Forensic Science International 354 (2024) 111890

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Fibres in the nasal cavity: A pilot study of the recovery, background, and transfer in smothering scenarios

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ARTICLE INFO

Keywords:
Forensics
Trace evidence
Evaluation
Interpretation
Activity level
Occurrence
Shedding
3D printed models

ABSTRACT

In cases where the suspected cause of death is smothering, fibre traces recovered from the nasal cavity are hypothesised to refute or support this proposition. In order to carry out such evaluations, an efficient recovery method must first be established. This pilot study tested five different recovery methods on 3D printed models of nasal cavities. Among which, the use of the transparent AccuTrans® polyvinyl Siloxane casts demonstrated the best recovery efficiency with a median of 90% of deposited fibres recovered. The efficacy of this method was then verified on cadavers. Apart from a reliable recovery method, an understanding of the background population of fibres in nasal cavities, as well as the mechanisms of the transfer from the purported smothering textile to the nasal cavity is essential to evaluate the findings in these cases of suspected smothering. Samplings of the nasal cavities of 20 cadavers were thus carried out to gather data on the background population of fibres. Results showed that nasal cavities are not void of fibres, but the quantities are expected to be low, with a mean of 3.8 fibres per cavity recovered. Information on generic fibre class, colour, and length of these background fibres were also obtained with the use of low and high-power microscopy. The frequencies found in this population of fibres closely align with data from other population studies where black cotton was the most common. Finally, transfer experiments using the 3D printed models fitted with a respiratory pump to simulate breathing were carried out, along with testing on live volunteers in-vivo. The results demonstrated a verifiable transfer of fibres into the nasal cavity in smothering scenarios. Textiles of various shedding capacities were used in these tests and the findings suggest an influence of this variable on the quantities of fibres transferred.

1. Introduction

The characteristic signs of asphyxia are difficult to detect in cases of smothering with textile implements [1]. This is often accompanied by the lack of any defensive traces, as victims of such events are often unable to offer up any resistance due to their age, disabilities, or incapacitation from illnesses or medications [2–4]. In a previous study, Schnegg et al. [5] proposed the consideration of trace evidence such as textile fibres to assist in such cases. Specifically, the consideration of fibres recovered from the facial region of the victim which are non-differentiable from the textile fibres of the purported smothering implement (such as a pillow). Schnegg et al. [5] suggested that the quantity of these fibres provides value in discriminating between a proposition of smothering with the implement from a proposition of legitimate activity, such as a night's sleep. The exploitation of quantities of non-differentiated fibres as support, or lack thereof, for various activity level propositions has already been well established [6–9]. The basis of such methods stems from the propensity of textiles to transfer fibres of varying quantities based on the intensity of a contact [10]. The number of fibres recovered on the surface is thus a vestige of the contact activity and provides insight to its intensity and occurrence. While the methods proposed by Schnegg et al. [11] are certainly useful, they rely on fibres deposited on a surface that is open to the external environment. Such conditions have the potential to result in significant drops in

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https://doi.org/10.1016/j.forsciint.2023.111890
Received 3 August 2023; Received in revised form 6 November 2023; Accepted 21 November 2023
Available online 24 November 2023

quantity due to exposure, contamination, as well as a noisy background unrelated to the propositions [6,10]. Furthermore, spatial distribution of these fibres may also play a key role according to the findings [12] and requires recovery to be effectuated as soon as possible at the scene with 1:1 taping methods [13]. Such efforts may not always be possible and different protocols may result in these steps being taken at a later stage of the investigation, for example during the autopsy where any valuable traces may already be lost or redistributed.

As a result, this present study aims to build upon the work by Schnegg et al. [5] and continue the exploration and exploitation of fibre traces in smothering cases. In essence, it aims to assess the feasibility and utility of fibre evidence found within the nasal cavity in substantiating the activity of smothering. In theory, any fibres found in the nasal cavity of a suspected smothering case would be more pertinent to the investigation as they are simultaneously better protected from loss and contamination due to the enclosed nature. In addition, during such an act, the victim's respiratory tract would be obstructed by the smothering textile, the attempts of inhalation would thus hypothetically result in a substantial transfer of fibres into the nasal cavity. However, these assumptions need to be empirically demonstrated and validated. In a proper evaluation of any non-differentiated fibres recovered from the nasal cavity, one must consider certain factors in order to establish the value of these results in supporting the propositions at hand [6–9]. Concretely, given two generic propositions, one where smothering had occurred and one where it did not, for these scientific findings to provide support for one over the other, the following criteria must be met:

1.1. The fibres in the nasal cavity need to be able to be recovered in a reliable manner

Any fibres deposited in the nasal cavity need to be first recovered to be analysed. If such a process is inefficient or inconsistent, the results cannot be evaluated in any meaningful way since the quantity of fibres cannot be properly interpreted with respect to any proposition at activity level [10]. A reliable method of recovery is thus required that preferably retrieves fibres with high efficiency and preserves the spatial distribution of these fibres in the nasal cavity. As far as the authors are aware, there have been no published methods or validations on the recovery methods of such an undertaking. While certain institutions may have their own protocol, these methods have not been tested for their efficacities in a peer-reviewed setting.

1.2. The background fibres in the nasal cavity need to be understood and preferably non-existent or sufficiently low in terms of quantity and occurrence

The background refers to any fibres that are present for reasons unrelated to activities explicitly disclosed in the propositions [6]. The main activity under consideration here, is one of smothering with a textile. As such, if background fibres already exist in the nasal cavity in quantities that overlap significantly with quantities of transferred fibres from the act of smothering, the value of the findings would reduce to only depend on the rarity of these fibres. If this is the case, for relatively common fibres, the result of non-differentiation with the purported smothering textile would not be unexpected whether a smothering had occurred or not, thus giving support to neither of the propositions over the other. Therefore, data on the size of foreign fibre groups and the occurrence of various measured characteristics need to be present to properly evaluate the results [6]. Data on the background fibre population in nasal cavities are also non-existent given the niche nature of such cases.

1.3. The number of transferred fibres in the nasal cavity need to be known and preferably sufficiently high for the activity of smothering

As with the background parameter, in order to distinguish between criminal and legitimate activities, the number of fibres that transfer during the criminal activity of smothering have to be significantly different from the quantity of fibres that would have been recovered if no such act took place. If not, the results would again offer no meaningful support for either proposition, especially if the fibres are relatively common. Data on the quantity of fibres transferred during such acts is thus required [10]. While some data exists in terms of the transfer of aerosols and asbestos fibres from inhalation [14–22], these studies are far from representative to the situation at hand. First, aerosols and asbestos fibres are expected to have major morphological differences compared to textile fibres and thus may behave differently in the respiratory tract. Second, these studies were not carried out under the conditions of smothering, which is an important parameter of interest in this case.

The present study thus aims to preliminarily address all three of these points based on smothering propositions. Due to the invasive nature of examining fibres in a nasal cavity, the use of 3D printed models was employed for the recovery and transfer experiments. The use of such models to simulate a nasal cavity have been previously explored and established in aerosol studies [16–18,23] and the medical field in general [24–28]. Additionally, they offer a high degree of control in terms of deposition of fibres as well as the ability to directly examine the interior of the nasal cavity models. Experiments were then also carried out on cadavers and living individuals to validate these results where applicable.

First, to obtain a suitable method of recovery, various methods were tested for their recovery efficiency of fibres deposited in a 3D-printed model. The method with the best results was then validated against the nasal cavities of cadavers by planting fibres in them. Following which, this method was then selected as the method of choice for the background fibre study, where fibres were recovered from the nasal cavities of 21 cadavers whose circumstances of deaths did not involve smothering. These recovered fibres were then classified by generic type and colour, such that an overall sense of the background population of fibres in the nasal cavity could be obtained. Finally, a series of transfer experiments were carried out, using textiles of variable shedding scores [29] on both pump assisted 3D printed models and live participants.

The results obtained for the background and transfer experiments could then be compared to evaluate if a significant difference exists between the number of fibres transferred during a smothering and the background levels of fibres that nevertheless exist in the nasal cavity. The results presented here represent a pilot study into the feasibility of such an approach. They give a preliminary appreciation of the quantity and occurrence of fibres that exist in the background of the nasal cavities compared to what can be recovered under the activity of smothering. This data may be used to carry out evaluations of findings that relate to fibres in the nasal cavity for cases of suspected smothering. However, a lot more work remains to be done to ensure that the data obtained is robust. Future and ongoing work that furthers the efforts here will be presented in the discussion section.

2. Materials and methods

2.1. Recovery method study

The recovery methods selected for testing could be broadly divided into two categories. The first involved swabs of varying material and length. These swabs were always inserted to the maximum depth before they were rotated three times, applying pressure to the nasal walls. The swabs were fully removed and inserted back into the nasal cavities at different angles for a total of three times. After the final removal, the swabs were carefully returned to their plastic tube. The following swab types were tested:

Cotton DNA Swab. Copan® cotton tipped DNA swab with a 13 cm wooden applicator stick and a plastic drying tube for storage and preservation.

Short Synthetic DNA Swab. Copan 4N6 FLOQ® Swab with Nylon fibre tips and a 5 cm applicator stick of plastic (Cat. No. 4504 C) with its own active drying system tube.

Long Synthetic DNA Swab. regular Copan FLOQ® Swabs (Code 552 C). These swabs come without the active drying system but are also tipped in nylon fibre and have a 13 cm applicator plastic stick.

The second category of recovery methods involved polymer casting using AccuTrans® Polyvinyl Siloxane from Coltene Holding AG. This is a silicone elastomer which rapidly solidifies after its addition reaction. It then takes an elastic and solid form when hardened. This method is frequently used in forensics science for the lifting of tool mark impressions to collect 3D casts for comparison. The polymers were introduced into the cavity from their 75 ml cartridge with the standard AccuTrans® dispenser and the universal mixing tips. Each tip was inserted to its maximum penetration depth before the polymer was slowly introduced. Once resistance was felt from the injected polymer, the dispenser was carefully pulled out of the nasal cavity slowly, making sure to fully fill the cavity. The polymer was then left to set for 10–30 min before removal. The resulting casts were then wrapped in clean A4 paper secured with paperclips until they were evaluated. Two different coloured polymers were tested:

Transparent AccuTrans®. Polyvinyl Siloxane from Coltene Holding AG (catalogue number 8516).

Brown AccuTrans®. Polyvinyl Siloxane from Coltene Holding AG (catalogue number 8501).

For both these products, manufacturer recommendations for minimum setting time are 8 min at 10 °C and 16 min at 0 °C. Room temperature storage of the resulting moulds was also recommended.

2.2. Testing on 3D model

The recovery methods of both categories were first tested on a 3D model which was created from the Carleton-Civic standardised nasal model files as described in Liu et al. [25]. The nasal cavity files were then converted into a negative mould with Autodesk Fusion 360. In order to facilitate test conditions such as the planting of target fibres and removal of polymer casts, the mould was split into four divisible parts. Each piece was fitted with registration pins for consistent assembly of the pieces. The pieces were printed with a Prusa i3 MK3s+ 3D printer using the PrusaSlicer Version 2.4.0, with the print profile set to 0.20 mm. The mould was printed in 1.75 mm flex semisoft transparent Thermoplastic polyurethane (TPU) filament provided by extruder, with a nozzle temperature of 240 °C and a bed temperature of 55 °C. The infill was set to 10%.

Bright pink, fluorescent polyester fibres obtained from the survey of Prod'Hom et al. [30] were utilised for deposition. These fibres exhibit an orange fluorescence under UV lighting at 350 nm. Two different lengths of fibres of 5 and 10 mm were chosen. 10 fibres of each length were used during each test for a total of 20 fibres in each replication. Each recovery method descibed was tested a total of 6 times with the same 3D printed nasal cavity model. Prior to each application, the nasal cavity model was first thoroughly washed and visually inspected to ensure no prior fibres remained. The fibres were then manually placed in the open cavity model in a positional distribution that was standardised (see Fig. 1) and covered all regions of the cavity. Once the fibres were deposited, the model was then closed securely, and the respective recovery methods were applied. When working with the casting methods, the two anterior nostril pieces of the model were removed to retrieve the Accutrans® mould and in certain instances, the model was be opened completely to prevent ruptures. After which, the cast was inspected once again under 350 nm light to observe the positional distribution of the fibres that were left behind.

The recovered fibres from both classes of methods were then visualised under 350 nm ultraviolet light conditions using a Rofin Polilight® PL500 combined with an orange filter. They were first counted with the unassisted eye and subsequently under a Leica M80 stereomicroscope with a 7.5–60x magnification and ring light illumination.

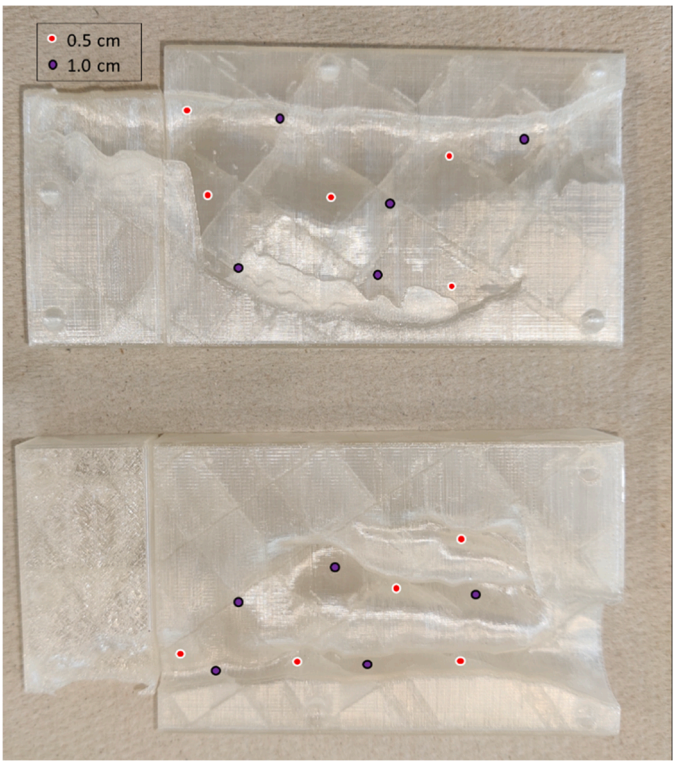

Fig. 1. Positional distribution of planted fibres in the nasal cavity 3D model.

2.2.1. Testing on human cadavers

In order to validate the results obtained from the 3D model tests, between 5 and 32 of the same fluorescent fibres were manually deposited in the nasal cavities of ten cadavers that had been previously blanked using the Accutrans® method. Two separate regions were chosen in the nasal cavities for deposition. First, fibres were planted as deep as achievable with a pair of tweezers (~4 cm), the remaining fibres were planted right at the nostril region. The transparent Accutrans® polymer was then introduced to each of these nasal cavities as described. For the removal, each polymer was first detached from the exterior skin and the nostril edges before it was gently pulled out. After the casting method was applied, swabs were used to check if more fibres could be recovered. The samples were then secured in plastic evidence bags and stored at − 17 °C to avoid the deterioration/rotting of the biological material. This temperature diverges from the manufacturer recommendations for storage at room temperature but was deemed necessary due to the nature of application. The resulting casts were then observed for the deposited fibres in the same method described above.

2.3. Background study

A background survey of fibres in the nasal cavity was carried out on human cadavers at the Institute of Forensic Medicine at the University of Zurich. The cadavers selected underwent the standard examinations of unnatural deaths but were not under the suspicion of having been smothered. A total of 21 cadavers were examined for their background fibre populations of the nasal cavity. The bodies generally reached the institute within 24 h of death/discovery and were kept refrigerated at 6–7 °C. Both nasal cavities were sampled with the AccuTrans® transparent polyvinyl siloxane polymer in the same manner as described in the recovery methods study.

Table 1
Fabrics utilised in the transfer experiments and their recorded properties.[2]

Fabric	Shedding score	Composition	Refs.
Burgundy pyjama	5 (high)	80% cotton / 20% polyester	Schnegg et al. [32]
Jogging shirt	2.5	91% polyester / 9% Elastane	Prod'Hom et al. [30]
Burgundy pillowcase	2	100% cotton	Schnegg et al. [5]
Orange pillowcase	1 (low)	100% cotton	Schnegg et al. [5]

The hardened polymer casts were then inspected for fibres under a Leica M80 stereomicroscope with a global magnification of 7.5–60x. The number of fibres of each colour was recorded and individual fibres from each of these identified groups were then mounted onto glass slides using glycerine solution. The microscopy examination was effectuated using a LEICA DMRX microscope with 10x, 20x, and 40x objectives under brightfield, darkfield, and polarised light conditions. The fibres were then identified based on characteristics outlined in Robertson et al. [31].

2.4. Transfer study

2.4.1. Testing on 3D printed model

In an effort to simulate the transfer of fibres during a smothering activity in a controlled manner, the 3D printed model from the recovery study was used. Breathing was simulated using a 1500 ml AERObag® PVC Manual Resuscitator for adults (Item Number HBB06-E2) from the "HUM Gesellschaft für Homecare und Medizintechnik mbH" affixed to the nostril end of the model as shown in Figure A1 in Appendix I. The Resuscitator was fixed with a custom 3D printed lid to seal all the intake valves, and the patient connection valve was disconnected. This ensured that all airflow, in both directions, surged through a single opening. Four different fabrics of varying shedding scores [29] sourced from previous studies (See Table 1) acted as the respective smothering textiles. They were individually pinned between the resuscitator and the nasal cavity model while the simulation was carried out. The nasal cavity model was then sealed with tape to make it as airtight as possible. 15 pumps of the AERObag® were administered over a 45–50 s period. For each pump, air was pushed in through the nostril end and allowed to flow back out before the next pump. The nasal cavity model was then carefully disassembled and examined for deposited fibres under a Leica M80 stereomicroscope with a 7.5–60x magnification and ring light illumination. Each fabric was tested a total of 5 times.

2.4.2. Testing on live volunteers

Testing on live participants was then carried out to validate the results from the 3D model tests. The same fabrics from Table 1, except for the jogging shirt, were provided to two volunteers. They were instructed to respectively press these fabrics against their nose and attempt to breathe through them. The participants were asked to do this for 45 s before their nostril regions were swabbed by themselves using the cotton swabs as previously discussed. These swabs were then examined for any transferred fibres under a Leica M80 stereomicroscope with a 7.5–60x magnification and ring light illumination. Each fabric was tested a total of 5 times. A control swab was taken between replicates to ensure no fibres remained from the previous trial.

[2] The shedding score is rated on a scale from 0 to 5 with respect to the amount of textile fibres removed after application of a standard tape at fixed pressure with 0 corresponding to (almost) no fibres removed and 5 corresponding to a very high number of fibres removed.

3. Results and discussion

3.1. Use of 3D printed model

The use of 3D printed models was intended to facilitate the placement of fibres and to avoid an overly invasive procedure on living participants. While the use of such models of nasal cavities has been established in other studies of a similar nature [16–18,24,25], clear physical differences do exist between a 3D printed model and an actual nasal cavity. The issues with constructing such an artificial model to replicate a nasal cavity must be taken into consideration. First of all, there were issues in replicating the texture of a nasal cavity, the polymer used for printing is not meant to simulate human tissue and is much more rigid. This is evidenced by the need in some cases to completely open the model to retrieve the Accutrans® cast intact, whereas when applied in a cadaver, the cast could be reliably pulled out in one piece without damage. This was despite efforts to increase the flexibility with filament selection and reduction of the infill during printing. As a result, the following considerations should be made when using or applying results from such models. It is recommended to always verify the results in-vivo if possible.

Anatomically and physiologically speaking, there is also the lack of nose hairs and body fluids in the printed model which presumably would result in differences in fibre retention, transfer, and interaction with the recovery method. The lack of mucus was addressed by introducing lubricants such as polydimethylsiloxane (PDMS) in early preliminary testing. However, there appeared to be no significant difference in the number of fibres recovered between the lubricant and non-lubricant condition.

The subsequent testing on nasal cavities of live participants or cadavers was thus intended to validate or invalidate the results from the 3D printed models. Although these tests are limited in nature due to the lack of control and penetration depth achievable using the prints, the results taken together with those from the 3D printed model gives a more complete picture.

3.2. Recovery method study

The results of the proportion (out of 20) of fibres recovered for each recovery method are summarised in Fig. 2. The recovery percentages of fibres for the long synthetic nylon swabs ranged from 0% to 35% from the total 20 planted fibres, whereas the short synthetic nylon swab recovery method recovered between 10% and 25%. The recovery for the cotton swab method ranged from 15% to 50%. For the brown AccuTrans® polymer, the recovery percentage ranged from 25% to 55%. Lastly, for the transparent AccuTrans® method, the recovery ranged from 85% to 100%.

The recovery rate of the swab methods plateaued around 10–20%, as can be observed in Fig. 2, with some variation between the types of swabs used. The long synthetic DNA swab method was introduced to correct for the length difference between the short synthetic swabs and the longer cotton swabs. Due to the more fibrous nylon tips, it was initially hypothesised that the fibres would generally adhere better to the synthetic swabs than the cotton swabs. However, this does not seem to be the case according to the results. One potential explanation for this is the rigidity of the application stick that the swabs are attached to. For the long synthetic swabs, these 13 cm long sticks were a flexible plastic whereas the cotton swabs were attached to 13 cm long rigid wooden sticks. This therefore resulted in a major difference in the pressure that could be effectively applied to the nasal cavity walls, which could have affected the transfer of the fibres onto the swab tip. Hence, if swab methods are to be utilised for recovery of fibres in the nasal cavity, the rigidity of the applicator stick could be a potential factor for selecting the type of swabs. However, it appears that swab methods do not reliably recover the fibres from nasal cavities and should only be used if no other options exist, or if the situation does not permit the use of casting

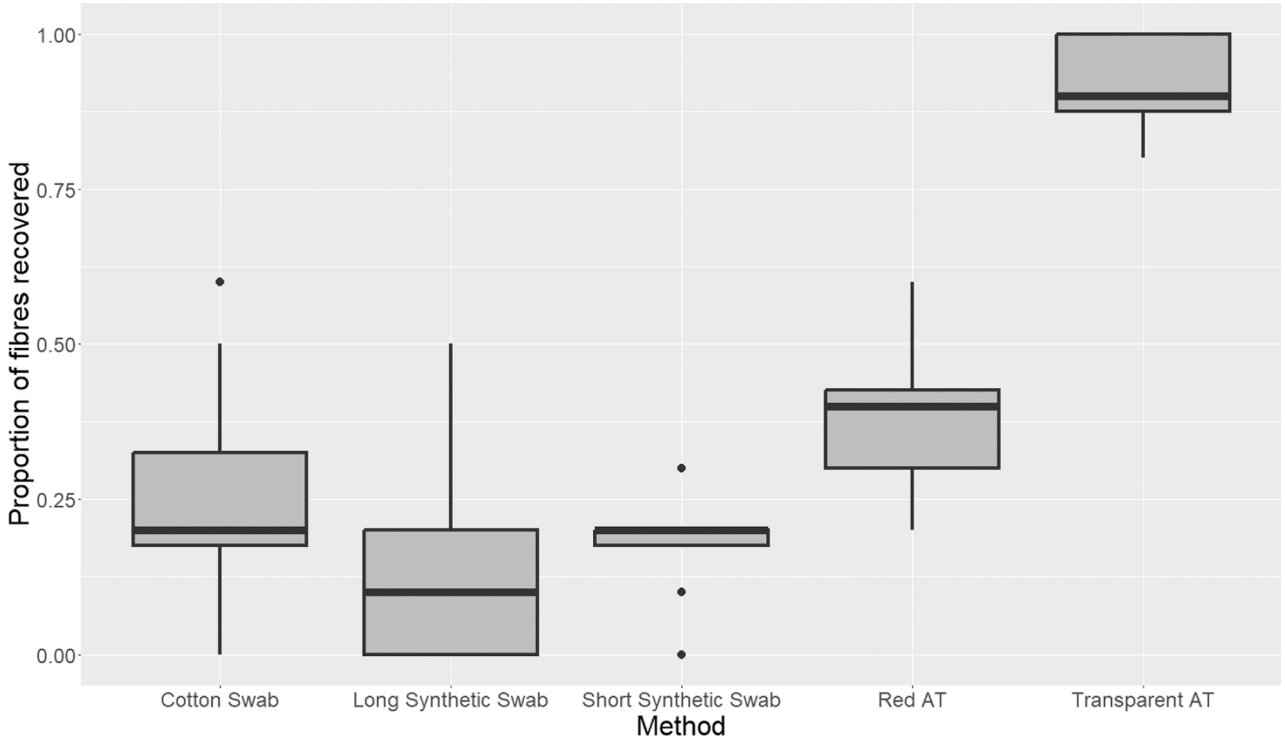

Fig. 2. Boxplot of number of planted fibres recovered using each collection method during testing on the 3D printed model, note that each length category of fibres was considered as separate replicates.

methods, for example, if the subject was still alive.

Based on the results on the 3D printed model, the transparent AccuTrans® polymer method is by far the most efficient in removing, and later locating fibres, with a median of 90% fibres recovered. The next best recovery method is the brown AccuTrans® polymer method, which has a median recovery efficiency of 40%. Given that both AccuTrans® methods are identical other than colour, it is likely that the difference observed between the two polymers used is most likely a function of detection of the fibres, rather than their recovery. This was supported by the fact then when the 3D model was examined after application of the brown AccuTrans®, the fibres appeared to be removed at similar rates to that of the transparent AccuTrans®. The transparent polymer thus offers better contrast, especially for darker fibres, which allows for more reliable detection and extraction. Moreover, the transparency also allows for the detection of fibres that were embedded in the polymer itself, which, as will be discussed in the background study, was the case for the majority of fibres. Although transparent and pale fibres are more likely to be missed, these fibres are often disregarded or of lower interest due to their low evidentiary value [6].

When used on cadavers, the transparent AccuTrans® method had a 78–100% recovery rate. Swabs applied after this method was utilised and recovered no additional fibres in the nasal cavities of the cadavers. These results appear to validate the findings in the 3D printed model and reaffirm the reliability of this recovery method. A comparison between these two results can be found in Fig. 3. Certain limitations do exist when testing on the nasal cavities of cadavers. First, only the nostril and middle turbinate region could be accessed when planting fibres with a pair of tweezers, as such it is difficult to generalise these results on human cadavers to more posterior regions of the nasal cavities. Secondly, the planting of the fibres was less reliable as there was no way to verify if these fibres were indeed deposited in the nasal cavity and not accidentally discarded during the removal of the tweezers from the cadaver nostrils. This resulted in a wide range of fibres (5 – 32) that were planted in this set of cadaver experiments. However, this implies that the results obtained are a lower bound as it may very well be the case that a portion of the fibres were not successfully planted in the nostrils and thus could not have been recovered anyway. As such, the recovery rates obtained at worst underestimate the recovery rates of the method. The results obtained on these cadavers should not be taken in isolation, but rather viewed as a corroboration of the previously obtained result from the 3D printed model. Essentially, they demonstrate that the transparent AccuTrans® polymer method was able to obtain comparable recovery efficiencies in cadavers as those obtained in the 3D printed models.

In terms of the positional distribution of fibres, swabbing methods do not permit the localisation of fibres within the nasal cavity. The use of swabbing methods was also observed to push unrecovered fibres deeper into the 3D printed nasal cavities. Hence, proximity to the nostril end of the model did not necessarily guarantee that the fibre would be successfully extracted. Such phenomena were observed for all three swab methods. As expected, the short synthetic swabs displayed less disruption and fibre rearrangement due to their lessened ability to penetrate the cavity. Finally, it was extremely difficult to accurately manipulate the precise location of the swab during its insertion into the nasal cavity. With the high degree of variation in nasal geometries [33,34], it would be difficult to standardise a swabbing method effectively to ensure complete coverage and replicability.

When comparing the positions of fibres between their deposition in the 3D model and the casts made by the Accutrans® polymers, the fibres appear to adhere to the polyvinylsiloxane AccuTrans® polymer with very little redistribution. Although some slight movement was observed, the general location remained largely unchanged. The areas with the largest discrepancies were areas where the AccuTrans® dispenser tips could reach, around the nostril and the middle airway region at the middle turbinate. In the former, the fibres were repositioned on the cast but remained in the same general region. The latter zone displayed a bigger displacement as fibres were recovered much further in towards the nasopharynx region of the cast. This is the region where the initial

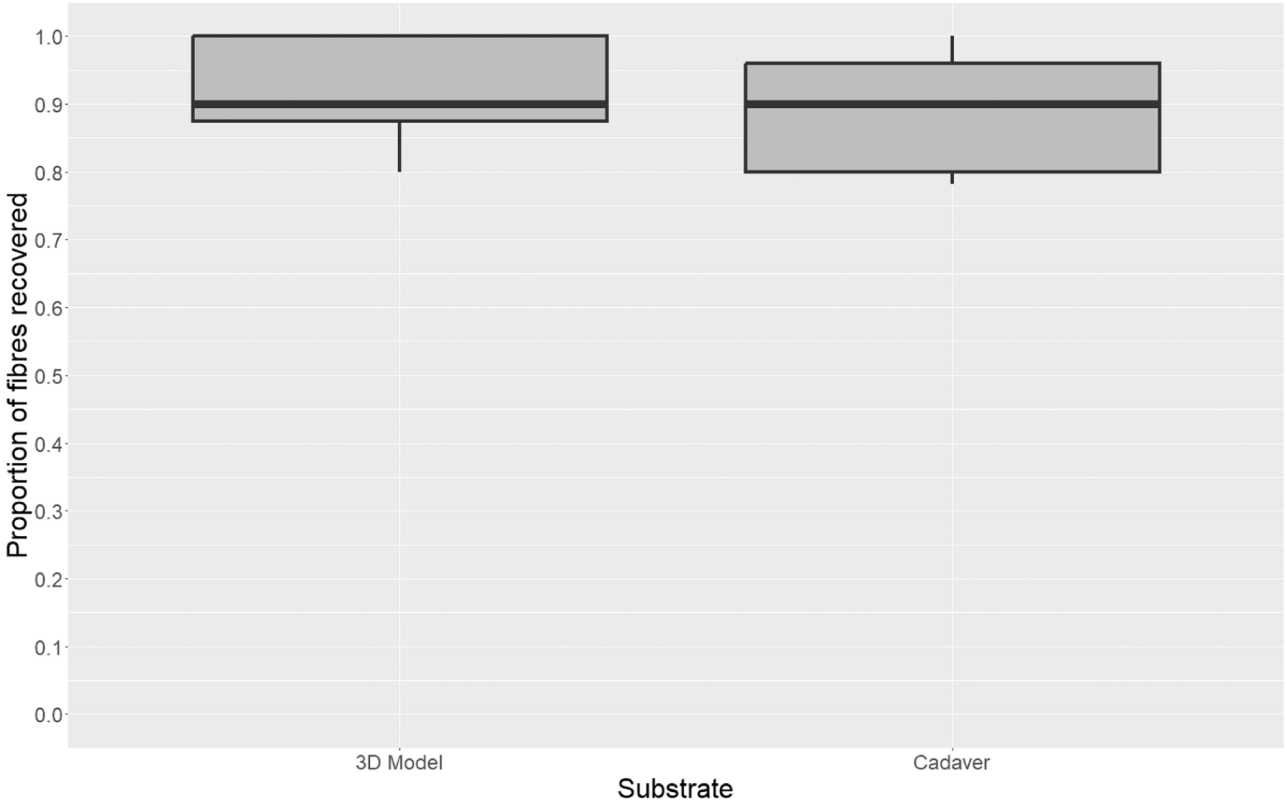

Fig. 3. Comparison between proportion of fibres recovered using the transparent AccuTrans® method on the 3D model and cadavers.

insertion of the AccuTrans® dispenser reaches its furthest depth, and where the injection of polyvinyl siloxane would be initiated. Possible explanations of this fibre redistribution are therefore either the direct mechanical interaction with the AccuTrans® dispenser itself or a washing effect of the liquid injection of the AccuTrans® polymer.

When applied to nasal cavities of cadavers where fibres were planted in both the nostril and middle turbinate region, some fibres were found on the posterior superior turbinate region and the posterior inferior turbinate region on the polymer casts, much deeper than where they were initially planted. In two trials, two fibres were pushed all the way towards the nasopharynx region. Other fibres were recovered outside the nostril area in the AccuTrans® polymer as it was drying and dripping out of the nasal cavity. However, it was never entire fibre groups that were displaced, but only between 1 and 3 fibres at a time. This shows that there can be a high discrepancy between where a fibre was originally deposited and where they are recovered on the AccuTrans® polymer extraction. Nonetheless, the majority of the fibres preserved their general spatial location.

To conclude this section of the present project, the transparent AccuTrans® method appears to be the most optimal recovery method to retrieve fibre evidence from the nasal cavities. Its advantages were not only in terms of the absolute number of fibres recovered, but also with respect to its preservation of possible positional distribution. As a result, this method was chosen as the collection method used in the background studies. However, it is still important to continue to validate these findings with in-vivo conditions and to establish a broader understanding of fibre prevalence in the nasal cavity to facilitate the interpretation of such potential evidential value.

3.3. Background study

3.3.1. Fibre counts

A total of 215 fibres across the 40 samples from 20 cadavers were recovered. The number of fibres recovered from each nasal cavity (right and left) of respective cadavers were recorded. Data from one cadaver was removed from the dataset due to the large amount of soot and debris present (the cadaver was a burn victim), which impeded upon the identification of fibres. The distribution for the number of fibres recovered per nasal cavity can be found in Fig. 4. The number of fibres in each cavity ranged from 0 to 58, with a clear single outlier of 58. Removal of this data point provides a mean of 3.8 fibres recovered per nasal cavity.

To put these numbers into some context, other background studies on comparable living surfaces, such as fingernail clippings of adults and children, report significantly higher numbers of fibres, with means of 46 and 84 fibres respectively [35]. Palmer and Burch [36] reported finding a mean of 241 and 458 fibres on the bare skin of women and men, respectively. These findings therefore indicate that the nasal cavities rank among the least polluted surfaces when it comes to background fibres. This is in accordance with the intuitive assumption that the nature of the nasal cavity anatomy serves to protect against frequent fibre deposition. Furthermore, the nose serves as a significant filter system for foreign particles, including textile fibres [37]. Nonetheless, the present findings still emphasise that the nasal cavities are not entirely void of textile fibres.

It was observed in this study that more fibres were recovered on average from the right nasal cavity compared to the left (see Figure A2 in Appendix I). Presumably in most cases, both nostrils would be exposed to fibres in equal proportions and this result was unexpected. This observation may be possibly due to the fact that all cadavers at the Institute of Forensic Medicine were initially tested for the coronavirus with nasal swabs. Although their standard operating procedures did not dictate which nostril had to be utilised, the cadavers were always in the same orientation and location when examined. As such, it is plausible that nostrils of a particular side were favoured and the swabs either introduced or removed a number of fibres from the nasal cavity. Since this cannot be validated, it is important that more research is conducted to help understand the effect of these coronavirus test swabs should they

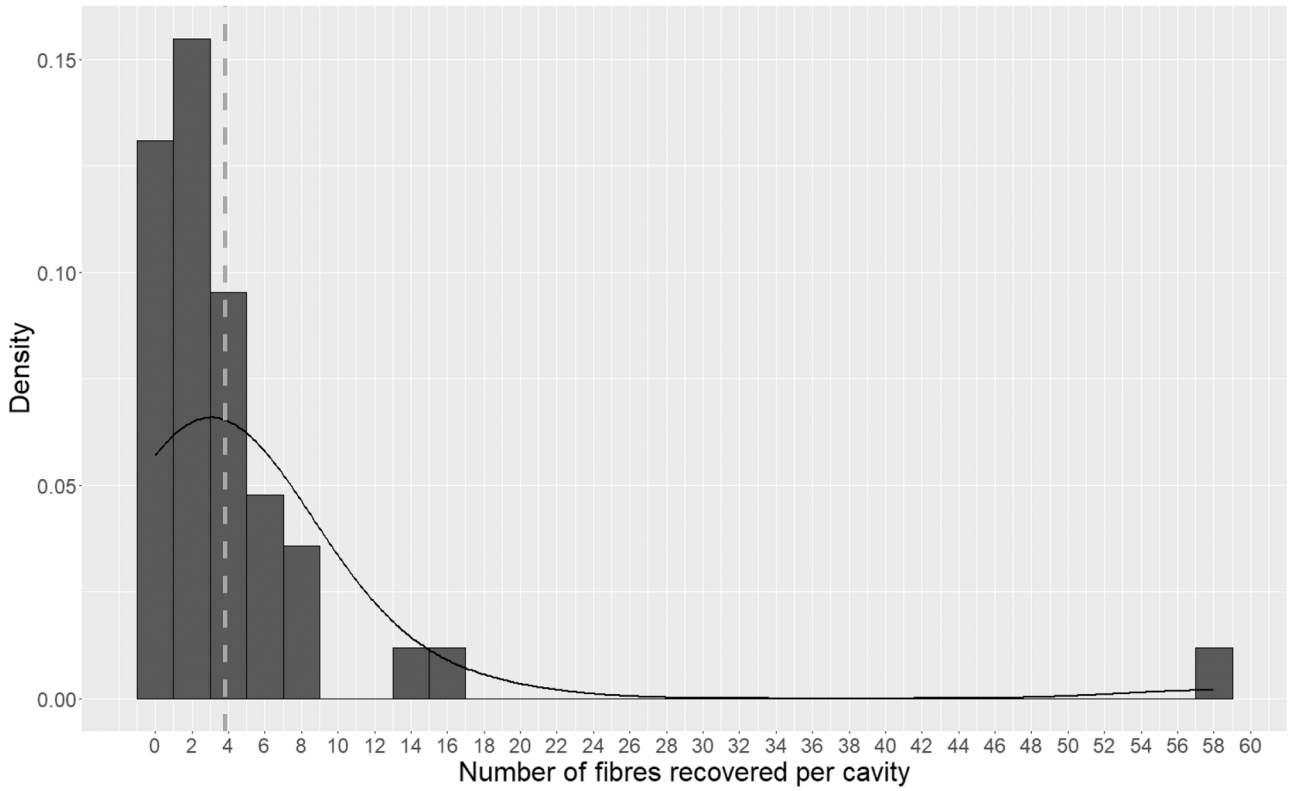

Fig. 4. Histogram of the number of fibres recovered per cavity with a fitted distribution. The dotted line at 3.8 represents the mean after removal of the outlier data point at 58 fibres recovered (n = 20).

be continued to be used in routine. Furthermore, examiners should be wary of any procedures that could potentially affect the recovery of fibres from the nasal cavities before recovery is carried out and reflect upon the sequence of methods.

3.3.2. *Population survey*

The 215 recovered fibres were identified and categorised according to their length, generic type, and colour as perceived under the stereomicroscope with reflective light. The resulting findings of these examinations are discussed in this section.

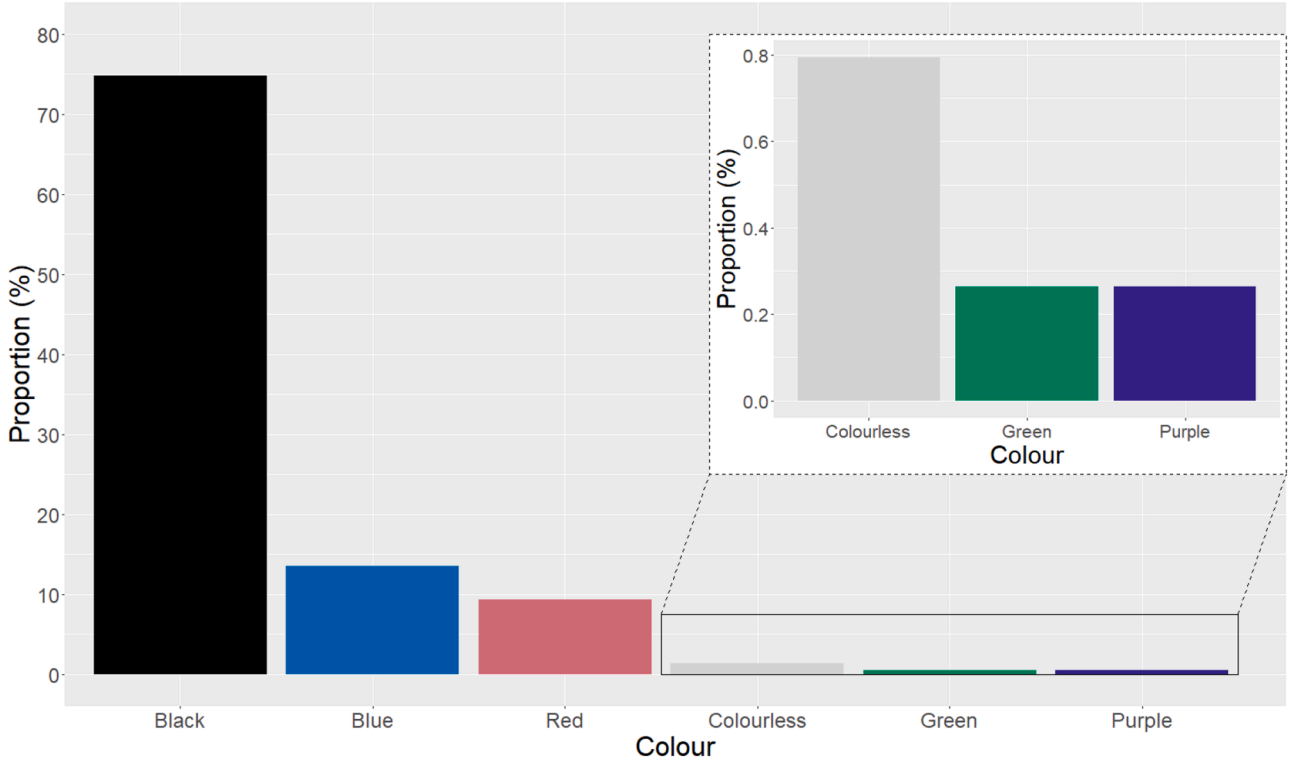

Fig. 5. Proportion of each colour of fibres recovered in the nasal cavities of cadavers (n = 215).

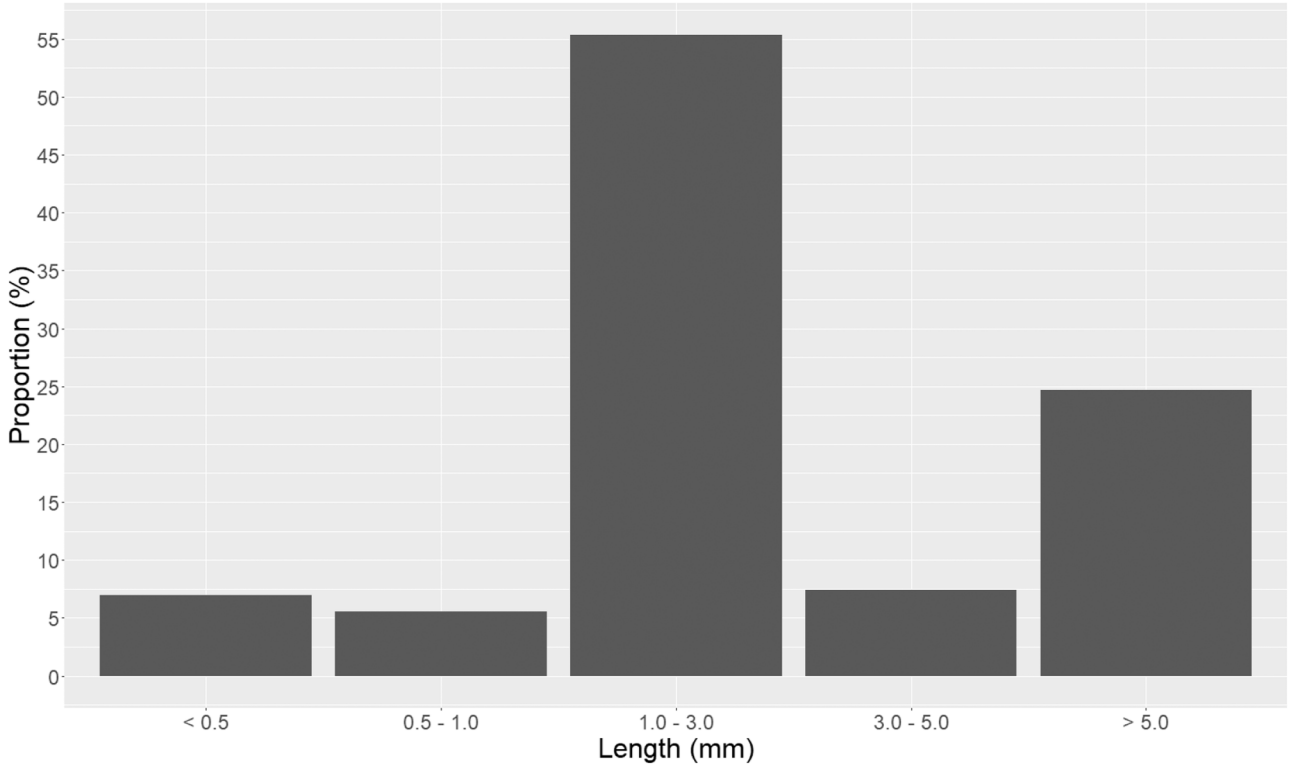

Fig. 6. Distribution of lengths of fibres recovered from the nasal cavities (n = 215).

Colour. The proportion of each colour of fibres recovered are depicted in Fig. 5. All colour categorizations were based on the colours as they were perceived under the stereomicroscope with reflective light. Black and blue fibres being the overwhelming majority of fibres is largely consistent with already existing literature [6]. These studies tend to disregard colourless fibres due to difficulty in detection and low value of these fibres. Additionally, the darker tones of black and blue would naturally provide a higher contrast from the colourless AccuTrans® polymer resulting in a higher detection rate. Consequently, transparent fibres and ones which possess brighter hues are most likely underrepresented in this study.

It is important to note that 75% (15) of the red fibres were recovered from only two cadavers, 50% (10) from one and 25% (5) from another. Omitting these two cadavers would therefore lower the red fibre frequency from 9% to 2%. This highlights the high variability of the data due to the small sample size.

Length. As shown in Figs. 6, 7% (15) of the fibres were equal to or smaller than 0.5 mm in length. The smallest measured fibre in this category was 0.35 mm long. It is possible that smaller fibres were present on the samples but were undetected due to the limits of stereomicroscopy. The most common fibre length was between 1.0 mm and 3.0 mm, with 55% (119) of the fibres falling in this range. This is comparable to other background surveys [36,38,39] which report the 1.0–3.0 mm range to be the most frequent. The largest fibres with lengths greater than 5.0 mm made up 25% (53) of the background population. When compared to the same background studies [36,38,39], fibre lengths of smaller than 0.5 mm seem to be less prevalent in the nasal cavity as compared to the other surfaces. On the other hand, the larger fibres of 5.0 mm length or greater are more present in the nasal cavities.

Generic class. No additional fibre type categories were observed other than the ones reported here in Fig. 7, and no further analysis of the man-made fibres were pursued at this moment. The most prevalent fibre observed was cotton and although this general trend is in agreement with most published literature [6], its frequency at 96% is significantly higher than previous reports. Out of the 8 man-made fibres recovered, 63% (5) came from one cadaver (P013). This indicates that the removal of this cadaver from the dataset would further lower the frequency of man-made fibres to below 1%.

Such an overwhelming frequency of cotton fibres combined with the absence of effectively any other class raises important questions. As small changes in cadaver numbers at this state can still have a sizable influence on fibre population, perhaps a larger sample population is still required. This small sample size of 20 should only serve as a preliminary study. In order to combat the high uncertainties associated with this low sample size, an ongoing study is being conducted with a larger sample size which includes analysis of these background fibres with instrumental methods. The higher discriminatory power in these studies serves to provide more useful data in terms of background group sizes. Additionally, since not all fibres were mounted for microscopy, the identity of the fibres were made primarily using stereomicroscopy only and is subjected to some error.

Type and Colour Combination. The most common colour and fibre type combination was black cotton, with 74.4% (160) of the fibres falling in this category. 13.5% (29) of the fibres were blue cotton and 7.0% (15) fibres were red cotton. This is consistent with the literature on background fibre populations [6]. The overall distribution is shown in Fig. 8.

3.3.3. Spatial distribution

The anatomical location of each fibre was also recorded and summarised in Fig. 9 based on their position in the polymer cast. The nasal cavity was divided into 8 different zones to facilitate the assigning of each fibre to a specific section of the cavity. This includes the nostril, the three turbinate regions divided into anterior and posterior zones, as well as the nasopharynx. Three fibres found were within the AccuTrans® polymer but outside the nasal structure itself.

It is important to note that the nasal zones as outlined were not divided based on exact threshold measurements, but proportional sections depending on the sample size. This was due to the large variation in

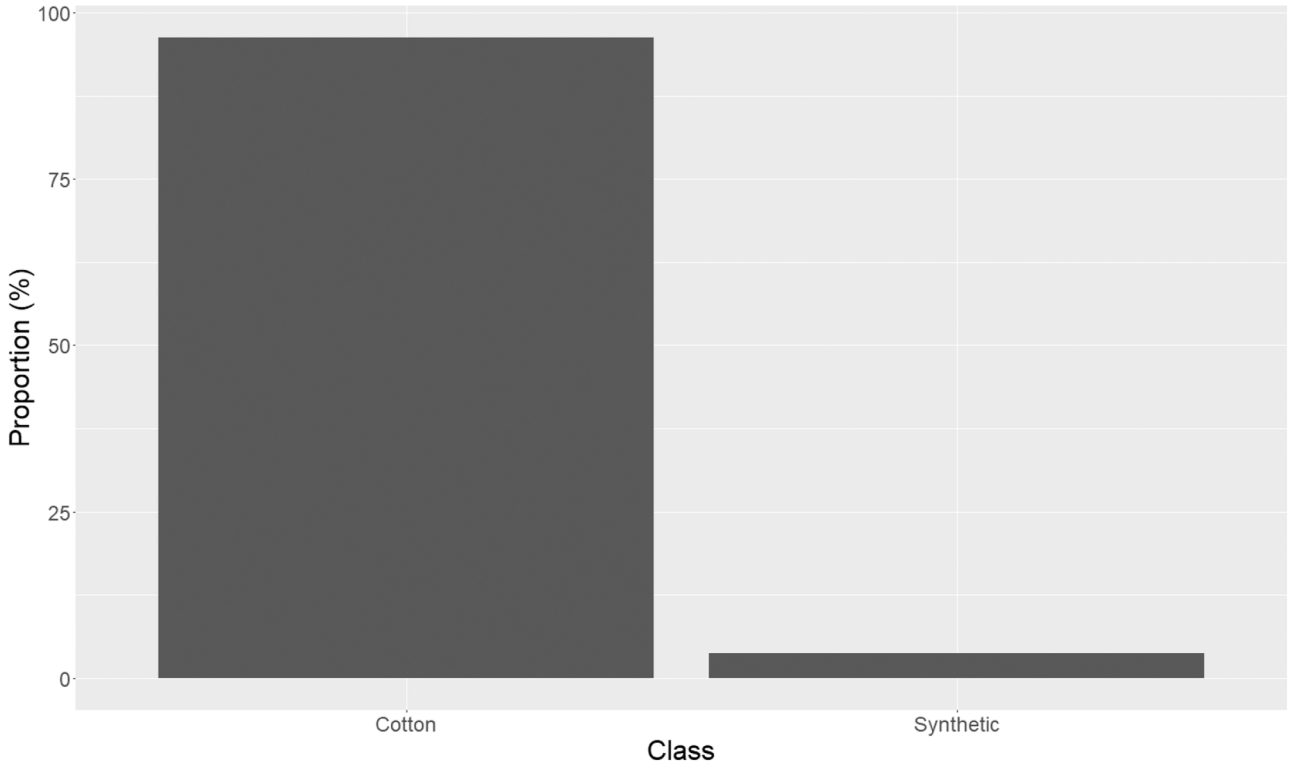

Fig. 7. Distribution of generic class of fibres recovered from the nasal cavities (n = 215).

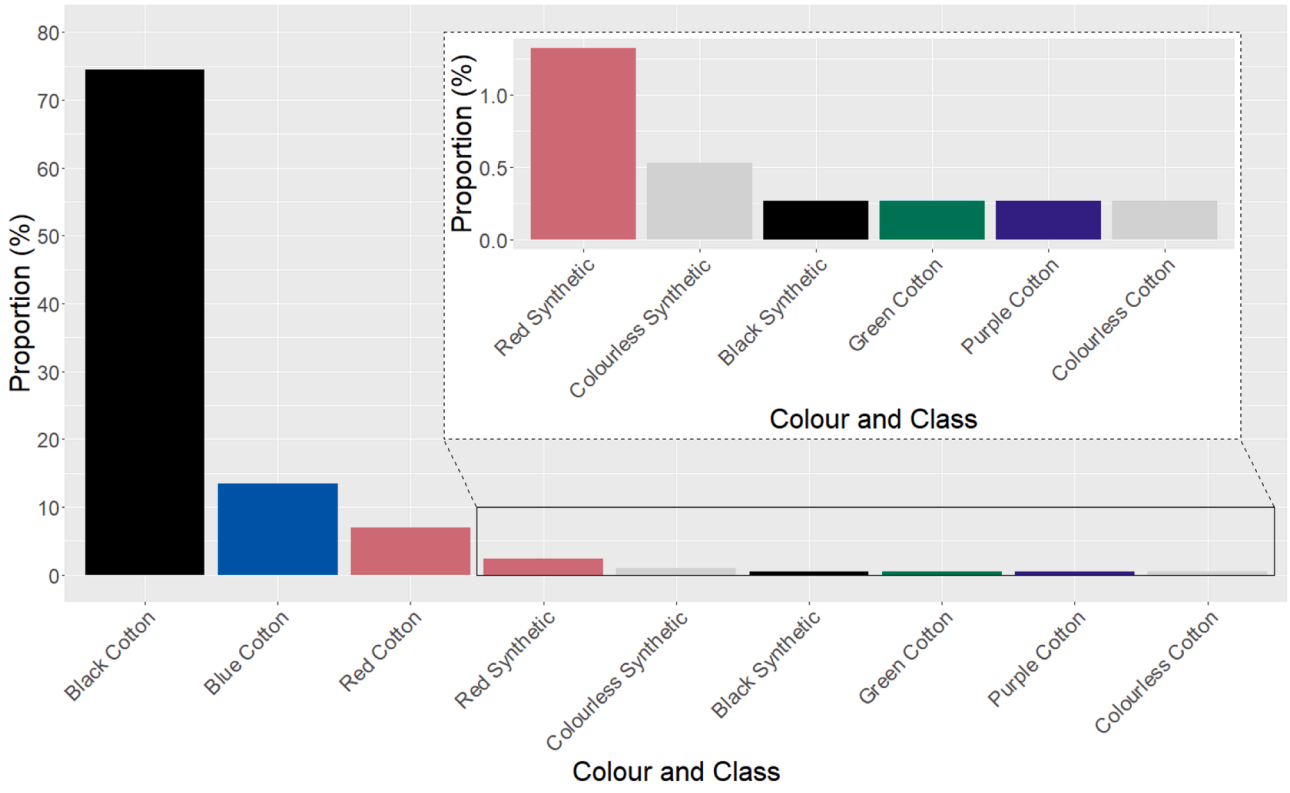

Fig. 8. Distribution of colour and generic class of fibres recovered from the nasal cavities (n = 215).

nasal cavity sizes. It is evident from Fig. 9 that the majority of the background fibres, 57.7%, were recovered from the nostril region. These findings are congruent with literature from the medical field regarding fibre deposition in the nasal cavity [21,22]. In particular, larger fibres appear to be more likely to deposit in the nostril region while smaller fibres penetrate deeper into the nasal cavity towards the nasopharynx region. However, these publications dealt with significantly smaller fibres with lengths ranging from 1 μm to 100 μm. One explanation for the

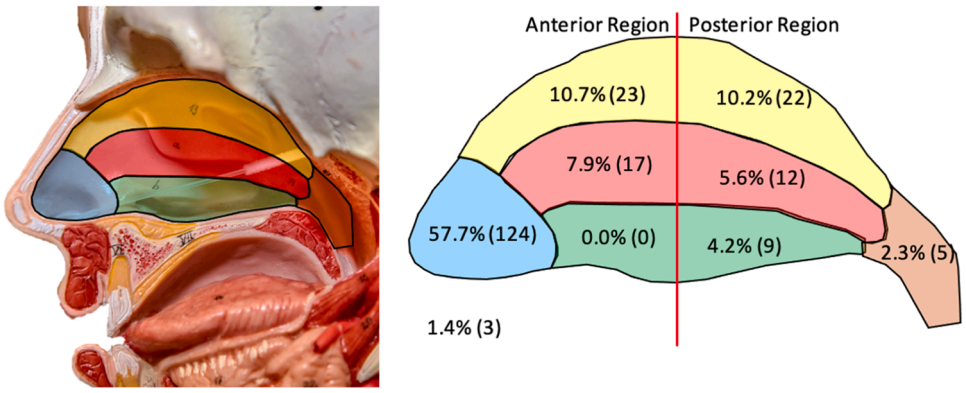

Fig. 9. Positional distribution of recovered fibres from nasal cavities (n = 215). the blue zone represents the nostril region, the brown zone represents the nasopharynx, the yellow zone represents the superior turbinate region, the red zone represents the middle turbinate region, and the green zone represents the inferior turbinate region.

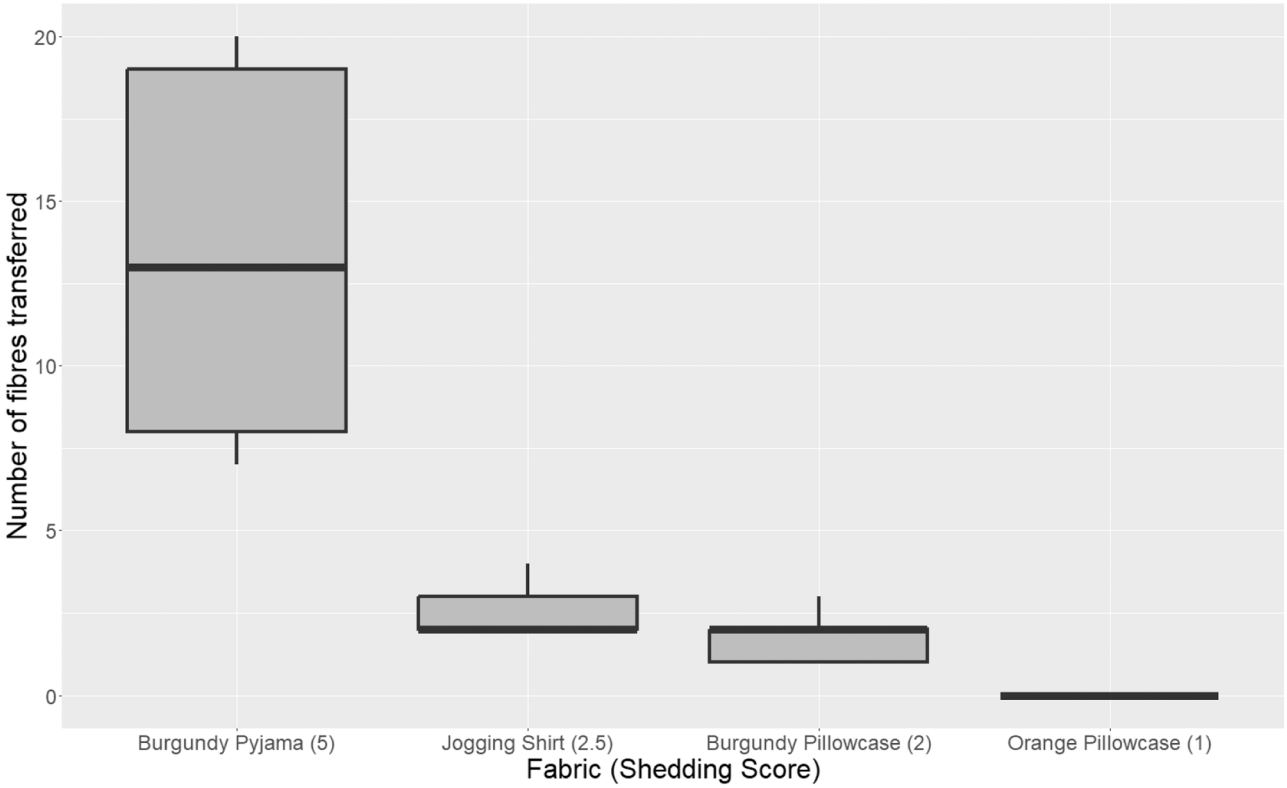

Fig. 10. Number of Fibres transferred for each textile tested using the 3D model.

observations may be due to the priorly functioning muco-ciliary clearance in living nasal cavities. This is an active transport system of beating cilia, conveying inhaled particles trapped in mucus towards the back of the nasal cavity into the throat [37]. It is therefore possible that fibres already in the nasopharynx region would be cleared out quite fast in in-vivo conditions compared to a model without such a filtering system. Across the rest of the defined zones, the fibres seem to be relatively evenly distributed. Another factor that seems to be affecting the fibre distribution is the aggregation of mucus into small structures which tended to clump fibres due to their adhesive properties.

Finally, it is important to acknowledge that, as mentioned in the recovery section, some fibre movement during the sampling process with the AccuTrans® polymer is inevitable. Hence, some error margins for these fibre distributions should be expected.

3.3.4. *Additional observations*

It was also noted that, during the search for fibres, 86% of the fibres were found embedded in the polymer whilst the remainder were found lying on top of the surface. This is an important feature of the AccuTrans® method when concerns for trace evidence contamination arise. After the polymer has hardened inside the nasal cavity, it is difficult to imagine how a foreign fibre would get itself embedded within the polymer. On the other hand, depending on laboratory procedures, the surface of the polymer can still be susceptible to contamination. This therefore provides a certain hierarchy of value of the fibre, depending on its nature of adhesion. The subsequent extraction of fibres did not pose any significant problems and were easily done with a pair of fine tweezers that could penetrate the polymer without any incisions.

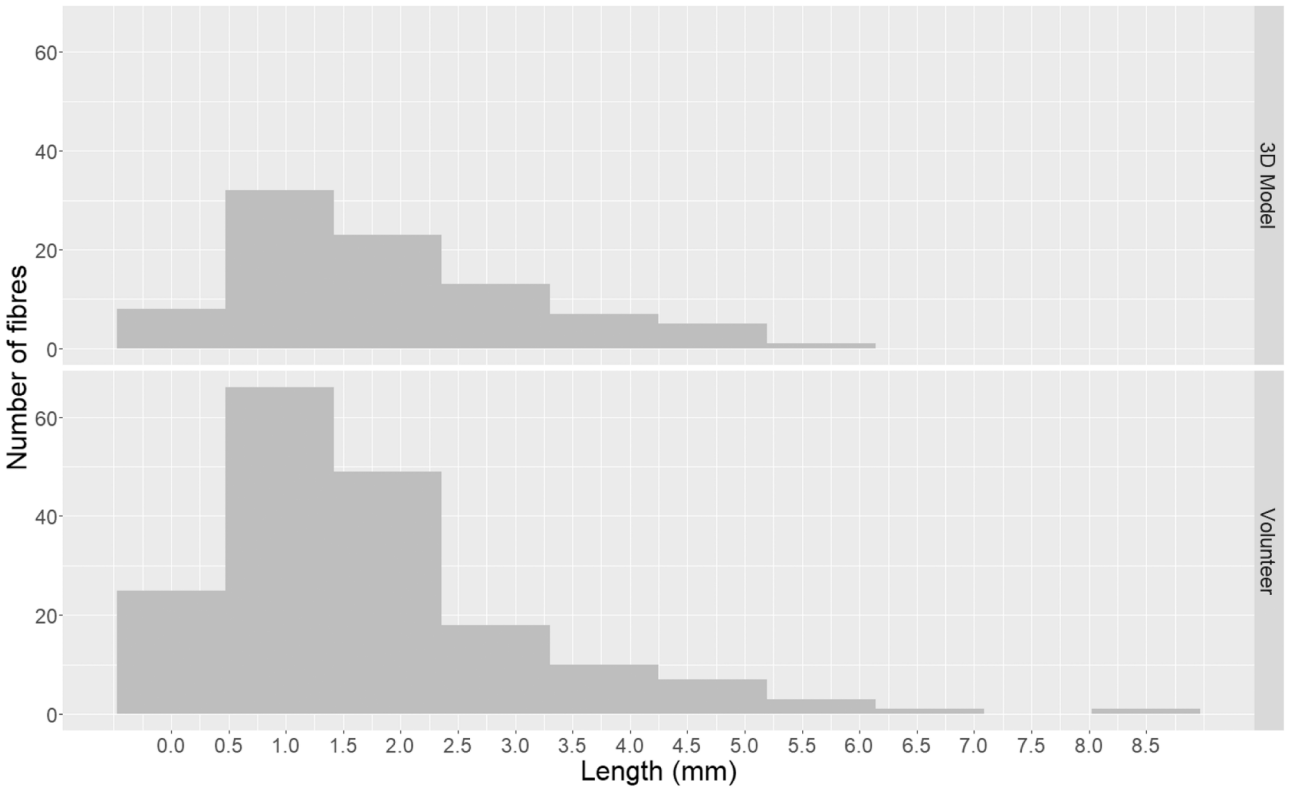

Fig. 11. Distribution of fibres lengths transferred in both the 3D model and volunteer tests.

3.4. Transfer study

In order to accurately simulate breathing under smothering conditions, it was important to properly select the total time and rate of breathing. Prinsen et al. [40] summarised that multiple sources report a need of between 3.5 and 5 min of smothering to reach unconsciousness or even death but justifies a 45 second standard smothering time for pragmatic reasons. As such, 45 s was chosen as the duration of each replicate in both the 3D model simulation and with live volunteers. It is expected that increased times would result in larger amounts of transfers, but the results do not allow for this extrapolation. In the 3D model simulation, 15 pumps of the 1500 ml AERObag® were carried out during the 45 ss. This was an augmentation from the recommended 12 pumps per minutes for normal use to reproduce breathing under smothering scenarios.

3.4.1. Testing on 3D model

Fig. 10 shows the number of recovered fibres of each fabric tested after the transfer experiment on the 3D model, along with their shedding scores [29]. In terms of the number of fibres transferred during the transfer simulation, the burgundy pyjama had a median of 13 fibres, the jogging shirt and the burgundy pillowcase have medians of 2 fibres respectively, and no fibres were transferred from the orange pillowcase. The quantity transferred appears to be correlated with the shedding score which is compatible with the findings of Schnegg et al. [5]. Under conditions of low shedding with the orange pillowcase, no fibres were transferred.

The length of all recovered fibres and their frequencies are presented in Fig. 11. The results are congruent with the fibre lengths found in the background survey where the majority of the fibres were in the range of 1.0–3.0 mm long. However, fibres longer than 5.0 mm seemed to be transferred in lower numbers as compared to fibres recovered in the background survey. Only 1.1% (1 of 89) of the transferred fibres fell into this category.

The positional distribution of the fibres on the 3D printed model was also recorded and summarized in Figure A3 in Appendix I. The majority of fibres (37.3–61.5%) went all the way through the cavity and were captured in the filter paper placed at the end of the model. These fibres represent those that would have penetrated deeper into the airways and would not have been recovered within the nasal cavity. Only a small proportion of the fibres were recovered in the nasal cavity itself. There seems to be no real consistency in terms of the positional distribution. For example, the number of fibres transferred to the nostril region were between 9.0% and 76.4%. Additionally, depending on the donor, fibres tend to deposit differentially in the anterior and posterior regions. The fibres from the burgundy pyjama showed greater deposition in the posterior regions while the fibres from the pink jogging shirt showed greater deposition in the anterior regions. Finally, the fibres from the burgundy pillowcase showed an equal distribution of fibres in both the anterior and posterior regions. This inconsistency may simply be attributed to the small number of fibres transferred, with single fibres having a substantial influence on the distribution proportions.

3.4.2. Testing on live volunteers

The number of fibres recovered from participant nostrils with the swab methods are summarised in Fig. 12. The median number of fibres recovered for the burgundy pyjama condition was 12, for the burgundy pillowcase condition it was 3.5, and for the orange pillowcase it was 2. In order to fairly compare these results to the ones obtained on the 3D printed model, Fig. 12 also includes the number of transferred fibres onto only the nostril region of the 3D printed model. It can be seen here that the results from live volunteers also reflect a positive correlation between shedding capacity and transfer.

However, the number of fibres transferred in the 3D models are significantly lower than on live volunteers. This difference is more marked considering the inefficiencies of a swab recovery method where fibres are most probably left unrecovered in the nostrils of the volunteers. It is thus evident that the simulation carried out on the 3D models

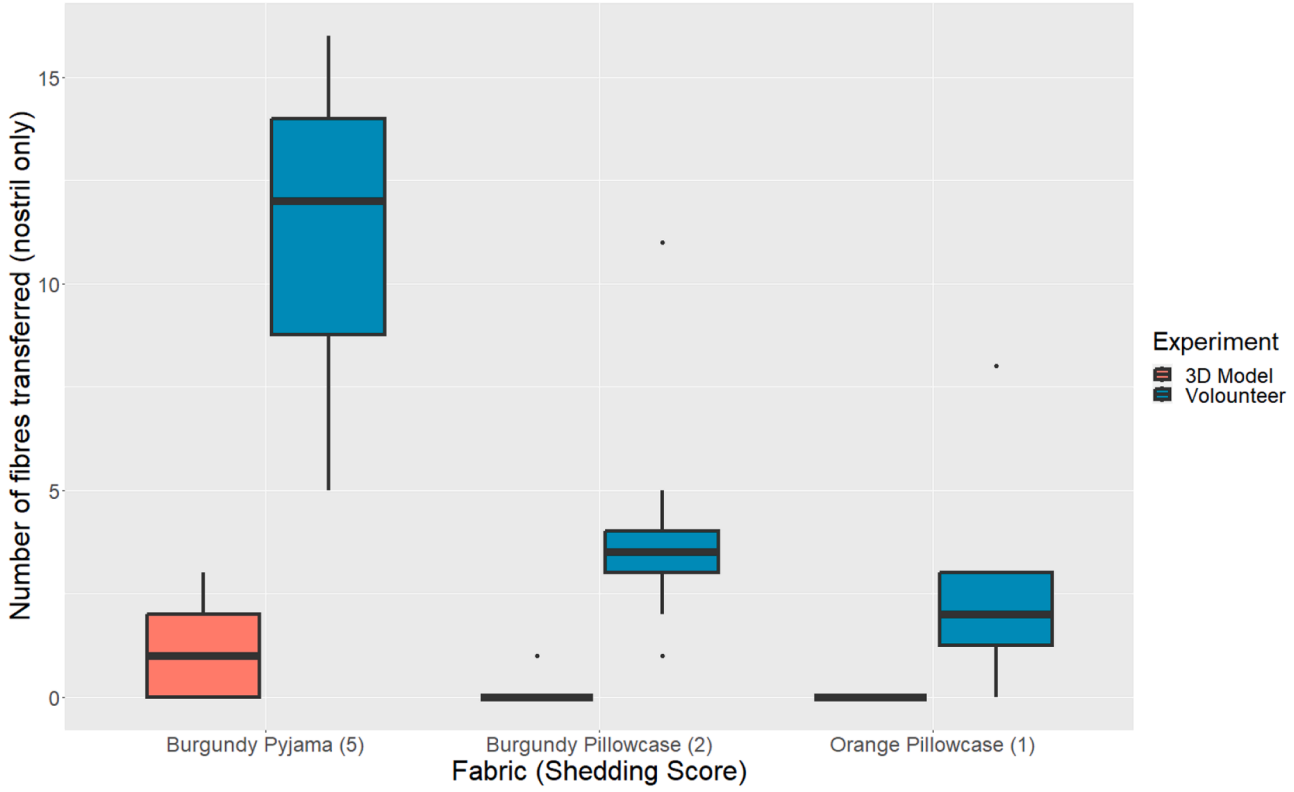

Fig. 12. Comparison of number of fibres transferred to the nostril region between the 3D model and live volunteer experiments.

does not accurately replicate true breathing conditions and likely underrepresents the number of fibres that would be transferred during an actual smothering. Higher numbers should be expected if recovery of fibres is carried out on the whole nasal cavity of a smothering victim. More accurate results would require a realistic replication of breathing conditions under duress. The use of live volunteers remains an option but continues to provide limited information on the number of fibres transferred as the results are confined to only the nostril region in addition to the use of inefficient recovery methods. Fig. 11 shows the frequency of each fibre length recovered during experiment 2 and appears to be comparable to the results of the 3D model, with differences only in the absolute quantity.

It is also worth noting that while simulations offer a practical way to study smothering, there are limits on their representativity of the situation. Breathing under duress would be largely limited through the mouth rather than the nose. As such other portions of the respiratory tract should be considered. Moreover, during a smothering, the intake of air is severely limited, which may limit the airflow despite increased rates of breathing. This could have an effect on the total number of fibres transferred, while simulations in this study (both 3D model and volunteer) assume that the airflow is proportional to the rate of breathing.

3.5. *Future work*

Following the results of this preliminary study, it would appear that the consideration of fibres in the nasal cavity for cases of smothering appears promising. However, more data is clearly required for robust interpretation of such cases. As such, additional work is already underway to augment the sample sizes for the background study. Other organs, such as the trachea and oral cavities, are also being considered. In terms of transfer, it is clear that accurate simulation of breathing conditions are difficult to carry out, future work will consider the use of breathing aids on cadavers, and further simulations with live volunteers, in order to obtain more data on the transfer of fibres. Some other considerations that could be explored include practical implementation of recovery methods into routine, be it during autopsy or on the scene, as well as persistence within the nasal cavity as well as. The latter is assumed to be good due to the closed nature of nasal cavities but remains to be validated with practical experiments under different conditions.

4. Conclusion

The results from this preliminary study provide insight into the methodologies as well as evaluative aspects of fibre traces in the nasal cavity. Firstly, the efficiency of various recovery methods were demonstrated, with the use of transparent AccuTrans® polymers performing the best. The recovery efficiencies of this method were 78–100% of the fibres when tested on cadavers and 85–100% on the 3D printed models, making it well suited for casework applications.

Secondly, the results also showcased that although nasal cavities are not entirely void of textile fibres. They are usually low in quantity with the mean number of fibres per nasal cavity being around 4. Rare occasions do crop up where a larger group of fibres are observed. The population study of these recovered fibres show agreement with other background surveys with respect to expected colour, type, and length characteristics. Such low numbers give hope for recovered fibres to differentiate between smothering and non-smothering scenarios.

Lastly, the transfer experiments demonstrated that the simulation of smothering scenarios does indeed result in the verifiable transfer of fibres into the nasal cavity and that the shedding capacity appears to be an important variable to consider. The transferred quantities show potential to be discriminated from the background. However, the simulations using 3D printed models and artificial pumps were preliminary and have potential for improvement. As such, more work needs to be done before stronger conclusions can be drawn.

The congregation of these findings provides promising insight into the possibility of forensically investigating fibres in the nasal cavities in cases of suspected smothering. Further research is required to

empirically validate and substantiate this difference in fibre frequencies between smothering cases and alternative, legitimate activities in order to facilitate the interpretation of such findings. This may thus provide an additional investigative avenue for cases where not much information is available and allow for a careful reconstruction of events and ultimately infer the causal activities.

CRediT authorship contribution statement

Nick Glauser: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Yu Chen Lim-Hitchings:** Conceptualization, Methodology, Formal analysis, Validation, Visualization, Writing – original draft. **Stefan Schaufelbühl**: Resources, Methodology **Sabine Hess:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing. **Kyra Lunstroot:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Geneviève Massonnet:** Conceptualization, Methodology, Supervision, Project administration, Writing – review & editing.

Declaration of Competing Interest

None.

Acknowledgements

The authors would like to extend their gratitude to the following people: Stephan Bolliger and Daniel Huber at the Institute of Forensic Medicine (Institut für Rechtsmedizin) of the University of Zurich for their collaboration in the background study. Following, Professor Matthew Johnson and Professor Edgar Matida from the department of Mechanical & Aerospace Engineering at Carleton University, for graciously providing us with the raw 3D model files for recreation.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2023.111890.

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