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The effect of reactive dyeing of fabric on the morphology of passive bloodstains

L. Dicken[a], C. Knock[a,*,1], D.J. Carr[b], S. Beckett[a]

[a] *Cranfield Defence and Security, Cranfield University, Shrivenham, Swindon, Wilts SN6 8LA, UK*
[b] *Previously a, now Defence and Security Accelerator, Porton Down, Salisbury SP4 0JQ, UK*

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ABSTRACT

The majority of fabrics at crime scenes have been coloured in some way. The effect of such treatments on resultant bloodstains has not been considered. In this work, horse blood was dropped onto reactively dyed calico fabrics (100% cotton, plain woven) with three different masses of 91 g m^{-2}, 171 g m^{-2} and 243 g m^{-2} and the results compared to previous work on the not-coloured calico fabric. Five impact velocities were used from 1.7 ms^{-1} to 5.4 ms^{-1}. The use of reactive dye increased the thickness (from 0.38 – 0.56 mm to 0.39 – 0.6 mm) and mass per unit area (from 85.1 – 224.6 g/m^2 to 91 – 243 g/m^2) of the calico fabrics. The reactively dyed fabrics had larger bloodstains (e.g. lightest calico 41.2 – 78.6 mm^2) compares to the not-coloured fabrics (e.g. lightest calico 21.4 – 67.5 mm^2) across all three mass per unit areas. The dyeing of the fabrics altered the intra-yarn spaces to a more optimum size for wicking blood, increasing the ease with which the blood could wick along the yarns in the dyed calico. The amount of wicking varied depending on individual variations within the fabrics and yarns. More variation in dry bloodstain area was seen among dyed calico specimens than for the not-coloured fabric. The amount of wicking which was seen on the dyed calico meant there was no correlation between dry bloodstain area and impact velocity, a correlation which was seen on the medium and heavy not-coloured calico in the previous work.

1. Introduction

Within blood pattern analysis (BPA), the earliest comprehensive study on bloodstains on fabric in 1986 [1] indicated the complexity of the subject. Subsequent work has shown that blood pattern analysis (BPA) requires studying many factors including the difference between contact and projected drops [2,3], satellite stains [4], the effect of impact velocity and fabric structure [5,6], backing materials [7] and angle of impact [8].

Yarn type has been found to affect blood stain morphology [6,8]. 100% cotton plain woven created smaller stains than those on 100% polyester plain woven. Even larger bloodstains were formed on a thin and low mass per unit area blended fabric (65% cotton / 35% polyester) where the blood spread laterally on the surface of the fabric rather than through the depth of the fabric.

Studying fabric structure, it was found that plain woven fabrics resulted in larger bloodstains than single jersey knit fabrics [6,8], most likely due to the higher compliancy of the latter resulting in less spreading across the surface of the fabric as it recovered elastically from the impact. The single jersey knit was also found to have a greater surface roughness than the plain woven, altering the wetting and wicking characteristics of the fabric which may also have led to a decrease in dry bloodstain area.

Yarn structure also affects bloodstain morphology. Six 100% cotton plain woven fabrics were created from yarns made using three different methods; ring spun, open end and murata vortex [9]. The yarn linear density and the twist multiplier were the same for the ring spun and open end yarns. The yarns were each woven into two different fabrics with different sett counts; 100 × 100 yarns per inch (39.4 × 39.4 yarns per cm) and 130 × 70 yarns per inch (51.2 × 27.6 yarns per cm). Porcine blood was dropped onto the fabrics from 500 mm. The resulting bloodstains revealed that while the blood wicked into the fabric woven from ring spun yarns, it did not wick into the fabric woven from either the open end or vortex spun yarns. Instead, the blood largely remained on the surface of the fabric with very little increase in the bloodstain area (5% for open end spun yarns, 15 – 20% for vortex spun yarns). On commercial bed sheeting, which was found to have ring spun weft yarns and Murata vortex spun warp

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[*] Corresponding author.
E-mail address: c.knock@cranfield.ac.uk (C. Knock).
[1] ORCID 0000–0002-5786–7508

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Table 1
mean fabric properties and standard deviation.

	Light dyed	Light not-coloured[a]	Medium dyed	Medium not-coloured[a]	Heavy dyed	Heavy not-coloured[a]
Thickness (mm)	0.39 ± 0.02	0.38 ± 0.03	0.51 ± 0.01	0.46 ± 0.02	0.6 ± 0.04	0.56 ± 0.03
Mass per unit area (g/m²)	91 ± 1.84	85 ± 1.54	171 ± 3.71	164 ± 2.26	243 ± 1.77	225 ± 1.56
Sett (yarns per 10 mm)	27 × 23	27 × 23	25 × 27	25 × 26	26 × 26	26 × 26

[a] taken from [10].

yarns, elliptical-shaped bloodstains were created owing to the uneven wicking with the different methods of yarn creation [9].

Dicken et al. [10] investigated three different mass per unit areas of calico (100% cotton, plain woven). The mean dry bloodstain area increased with increased impact velocity. The correlation was greatest for the medium and heaviest fabrics for which lateral spreading on the fabric surface was the key mechanism determining the blood stain size. Their greater yarn linear density meant a greater volume of blood was required to fully wet and wick into the intra-yarn spaces, therefore less blood was available for wicking along the yarns. The fabric with the lowest mass per unit area created the largest bloodstain. The low yarn linear density of this fabric meant the blood could easily fill the volume of the yarn, and therefore wick along the intra-yarn spaces, drawing from the reservoir of blood pooled in the inter-yarn spaces. The smallest bloodstains were seen on the fabric with the middle mass per unit area which had the highest twist. The higher twist rate meant that wicking along the yarns was reduced owing to the tighter yarn structure, resulting in a smaller bloodstain area.

The majority of bloodstained fabrics found at crime scenes will have had a finishing treatment, which is known to alter fabric properties. Fourteen 100% cotton single jersey knit samples were produced and subjected to bleaching, dyeing and softening [11]. The dyeing of the fabrics was seen to affect rigidity, tensile energy and resilience, mass per unit area and geometric roughness due to the shrinking of the fabric during dyeing, as well as the disruption of surface fibres and creation of surface irregularities. Bleaching has been seen to increase the amount of absorption in cotton fibres owing to physical changes in the fibre structure [12]. However, there is little discussion of finishing treatment within the literature on bloodstains. The effect of Scotchgard (a water repellent) has been mentioned in terms of preventing the blood from absorbing into the fabric, and the blood drop drying on the surface of the fabric [13]. Although fabric used in many studies [6,8,9] may have been treated in various ways (e.g. optically brightened, bleached [7]), the effect of these treatments is not considered.

The work in this paper was undertaken in order to ascertain whether the dyeing of 100% cotton fabrics affected the resultant bloodstain morphology. Passive blood drops were allowed to fall from a number of heights onto three different mass per unit areas of dyed calico. The fabrics under consideration had been previously used in a not-coloured state [10] to allow direct comparison of the bloodstains on the fabric in a dyed and not-coloured state.

2. Materials and method

2.1. Materials

To study the effect of reactively dyeing fabrics on bloodstains, three masses of calico, a 100% cotton plain woven fabric, were used. Due to the use of a 100% cotton fabric, a reactive dye was used. Reactive dyes have become the most popular dye for dyeing cotton since their creation in the 1950 s. Around 38% of the total cellulose dye market uses reactive dye [14]. Reactive dyes are the only water-soluble dyes which produce a dye-fibre covalent bond [15], meaning that they are easy to apply, have extremely good wash-fastness, and are available in a wide shade gamut.

Table 2
impact velocities.

Drop height (mm)	Impact velocity and standard deviation (ms^{-1})
200	1.7 ± 0.02
500	2.8 ± 0.04
1000	4.1 ± 0.04
1500	4.8 ± 0.03
2500	5.3 ± 0.07

All three fabrics were dyed using Dylon Sunflower Yellow 05 fabric dye. This contains Colour Index (C.I.) reactive yellow 125. The fabrics were dyed according to the packet instructions; fabrics were washed with detergent[2] before the dye was added to the washing machine,[3] which was then run on a 40 °C cotton cycle. The fabric was then washed again with detergent[2] to remove any excess fabric dye and washed a further four times to create a dimensionally stable fabric [16]. The fabrics were line-dried and cut into 100 mm × 100 mm specimens (n = 87), minimising the repetition of warp and weft yarns. The specimens were then pressed using a digital fabric steam press model PSP-202E on the 'cotton' setting, before conditioning to 20 ± 2 °C and 65 ± 4% relative humidity for 24 h [17].

Following conditioning, the thickness (mm) [18], mass per unit area (g/m²) [19] and sett [20] were measured, Table 1.

The bloodstains were created using defibrinated horse blood, obtained from Southern Group Laboratory.[4] The blood was stored below 4 °C and used within one week of acquisition.

2.2. Method

In order to simulate a blood-letting event at body temperature, the horse blood was heated to 37 °C. The blood was dropped from five heights onto the calico to create passive bloodstains, Table 2. A Phantom V7 high-speed video filmed all the drops (256 × 256 resolution, 4796 fps and 80 µs exposure). Phantom Camera Control software[5] was used to analyse the high speed videos to measure impact velocity and droplet diameter. The mean droplet diameter was 3.4 ± 0.1 mm. Five repeats were taken at 200 mm, 500 mm, 1000 mm and 1500 mm on each fabric and three repeats at 2500 mm on each fabric resulting in a total of 69 specimens.

A second experiment was undertaken as per Dicken et al. [21] to film the technical face and technical rear of the fabric at impact. Blood was dropped from three heights; 200 mm, 1000 mm and 2000 mm. This resulted in velocities of 1.8 ms^{-1}, 4.2 ms^{-1} and 5.7 ms^{-1} respectively. The blood drop diameter was 3.6 ± 0.24 mm². Two repeats on each fabric from each height were undertaken resulting in a total of 18 specimens.

The wet bloodstains were photographed less than 30 s after impact. The dry bloodstains were photographed on both the

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[2] Persil non-bio small and mighty

[3] Samsung Ecobubble at 40 °C cotton cycle

[4] E-H Cavendish Courtyard, Sallow Road, Weldon Industrial Estate, Corby, Northants. www.sglab.co.uk

[5] https://www.phantomhighspeed.com/resourcesandsupport/phantomresources/pccsoftware
Page accessed 14th March 2022.

Table 3
µCT scanner parameters.

Scanning values						Reconstruction	
Target	Voltage (kV)	Current (µA)	Exposure (ms)	Projections	Frames per projection	Beam hardening	Noise reduction
Tungsten	50	150	500	1080	2	1	1

technical face and technical rear at least 24 h later. All photographs were taken using a Nikon D3300 camera.

A Nikon XTH225 micro computed tomography (µCT) scanner was used to analyse all the specimens (Table 3). Following scanning, the data was manually reconstructed in CT Pro 3D (Table 3). Using VGStudio Max, the reconstructed data was analysed and a 2D image of the 3D reconstruction and 2-dimensional cross-sectional data were saved [10].

A Hitachi SU3500 scanning electron microscope (SEM) and EDAX TEAM microanalysis system[6] was used to examine two specimens from each velocity and fabric combination (15 kV, 60 Pa).

The bloodstain areas from the technical face wet and dry and technical rear dry external photographs were measured using ImageJ.[7]

Analysis of variance (ANOVA) (IBM SPSS statistics version 22) was carried out on the data to assess whether drop impact velocity or fabric type had a statistically significant effect on the dry technical face bloodstain area. To identify which variables contributed to any significant effects, Tukeys HSD analysis was carried out. Interactions were only reported if they were found to be significant. Equality of variances and normality of data were checked. An independent t-test was used to assess whether there were any statistically significant differences in external wet and dry technical face and technical rear bloodstain area between the dyed and not-coloured [10] calicos. Equal variances were assumed, unless Levene's test was significant.

3. Results and discussion

The resultant bloodstains from the passive blood drops were examined to determine the effect of fabrics mass and impact velocity on bloodstain size and shape.

3.1. Overall trends

Bloodstains formed on the fabrics in two stages. First, the dynamics of the blood drop immediately following impact, referred to in this paper as impact dynamics, and secondly wicking into and along the yarns.

The high speed camera showed that following impact, the blood initially penetrated through to the technical rear of the light calico at all velocities (1.8, 4.2 and 5.7 ms^{-1}) as quickly as 0.067 ms after impact. For the medium and heavy calico, no blood penetrated through to the technical rear of the fabric as a result of the impact of the blood drop within the 50 ms of high speed video. For all three fabrics, the blood spread laterally on the surface of the fabric for between 2 and 4 ms, taking longer as the impact velocity decreased. The blood drop then retracted, and satellite stains and ligaments formed. By between 8 and 14 ms after impact the impact dynamics had ceased and the blood drop had settled on the surface of the fabric.

Once the blood drop had settled on the surface of the fabric, any blood remaining on the surface then wicked vertically into the yarns and then along the intra-yarn spaces. If the blood had already penetrated the yarns, the blood then wicked along the intra-yarn spaces. The greater yarn linear density of the medium and heavy than the light calico meant a greater volume of blood was required to fully wet and wick into the yarns, resulting in a smaller volume of blood being available to wick along the yarns. This resulted in less wicking and smaller dry bloodstain areas than for the light fabric. The amount of wicking within a fabric can vary with variations within the fabric, yarns and fibres [22]. As a result, the amount of wicking was not consistent across specimens for any given fabric, which created the variation in the data.

Univariate analysis of variance (ANOVA) showed fabric density ($F_{14,54}$ = 16.931, $p \leq 0.01$) affected dry bloodstain area. Tukey's HSD analysis revealed the mean dry bloodstain area on the light calico (64.4 mm²) was statistically significantly larger than that on the medium (50.9 mm²) and heavy (48.4 mm²) calicos.

3.2. Impact velocity

For all three fabrics, as the impact velocity increased, the amount of lateral spreading which occurred following impact also increased, Fig. 1. Plotting impact velocity against blood stain size showed, Fig. 2, that there was a wide variance in the data with variations in area of up to 30 mm² (the dry bloodstain area for the medium calico for 1.7 ms^{-1} impacts varied from 29.7 mm² to 59.6 mm²) and coefficients of variation (CVs) of up to 28%. Due to the large variance, there was no observed correlation between dry bloodstain area and impact velocity, although there was a general trend for the dry bloodstain area to increase as the impact velocity increased. However, for the light dyed calico, the dry bloodstain areas did not increase consistently with impact velocity with a decrease in bloodstain area from 1.7 ms^{-1} to 2.8 ms^{-1} (mean areas: 63.3 mm² and 55.7 mm² respectively). The area then increased as the impact velocity increased to 4.1 ms^{-1} (69 mm²) before decreasing with an impact velocity of 4.9 ms^{-1} (64.9 mm²). Generally, the dry bloodstain areas were largest on the light calico, with overlap between all three fabrics, Fig. 2.

Univariate analysis of variance (ANOVA) showed that impact velocity ($F_{14,54}$ = 9.855, $p \leq 0.01$) did affect dry bloodstain area. The dry bloodstain area on the specimens from an impact velocity of 1.7 ms^{-1} (46.4 mm²) were statistically significantly smaller than those from 4.1 ms^{-1} (56.5 mm²), 4.9 ms^{-1} (63 mm²) and 5.3 ms^{-1} (63.4 mm²). The dry bloodstain area on the specimens from an impact velocity of 2.8 ms^{-1} (47.1 mm²) were statistically significantly smaller than those from 4.9 ms^{-1} and 5.3 ms^{-1}.

3.3. Low velocity impacts

At low velocities on the light calico, the mean dry bloodstain area from impacts of 1.7 ms^{-1} (63.3 mm²) was larger than that from impacts of 2.8 ms^{-1} (55.7 mm²). This decrease in dry bloodstain area with increased velocity was likely caused by the larger amount of blood remaining on the surface at impact for an impact velocity of 1.7 ms^{-1}, Fig. 3a, compares to 2.8 ms^{-1}, Fig. 3b.

At 1.7 ms^{-1} the lack of lateral spreading at impact owing to the low kinetic energy resulted in a pool of blood remaining on the surface of the fabric. This resulted in a reservoir from which wicking occurred preferentially along the warp yarns around the edge of the bloodstain, Fig. 3a and c, marked 'A'. The wicking was preferential

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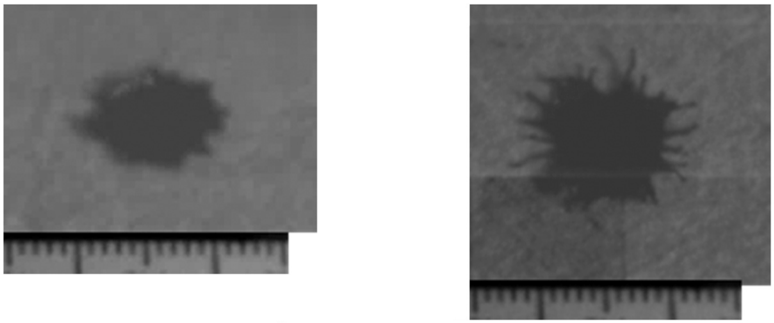
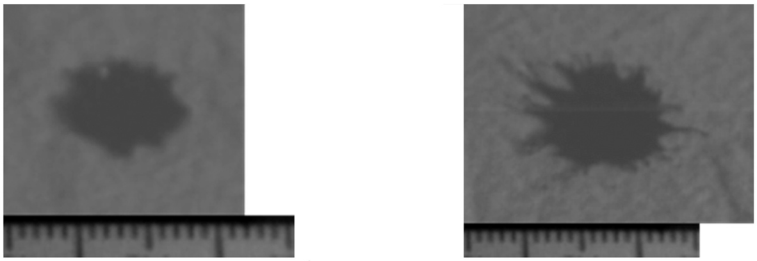
[6] https://www.edax.com/products/eds/team-eds-system-for-the-sem Page accessed 27th September 2018

[7] ImageJ https://imagej.nih.gov/ij/ Page accessed 14th March 2022.



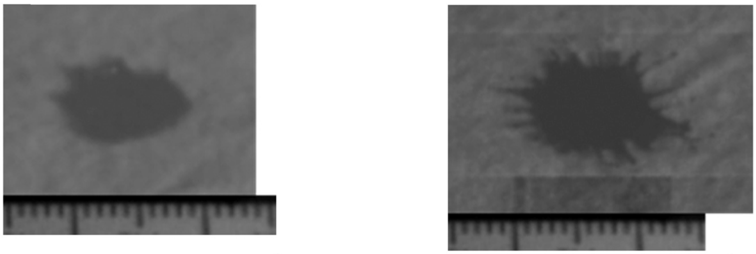
a: light calico from 1.8 ms^{-1} at 3ms

b: light calico from 4.2 ms^{-1} at 2ms



c: medium calico from 1.8 ms^{-1} at 3ms

d: medium calico from 4.2 ms^{-1} at 2ms



e: heavy calico from 1.8 ms^{-1} at 3ms

f: heavy calico from 4.2 ms^{-1} at 2ms

Fig. 1. stills from high speed video of the fabric technical face. Scale is 2 cm.

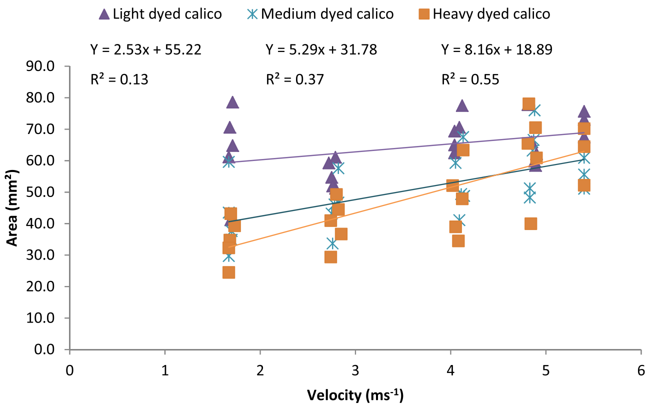


Fig. 2. dry bloodstain area plotted against impact velocity.

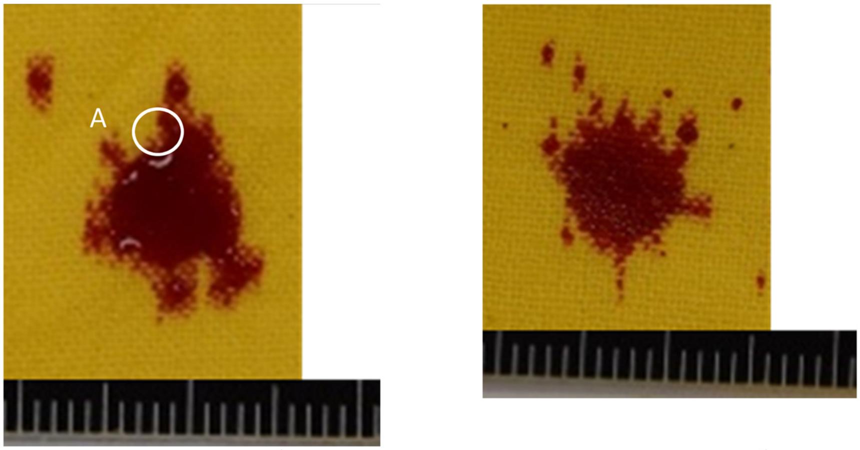
along the warp yarns around the edge of the bloodstain (Fig. 3a and c, marked 'A′) due to differences in yarn linear density between the warp (14 tex) and weft (18 tex) yarns. The lower yarn linear density of the warp yarns meant less blood was required to fill the volume of the yarns, and therefore more blood was available for wicking along them. This altered the shape of the bloodstain from a typical 'circular' passive bloodstain [23] to a bloodstain which was a more irregular shape, Fig. 3c.

The increase in kinetic energy at impact from 1.7 ms^{-1} to 2.8 ms^{-1} for light calico resulted in blood penetrating through to the technical rear of the fabric, Fig. 3f and hence no blood being available to pool on the surface after impact, Fig. 3b. At 2.8 ms^{-1}, a small amount of blood wicked preferentially along the warp yarns at the edge of the bloodstain, Fig. 3d, marked 'A′, but with no reservoir of blood on the surface this was less than for the 1.7 ms^{-1} impacts, resulting in smaller stains for the 2.8 ms^{-1} impacts.

On the medium and heavy calicos, as for the light calico, the low kinetic energy at a 1.7 ms^{-1} impact velocity resulted in a lack of lateral spreading at impact, Fig. 1c and e, and blood remained pooled on the surface of the fabric, Fig. 4a and e. In the middle of the bloodstain the blood wicked vertically into both the warp and weft yarns, Fig. 4d and h, marked 'A′. The blood which was inside the yarns, Fig. 4c and g marked 'B′, was then able to wick along the intra-yarn spaces. However, some blood remained on the surface of the fabric and dried before it was able to wick away along the yarns. This is shown in the large rim of dense, dry blood, as in the coffee ring effect [24], around the edge of the bloodstain, Fig. 4b, c, f and g, marked 'C′.

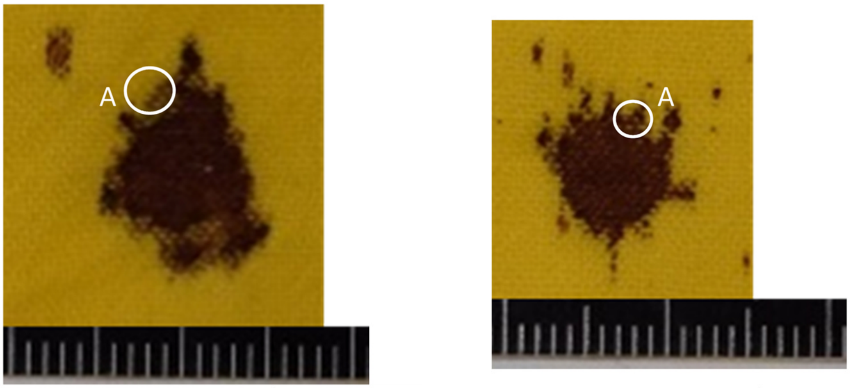
3.4. High velocity impacts

On the light calico, the greater amount of lateral spreading of the blood on the surface of the fabric with the increase in impact



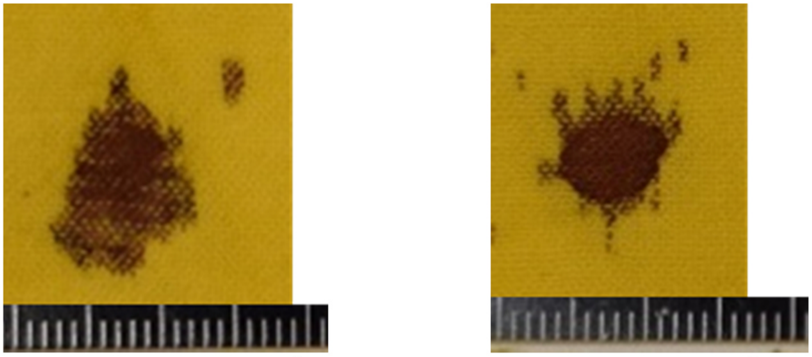
a: wet bloodstain 1.7 ms^{-1}.

b: wet bloodstain 2.8 ms^{-1}.



c: dry bloodstain 1.7 ms^{-1}.

d: dry bloodstain 2.8 ms^{-1}.



e: dry technical rear, 1.7 ms^{-1}.

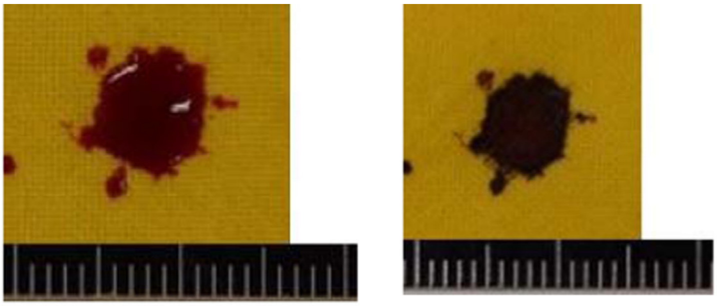
f: dry technical rear 2.8 ms^{-1}.

Fig. 3. typical examples of a wet and dry bloodstain on the dyed light calico. 'A' indicates where the blood has continued to wick along the warp yarns beyond the main bloodstain. Scale is 2 cm.

velocity resulted in an increase in mean dry bloodstain area from 2.8 ms^{-1} (55.7 mm²) to 4.1 ms^{-1} (69 mm²).

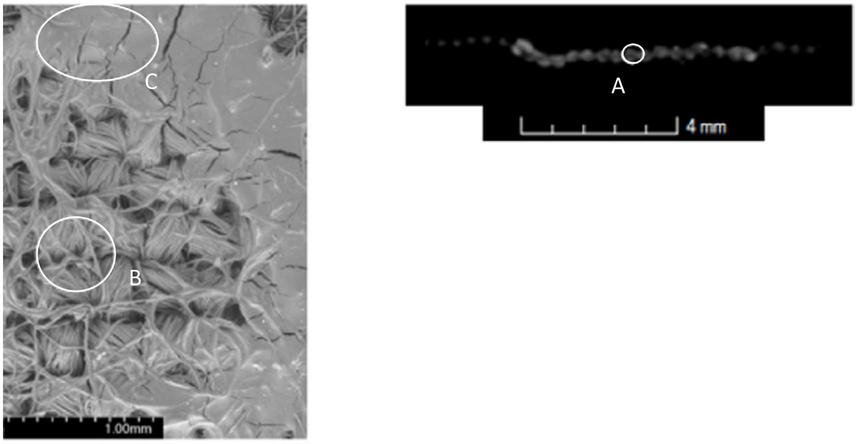
For the light calico at an impact velocity of 4.1 ms^{-1} the blood is patchy in the centre of the bloodstain as the blood wicked into and along the yarns, Fig. 5a. The mean dry bloodstain area decreased from 69 to 64.9 mm² as the impact velocity increased from 4.1 to 4.9 ms^{-1}. The higher impact velocity forced the blood into the yarns, spreading evenly along the warp yarns throughout the entire bloodstain, Fig. 5b. This reduced the amount of blood which pooled in the inter-yarn spaces. The reservoir of blood from which wicking along the intra-yarn spaces could occur was therefore reduced resulting in less wicking as shown by the similarity between the mean wet (68.3 mm²) and dry (64.9 mm²) bloodstain areas.

The mean dry bloodstain area on the light calico specimens increased to 72.1 mm² with the increase in impact velocity to 5.3 ms^{-1}. The increase in impact velocity resulted in a greater amount of



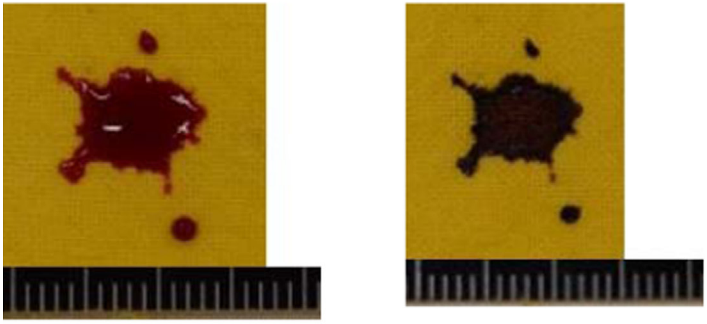
a: medium dyed calico wet bloodstain

b: medium dyed calico dry bloodstain



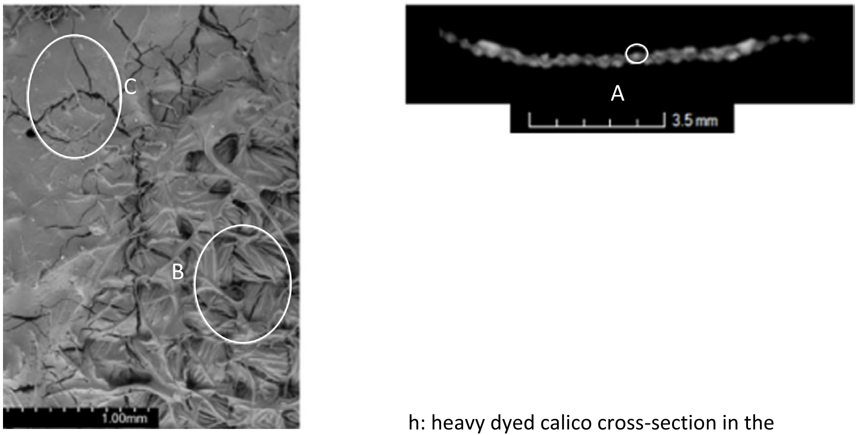
c: medium dyed calico SEM image

d: medium dyed calico cross-section in the weft direction



e: heavy dyed calico wet bloodstain

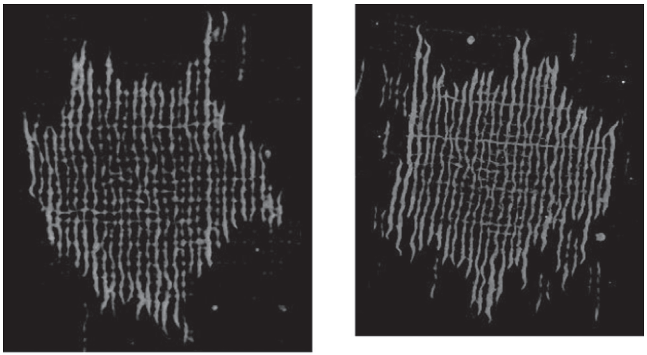
f: heavy dyed calico dry bloodstain



g: heavy dyed calico SEM image

h: heavy dyed calico cross-section in the weft direction

Fig. 4. examples of a wet and dry bloodstain (scale: 2 cm) and SEM image at 42x magnification and CT cross section on the dyed medium and heavy calicos from an impact velocity of 1.7 ms^{-1}. 'A′ is an example of blood in the warp and weft yarns at the centre of the bloodstain. 'B′ is the blood in the centre of the bloodstain inside the yarns. 'C′ is the blood at the edge of the bloodstain which remained on the surface of the fabric.



a: 4.1 ms^{-1} b: 4.9 ms^{-1}

Fig. 5. examples of a 2D image of the 3D CT reconstruction of a light calico.

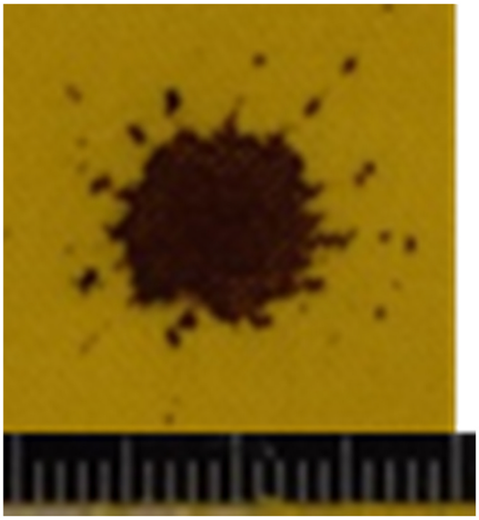


Fig. 6. examples of a dry bloodstain on the light calico at 5.3 ms^{-1}. Scale is 2 cm.

lateral spreading at impact, and then a large amount of wicking. The amount of wicking which occurred following impact at 5.3 ms^{-1} was shown in the largest increase in light calico bloodstain area between the mean wet (64.7 mm²) and dry (72.1 mm²) bloodstains among the velocities investigated. The wicking occurred in both the warp and weft directions, evidenced by the bloodstains from 5.3 ms^{-1} impacts being the only specimens where no wicking occurred along the warp yarns beyond the edge of the main bloodstain, Fig. 6.

On the dyed medium and heavy calicos, for 4.1 ms^{-1} impacts the wet blood did not pool on the surface of the fabrics (Fig. 7a and b), owing to the increased lateral spreading at impact. Although the blood may have penetrated slightly into the yarns, the high speed video showed the blood did not penetrate through to the technical rear of the fabric following impact. Later the blood did wick into the yarns and through to the technical rear of the fabric in the medium, Fig. 7e and heavy, Fig. 7f, calicos. The blood wicked through, rather than around, the yarns, Fig. 7g and h. Only a small amount of blood remained to dry on the surface of the fabric resulting in only a small area of dense blood around the edge of the bloodstain, Fig. 7c and d.

For the medium and heavy calicos, these trends continued for the higher velocities (4.9 and 5.3 ms^{-1}).

3.5. *Effect of dyeing on fabric*

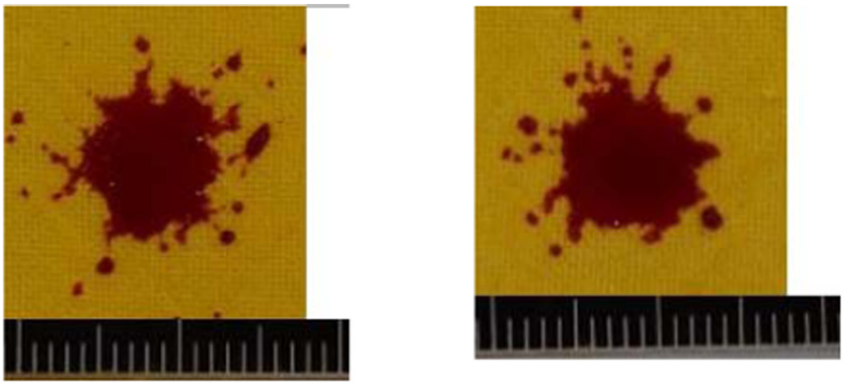
The use of reactive dye resulted in chemical and physical changes to the fabric. Dyeing increased the thickness by 2.6 – 7%, and mass per unit area by 7 – 8%, Table 1. The sett of the three fabrics is almost identical before and after dyeing, Table 1, so the increase in thickness and mass per unit area was not owing to the yarns being closer to each other. Therefore, the size of the yarns themselves must have increased.

SEM images showed that the dyed fabric, Fig. 8a, was more tightly compacted than the not-coloured fabric (Fig. 8b), with less evidence of inter-yarn spaces. This was due to the swelling of the fibres, and therefore the yarns during the dying process when the dye molecules enter the polymer structure of the fibre. The structure of a cotton fibre is partly crystalline and partly amorphous. The crystalline regions do not alter when immersed in water [25] but the amorphous regions of cotton fibre swell, allowing the dye molecules entry into the fibre [25]. For a reactive dye, the molecules form a covalent bond with the cotton. Upon drying, owing to the addition of the dye, the cotton fibres will remain swollen, increasing the diameter of the yarns, and altering the intra-yarn spaces between the fibres. As long as the intra-yarn spaces were changed to a more optimum size for wicking [26], wicking both into and along the yarns would increase. Further as the amorphous regions of the cotton fibre reacted with the dye, the blood is less likely to be absorbed into the fibres. This would increase the amount of blood available to wick along the intra-yarn spaces, as the blood would only be able to wick between the fibres, not into them.

3.6. *Comparison to bloodstains on not-coloured fabric*

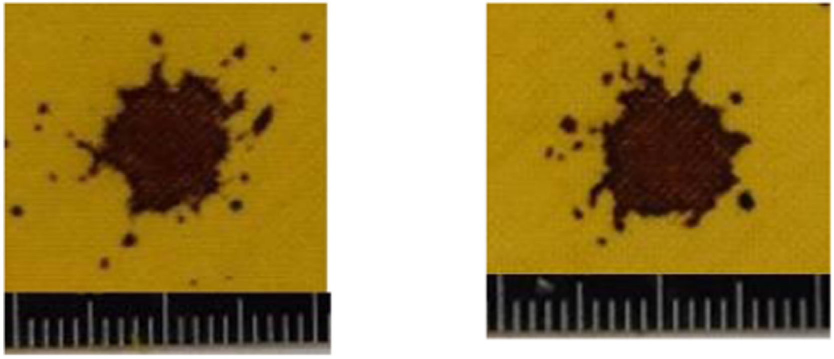
Comparisons of bloodstains from passive drops were carried out between the dyed fabric and previous work on the fabric from before it was dyed (not-coloured) [10].

There is some overlap in the areas of the dyed and not-coloured fabric, Fig. 9. However, an independent t-test showed that the dry bloodstain areas for the dyed calico were statistically significantly larger than the not-dyed calico for all fabrics (light: t_{46} = 6.219, $p \le 0.01$, medium: t_{46} = 5.340, $p \le 0.01$, heavy: t_{46} = 2.33, $p \le 0.05$) and all velocities (1.7 ms^{-1}: t_{28} = 5.046, $p \le 0.01$, 2.8 ms^{-1}: t_{28} = 3.699,



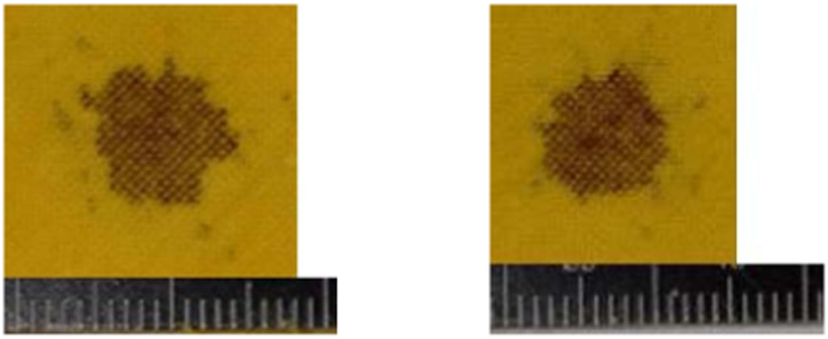
a: medium calico wet bloodstain

b: heavy calico wet bloodstain



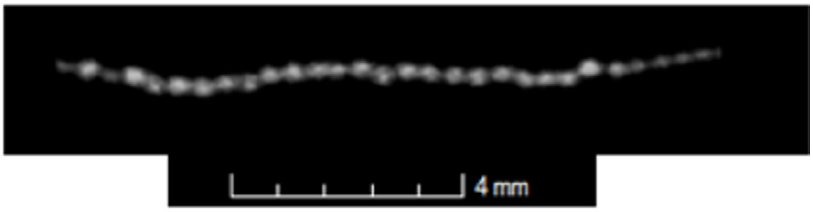
c: medium calico dry bloodstain

d: heavy calico dry bloodstain

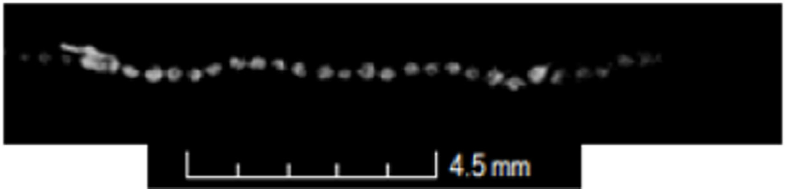


e: medium calico dry technical rear bloodstain

f: heavy calico dry technical rear bloodstain

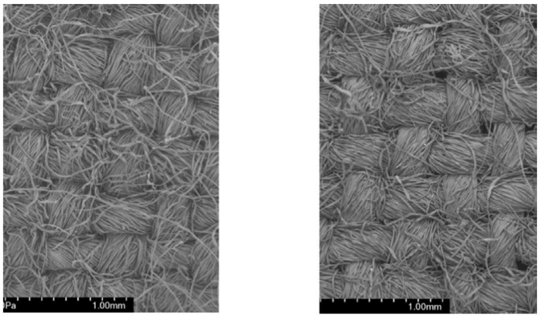


g: medium calico CT cross-section



h: heavy calico CT cross-section

Fig. 7. examples of a wet and dry bloodstain (scale: 2 cm) and CT cross-section in the weft direction from 4.1 ms^{-1} impact.



a: dyed calico at 42x magnification b: not-coloured calico at 42x magnification

Fig. 8. SEM images of the dyed and not-coloured [10] medium calico.

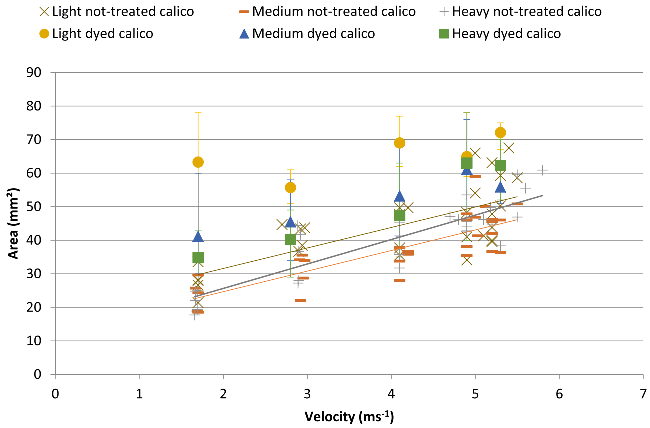


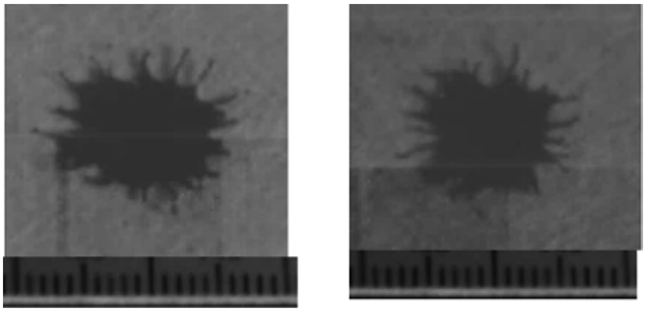
Fig. 9. Dry bloodstain area for the not-coloured calicos [10] and the mean dry bloodstain area for the dyed calicos plotted against impact velocity.

$p \leq 0.01$, 4.1 ms^{-1}: t_{28} = 4.533, $p \leq 0.01$, 4.9 ms^{-1}: t_{28} = 4.985, $p \leq 0.01$, 5.3 ms^{-1}: t_{22} = 2.956, $p \leq 0.01$).

The only slight correlations between dry bloodstain area and velocity are for the medium and heavy not-coloured fabrics, Table 4. For the medium and heavy not-coloured calico, the greatest influence on the dry bloodstain area was the amount of lateral spreading of the blood drop on the fabric surface at impact [10], which increased with higher velocities. This resulted in a slight correlation between dry bloodstain area and velocity, Fig. 9, Table 4. The blood was not able to wick along the intra-yarn spaces in the not-coloured calico to the same extent as in the dyed fabrics, as dye entering the amorphous regions in the cotton fibres had altered the intra-yarn spaces. This resulted in more wicking for the medium and heavy dyed fabrics and increased the variation among specimens and as discussed earlier therefore reduced the correlation with velocity.

Table 4
the correlation coefficients using a least square fit, R², of area against impact velocity for the not-coloured [10] and dyed fabrics.

	Not-coloured	Dyed
Light	0.51	0.13
Medium	0.68	0.37
Heavy	0.76	0.55

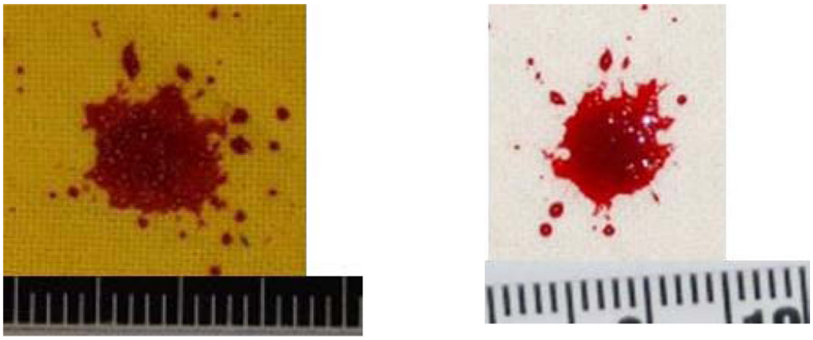


a: not-coloured, area 104 mm² b: dyed, area 103 mm²

Fig. 10. examples of a high speed video still from 2 ms following impact on a light dyed and not-coloured calico [20] from 4.2 ms^{-1}. Scale is 2 cm.

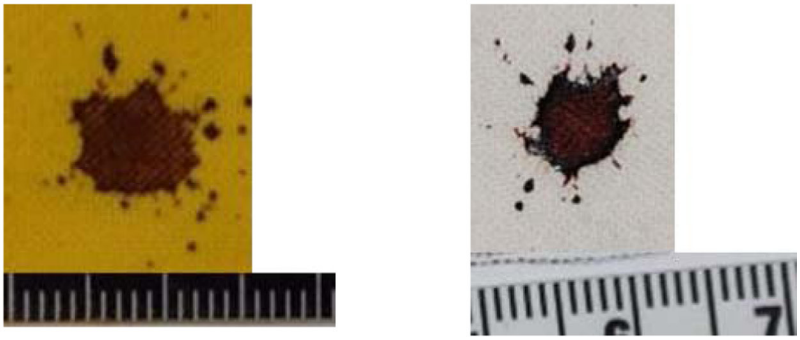
Initially at impact, the amount of lateral spreading which occurred was comparable between the dyed and the not-coloured calicos (e.g. Fig. 10, areas of spreading were 103 and 104 mm² 2 ms after impact respectively). However, a greater amount of blood remained pooled on the surface of the not-coloured calico [10] than the dyed calico. On the not-coloured calico, pooled blood was present on the surface of the medium and heavy fabrics up to an impact velocity of 4.1 ms^{-1} [10] (Fig. 11b), while pooling only occurred on the dyed calico up to 2.8 ms^{-1} impacts.

On the not-coloured calico, the blood which pooled on the surface was not able to wick into the yarns before drying occurred, resulting in a rim of dense, dry blood [10], Fig. 11d and f. On the dyed calico the blood was able to wick into the yarns before it dried on the surface of the fabric. This resulted in a smaller rim of dense blood around the edge of the bloodstain (Fig. 11c and e). The blood was inside the yarns in the dyed calico; both the warp and weft yarns are blood soaked for the dyed calico, Fig. 11g, while they are patchy for the not-coloured calico [10], Fig. 11h. The blood which was inside the yarns in the dyed calico was then able to wick along the intra-yarn spaces, resulting in a larger dry bloodstain area for the dyed than the not-coloured fabrics. The greater ease with which the blood could wick into the yarns, resulting in less blood on the surface of the dyed calico, and along the yarns, creating larger dry bloodstain areas, suggests the alteration of the intra-yarn spaces following dyeing resulted in them being a more optimum size for wicking blood.



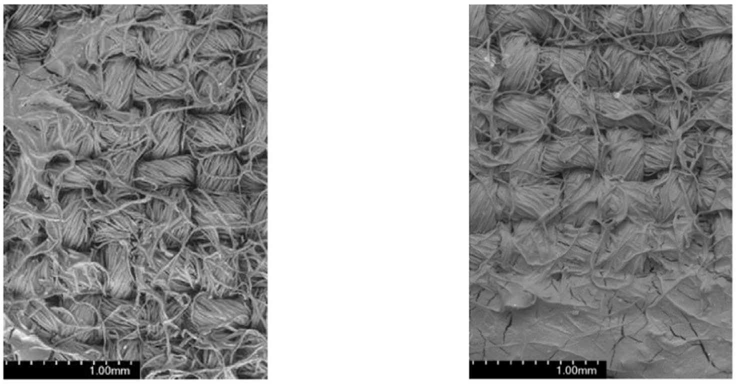
a: medium dyed calico wet bloodstain

b: medium not-coloured calico wet bloodstain



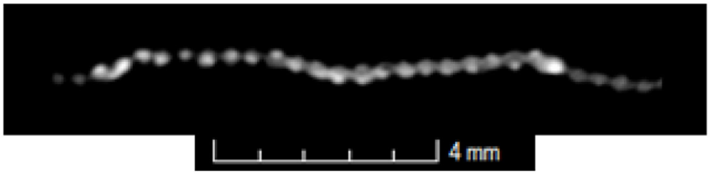
c: medium dyed calico dry bloodstain

d: medium not-coloured calico dry bloodstain

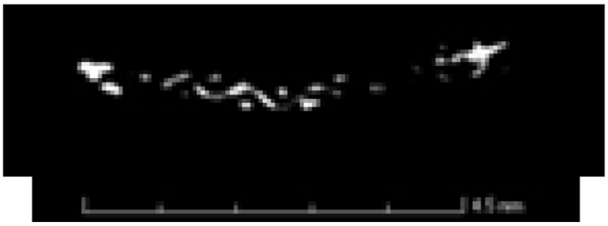


e: medium dyed calico SEM image

f: medium not-coloured calico SEM image



g: medium dyed calico CT cross-section



h: medium not-coloured calico CT cross-section

Fig. 11. examples of wet and dry bloodstain photograph (scale: 2 cm), SEM image at 42x magnification and CT cross-section in the weft direction on the medium dyed and not-coloured calico (images taken for research [10] but not used in publication) from 4.1 ms^{-1}.

4. Conclusions

To study the effect of the dying of fabric on blood stain morphology, passive bloodstains were created on three mass per unit areas of 91, 171 and 243 gm^{-2} of reactively dyed 100% cotton plain woven calico using five impact velocities (1.7 ms^{-1} to 5.3 ms^{-1}). The reactive dyeing of the fabric resulted in the blood being able to wick more easily along the yarns, resulting in a larger dry bloodstain area across all three dyed calicos than on not-coloured calico. The increase in wicking for the dyed calico also removed any correlation with velocity, as the wicking increased the variability in dry bloodstain area among specimens. The amount of wicking which was able to occur, most notably on the light dyed calico, altered the shape of the parent bloodstain from the typical ‘circular’ bloodstains seen on not-coloured calico to a more irregular shape. This has implications in assessing bloodstains at a crime scene, as typically passive bloodstains are thought to have an approximately circular shape, while bloodstains from an angled impact would be more elongated. The work undertaken in this paper shows an irregular-shaped bloodstain can occur without an angled impact, and indicates great care needs to be taken in analysing bloodstains on fabric when a lot of wicking can occur. Therefore, at a crime scene the manner in which the fabric has been processed would need to be taken into consideration when assessing the morphology and area of the dry bloodstains. Different surface treatments may alter the dry bloodstain area and the likelihood of a correlation with velocity.

Ethical statement

The horse blood used in this work was collected in an ethical and legal manner and ethical approval for the research was obtained.

CRediT authorship contribution statement

Dr Clare Knock: (Bloodstains and overall project) Supervision, writing – review and editing, conceptualization. **Dr Lisa Dicken:** Investigation, methodology, formal analysis, writing – original draft. **Dr Sophie Beckett:** (Use of CT scanner) Analysis, methodology, supervision, advice. **Dr Debra Carr:** (Fabrics) Supervision, methodology, writing – review and editing.

Conflicts of interest

None.

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