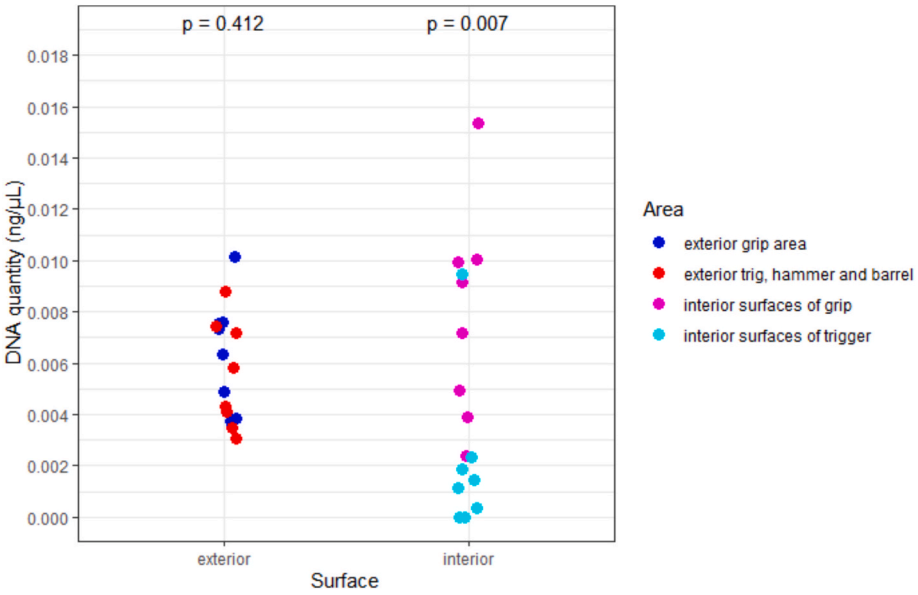

Fig. 7. Quantity (ng/ µL) of DNA recovered from the surfaces of the Harlot pistols after firing and grouped by sample area.

be measured for this study, effect sizes will not be reported.

There were no other significant differences observed across the number of fires or the interior and exterior surface, although post tests indicated a possible difference between exterior surfaces of the harlots across number of fires (Fig. 8). As there no trend could be discerned to correlate number of fires and quantity of DNA recovered from the external surfaces of the Harlots, effect size has not been reported. There were no other significant differences observed across the number of fires or the interior and exterior surface- ($p > 0.05$) (Fig. 8).

3.3. Contributor proportions of DNA profiles recovered from firearms

STRmix 2.7 was used to deconvolute DNA profiles and assign contributor proportions. The reference profiles for the assemblers and handlers were compared to the recovered profiles and LRs were calculated for each known contributor (assemblers and handlers). The contribution proportions for each contributor were calculated after deconvolution.

The external surfaces of the FGC-9 harboured the presence of both assemblers and handlers. The contribution proportions for the assembler and handler recovered from the FGC-9 (with $LR > 1$) were grouped by surface (interior/exterior), sample area, and times fired. These were

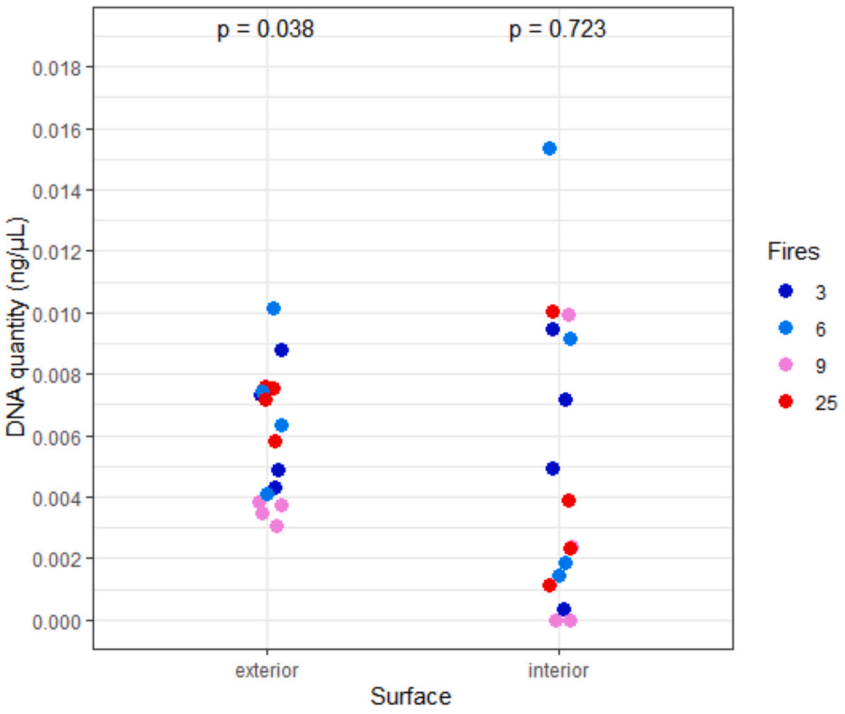

Fig. 8. Quantity (ng/ µL) of DNA recovered from the surfaces of the Harlot pistols firing and grouped by number of times fired.

found to be normally distributed after Shapiro-Wilk tests ($p > 0.05$) except for swabs of the interior surface ($p = 0.006$). This lone significant result is consistent with a false positive as a result of multiple comparisons and so an ANOVA was applied.

The ANOVA revealed significantly higher contributor proportions for assemblers on interior surfaces than on exterior surfaces (Fig. 9, $p = 0.007$), and a corresponding significantly higher contributor proportions for handlers on exterior surfaces (Fig. 10, $p = 0.006$). The effect of surface type was determined to be large ($\eta^2 = 0.62$ and $\eta^2 = 0.64$ respectively) which is to be expected as the handler only directly handled the exterior surfaces of the firearm. There were no significant differences in the contributor proportions obtained amongst the specific sample areas for either assemblers or handlers.

Profiles recovered from external surfaces of the Harlots contain a range of contribution proportions for the assembler (with $LR > 1$), ranging from 35% to 100% (Fig. 11). For the internal surfaces, the assembler contributed from 65% to 100%. All DNA profiles recovered from the internal trigger surface produced high LRs for the assembler with 100% contribution, the internal trigger sample after 9 fires produced no DNA (0% contribution). Only one profile (THB surface / 9 fires) recovered from the harlot pistols contained contributor proportions with an $LR > 1$ for the handler of the Harlots (35%) however its relative $\log_{10}LR$ was 0.4.

The assembler contribution proportions were grouped by surface (interior/exterior), sample area, and number of times fired. These groups were found to not be normally distributed after Shapiro-Wilk tests ($p > 0.05$). The number of times the firearms were fired did not appear to influence the contributor proportions for either assemblers or handlers.

The number of times the firearms were fired did not appear to

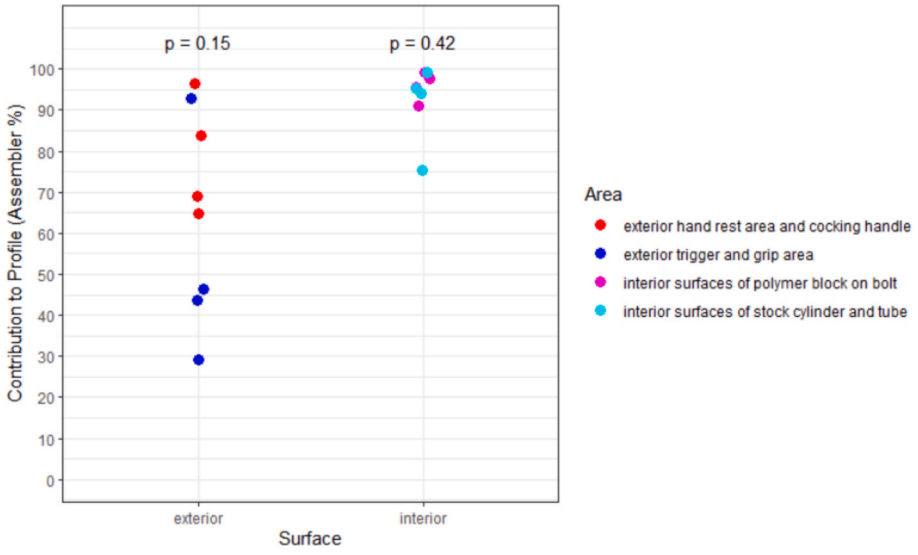

Fig. 9. Assembler mixture proportions for the FGC-9 after firing and grouped by sample area.

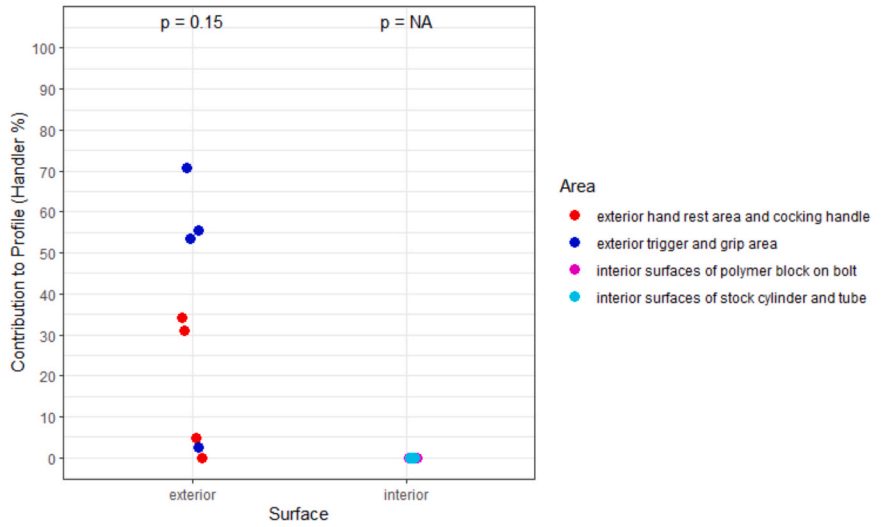

Fig. 10. Handler mixture proportions for the FGC-9 after firing and grouped by sample area.

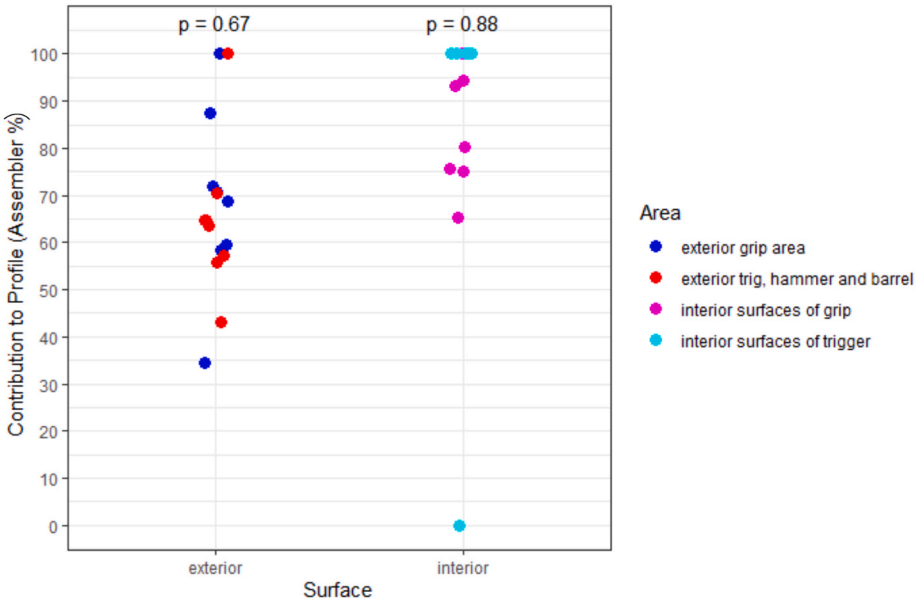

Fig. 11. Assembler mixture proportions for the Harlot pistols after firing and grouped by sample area.

influence the contributor proportions with high assembler or handler LRs recovered from any surface.

3.4. Probative value of DNA evidence

STRmix 2.7 was used to assign likelihood ratios (LRs) for the DNA profiles from the samples. The most probable number of contributors (NoC) was either 0, 1, 2 or 3. The person of interest POI was either the assembler of the 3D printed Harlot or the handler (firer). The magnitude of the LR indicated probative value according to the LR interpretation framework. LR > 1 and $\log_{10}$LR > 0 favours H_1; LR $= 1$ and $\log_{10}$LR $= 0$ favours neither H_1 nor H_2 (i.e. no probative value); and LR < 1 and $\log_{10}$LR < 0 favours H_2.

Fig. 12 shows that positive $\log_{10}$LRs were obtained for the assembler of the firearms on all but two of the examined surfaces for all firearms. Only two surfaces of the Harlot pistols yielded positive $\log_{10}$LRs for the handler's DNA: 0.15 (Harlot 1 – 3 fires) and 0.4 (Harlot 2 – 9 fires), both from the external THB surfaces.

For the FGC-9, only positive $\log_{10}$LRs for assembler DNA were recovered from internal surfaces, handler $\log_{10}$LRs were either negative or 0. For external surfaces the assembler $\log_{10}$LRs were always higher than handler $\log_{10}$LRs with one exception: the external trigger/grip surfaces after eight fires. The only external surface without a positive $\log_{10}$LR for handler DNA was the cocking handle/underbarrel surfaces after 3 fires. The assembler $\log_{10}$LRs from internal surfaces were consistently higher than those from external surfaces. The number of times the Harlot was fired did not appear to have an effect on assembler or handler $\log_{10}$LRs. However, the last round of firing (eight discharges) produced the lowest assembler $\log_{10}$LRs for external and internal surfaces.

The $\log_{10}$LRs from profiles recovered from each firearm were grouped by surface (interior/exterior), sample area, and times fired, these were found to not be normally distributed after Shapiro-Wilk tests ($p > 0.05$). The number of times the firearms were fired did not appear to influence $\log_{10}$LRs, recovered from profiles from any surface.

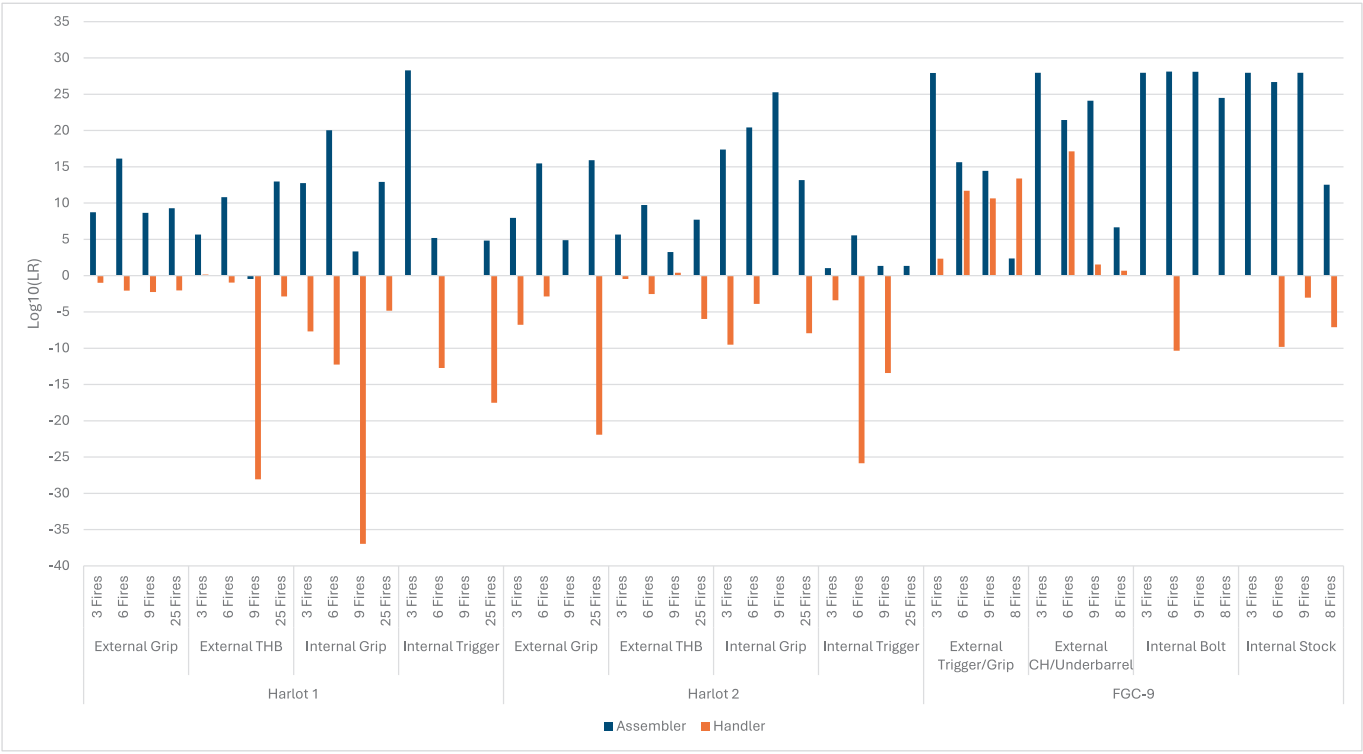

Fig. 12. Log_{10}LRs for assemblers and handlers from DNA profiles from the external and internal surfaces of Harlots and the FGC-9 after firing.

3.5. Shedder test

A [redacted for review] standard shedder test was performed for all of the participants (two assemblers and two handlers). The results (Fig. 13) suggest that all participants would be classified as "heavy shedders" (> 9.98 cells/mm^2).

4. Discussion

4.1. Recovery method

The results from the DNA recovery methods on 3D printed tiles align with expectations based on conventional procedures for recovering DNA from non-porous surfaces and support the use of double swabbing as the

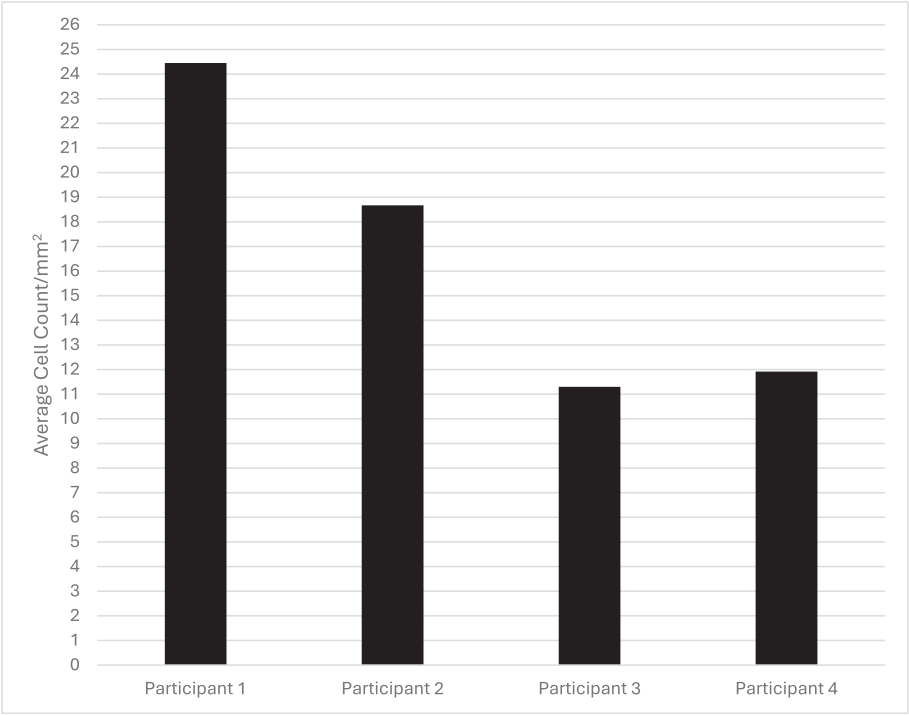

Fig. 13. Determination of individual shedding propensity for all participants.

DNA recovery method for the plastic surfaces of the 3DPFs. Significantly higher quantities of DNA were recovered from both surfaces of the tiles via double swabbing than tape lifting (Fig. 4). There is a noticeable difference between the recovery rates from the top and bottom surfaces, with yields from the top surface being typically higher than from the bottom surface (Fig. 4). This may be due to a difference in the textures of the surfaces. The grooved path lines of the top surface, caused by the printing process, may better harbour the deposited DNA.

Swabs of both sides of the tiles with no saliva deposit were also added to 1.5 mL plastic tubes and processed alongside the main samples. These were to be used as a manufacturing and cleaning control. No DNA was recovered from the control samples, suggesting that the cleaning methods of soaking the tiles in bath of bleach followed by soaking in a bath of ethanol and being left to dry was an effective method of sterilising the tiles between experiments. The same cleaning method was applied to the components of the Harlot pistols.

4.2. Shedder test

The handler of the Harlot pistols transferred a surprisingly low amount of DNA onto both Harlots. STRmix analysis of the recovered DNA profiles revealed that only negative $\log_{10}$LRs were produced for the proposition that the handler was a contributor to the mixed DNA profiles, aside from two positive $\log_{10}$LRs below 0.50. For DNA mixtures recovered from the Harlots, STRmix deconvoluted the profiles and attributed a contributor proportion to each presumed contributor. The handler was assigned the lowest (non −zero) contributor proportion in each case STRmix was forced to compare the proposed deconvoluted profiles with the assigned reference profiles. The mostly negative $\log_{10}$LRs suggest it is likely that the handler proportion was negligible in most cases and that the unassigned proportions belonged to unknown individuals for which a reference profile was not available. A reasonable explanation for these results is that secondary transfer of DNA from unknown individuals onto the Harlots had occurred. That fact that the transferred DNA likely returned higher mixture proportions than the handler suggests that the handler may have a low propensity to shed DNA, relative to the assembler and any other unknown contributors.

An [redacted for review] standard shedder test was performed with the individuals involved in the Harlot experiment to further investigate these results. The results of the shedder test (Fig. 13) suggest that the assemblers of both Harlots are higher shedders than the handler (handler 1).

Since the handler of the harlot pistols is a lighter shedder compared to the assemblers, the lower amount of DNA deposited may be harder to detect. This would typically result in lower LRs supporting their contribution to the DNA profile. It has been observed that there is a significantly higher probability of detecting DNA transferred from a high shedder than from low shedders to surfaces [17]. Although not classified as a 'low shedder', the handler cell counts were lower than both assemblers and the difference in the propensity for the participants to deposit trace DNA is clear. However, this does not completely explain why the handler deposited such small amounts of DNA on the firearm with most LRs < 1. Negative handler LRs and poor-quality handler profiles are further discussed in section 4.6.

4.3. Harlot pistols

The results suggest that the assembly of a Harlot by an ungloved individual can consistently deposit enough DNA on both internal and external surfaces to lead to identification. There are benefits from swabbing for trace DNA from various surfaces. The internal trigger surface yielded less DNA but was more probative for the presence of the assembler: 100% proportions with high $\log_{10}$LRs. Therefore, DNA recovered from the internal surfaces can be more useful for identifying an assembler than the external surfaces and should be targeted in casework. There was only one profile with a positive handler $\log_{10}$LR (and 35% contributor proportion), the THB surfaces from Harlot 2 after 9 fires, which was the only case where the $\log_{10}$LR for the handler was above 0 (0.4). It is still likely not an indication of how much DNA the handler actually contributed to the profile. In nearly all cases, the DNA mixture profiles were more likely if the handler was not a contributor to the mixtures than if the contributors were two random members of the population. No handler DNA was recovered from the internal surfaces of the pistols, however, it is also essential to recognise that the low levels of handler DNA on external surfaces may potentially be further reflected on internal surfaces. The results do not indicate when any DNA from unknown contributors was indirectly transferred to these internal surfaces, either during the assembly or firing of the firearm. Overall, the firing process, and an increase of discharges within a round of firing does not have an effect on the recovery of assembler DNA from either internal or external surfaces.

4.4. FGC-9

Similarly to the Harlots, the results suggest that the assembly of an FGC-9 by an ungloved individual can consistently deposit enough DNA on both internal and external surfaces to lead to identification. There was strong evidence to support the presence of assembler and handler DNA on almost all external surfaces of the FGC-9. There are benefits from swabbing for trace DNA from various surfaces. DNA recovered from the internal surfaces can be more useful for identifying an assembler than DNA from the external surfaces. The external trigger/grip surfaces produced the highest yields of DNA with high handler LRs, promoting this surface as a target when aiming to recover handler DNA from a fired 3DPF. There is no evidence to support the presence of handler DNA on internal surfaces suggesting the test firing process did not relocate handler DNA to internal surfaces of the firearm. Small proportions of unidentified DNA were recovered from all surfaces of the firearm. The firing process, and an increase of discharges within a round of firing does not have a noticeable effect on the recovery of handler or assembler DNA from either internal or external surfaces.

4.5. Effects of firing

The results indicate that the process of firing has no significant effect on the recovery of DNA from the firearms. The effects of firing on DNA recovery has been previously investigated. Prasad, Atwood, van Oorschot, McNevin, Barash and Raymond [9] sampled internal surfaces of firearms including the magazine and cylinder. These internal surfaces produced single source profiles from the owner of the firearm. In contrast, Horsman-Hall, Orihuela, Karczynski, Davis, Ban and Greenspoon [30] recovered very low yields of DNA from internal surfaces of firearms (barrel, breach face and ejection port) and they propose it originated from secondary transfer of DNA from loaded and fired cartridges. They suspected the cause of the low-quality profiles were a combination of low initial quantity and degradation from the heat and gunshot residue inhibitors. Both the firearms used in this study had surfaces in close proximity to the cartridge during discharge, yet no significant effect of firing was observed. The only results where a case could be made for a greater number of fires affecting DNA recovery would be the external trigger/grip surfaces of the FGC-9. Over the second to fourth rounds of firing, with 6, 9 and 8 fires, an increase in contributor proportions with high handler LRs was observed. Although the last round was stopped due to malfunctions in the mechanisms of the firearm, the handler had extended contact with the surface in an attempt to overcome the malfunctions. This process may have also removed assembler DNA from the firearm, contributing to the higher handler proportion. Prolonged contact over an extended period generally results in greater DNA transfer compared to brief contact durations [16]. Increasing contributor proportions with high handler LRs was also observed by Oldoni, Castella and Hall [31], although the handling durations were different.

4.6. Poor quality of handler profiles

It is likely the negative or low handler LRs were influenced by the quality of profiles recovered from the external surfaces of the Harlot pistols. Handler LRs were likely penalised at various sites due to the low RFU values and poor quality profiles. Low RFU values suggest the handler did not contribute a large amount of DNA to the profiles and as such we are unable to recover a large amount of their DNA. The cause for the low amount of handler DNA contribution is unclear, especially when acknowledging that although the handler can be considered a poorer shedder than either Harlot assembler, the difference in the individual shedding propensity tests was not substantial.

Persistence and transfer of DNA are two core factors of DNA transfer that potentially influenced these results. Neither of these have been thoroughly investigated on 3D printed firearms or surfaces. When comparing the persistence of assembler and handler DNA, it is possible that the textured plastic surface of the Harlots external surfaces more effectively harboured the assembler DNA. This assembler DNA may not have been removed by the handler and persisted throughout the firing process and transferring of the firearm to the examination room, thus contributing more to the recovered profiles. Handler DNA deposited "on top" of assembler DNA may have been more affected more by the remote firing process and transport to the examination room. It should be noted that some external surfaces of the Harlot pistols, such as the barrel and hammer, were smooth plastic (no ridges to harbour DNA) and not excessively contacted by the drop sheet during remote firing (Fig. 3).

These factors may have influenced the poor quality handler profiles from the Harlot pistols, which introduced the potential for exogenous DNA from unknown individuals, drop-in or other factors to outweigh the recovery of handler DNA.

4.7. Mixture inversion

Mixture inversion is a product of indirect transfer when an individual contacts a surface yet doesn't appear as the major contributor to the profile. Instead, DNA that is indirectly transferred to the surface, via the individual, has a stronger presence in the profile.

The presence of assembler DNA on the external surfaces of the Harlot pistols was supported by high $\log_{10}$LRs and high contributor proportions. However, there were considerable proportions of the DNA profiles from external surfaces that were unaccounted for. The negative handler $\log_{10}$LRs suggests that the unknown DNA is more likely to have originated from two random individuals than if the handler had contributed to the profile. This is strong evidence that handler DNA was not present in most profiles recovered from external surfaces.

Fig. 11 shows up to 65% of DNA from profiles from external surfaces of the Harlot pistols originated from unknown individuals, not involved in the assembling or handling of the firearms. These values are notably higher than in other experiments where significant mixture inversion has occurred. Cale, Earll, Latham and Bush [32] obtained a 25% mixture inversion rate on plastic knife handles, following "vigorous" hand shaking of two individuals for two minutes but this study has been criticized for employing an unrealistic transfer scenario.

The unknown DNA on the 3DPFs may have originated from any number of individuals. Analysts and laboratory personnel were excluded as the source. It is also important to consider that both the assembler and the handler were possible vectors for indirect transfer. However, considering that only in 2/16 profiles was there weak evidence to support the presence of handler DNA with $\log_{10}$LRs of 1.5 and 0.4, a potential explanation is that the assembler deposited their own DNA and the DNA of others, and the handler deposited (a very small amount of) their own DNA and the DNA of others. This promotes the presence of mixture inversion, where the handler transferred more DNA to the external surfaces of the Harlots, than their own. Of course, this is difficult to prove given the amounts of DNA indirectly transferred by each participant during the experiment is unmeasurable.

4.8. Limitations and Suggestions for further research

There were several limitations to the study's methodology. The primary one was the small data set (only three 3DPFs, two handlers and two assemblers). In addition, the modified firing process for the Harlots is different to what would happen at a real crime scene or forensic test fire examination. Future research into DNA recovery from 3DPFs should involve a larger sample size as the low number of samples collected in this study had high variance, and a larger data set may more accurately present trends and deduce more statistically meaningful findings. In regard to the experimental design, different/more handlers for the Harlot pistols would be of substantial benefit. Utilising a greater number of different assemblers and handlers would also be beneficial in limiting the influence of the individuals on DNA transfer, such as individuals shedding propensity. Further investigation into the persistence and transfer of DNA on all 3D printed materials is required to understand the circumstances that may lead to mixture inversion and better interpretation of resulting profiles at the activity level.

5. Conclusion

The main aims of this study were to determine the effect of the test firing process on the recovery of DNA from the internal surfaces of a 3DPF, and to recommend procedures for forensic examiners in the event of the examination of a 3DPF.

The results promote the efficacy of swabbing over tapelifts on non-porous surfaces such as rigid plastics. Significantly higher DNA quantities were recovered with swabs than tapelifts from both sides of the plastic tiles. These results support our choice to perform DNA recovery on the surfaces of the 3DPFs with double swabbing. The double swabbing quantity results from the surfaces of the plastic tiles are within the same order of magnitude and value range as quantities recovered from the surfaces of the 3DPFs. This suggests that the plastic tiles are an accurate model for applying the DNA recovery method results to 3DPFs.

The assembly of an FGC-9 by an ungloved individual can consistently deposit enough DNA on both internal and external surfaces to lead to identification. Although not as strong, evidence to support the presence of handler DNA can be recovered from external surfaces after firing an FGC-9. More handler DNA can be recovered from surfaces the handler comes into vigorous contact with, such as the trigger and grip. There was no evidence to support the presence of handler DNA on the internal surfaces of the firearm. Whilst assembler DNA was recoverable on external surfaces, internal surfaces should still be targeted when attempting to identify an assembler, as profiles recovered from the internal surfaces included a lower proportion of unidentifiable DNA.

There was no clear effect on DNA recovery from increasing the number of times the firearms were fired. This was true for both firearms, as well as internal and external surfaces. Regarding our main aims, the firing process does not appear to diminish the quantity or quality of DNA recoverable from internal surfaces.

This study presents an informative foundational study for DNA recovery from 3DPFs. However, there are many improvements that can be made in future research. Generation of additional data will be essential in strengthening the statistical power and reliability of the results. Introducing more variation in experimental design would also be useful, such as experimenting with exclusively modern 3DPFs and using a greater number of assemblers and handlers. Furthermore, an effort should be made for all forensic fields to stay updated with the rapidly advancing field of 3DPF technology.

6. Ethics Statement

This research was approved by the UTS Health & Medical Research Ethics Committee (MREC: Project ETH22-7601) and was conducted in accordance with the principles of the Declaration of Helsinki.

CRediT authorship contribution statement

Flynn Thomas Oltrogger: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Greg Howe:** Writing – review & editing, Resources, Conceptualization. **Dennis McNevin:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation. **Matthew Bolton:** Writing – review & editing, Supervision, Resources, Methodology, Investigation. **Caroline O'Driscoll:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization. **Stephen Woodcock:** Formal analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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