Int J Legal Med (2012) 126:739–746
DOI 10.1007/s00414-012-0734-2

ORIGINAL ARTICLE

Drying properties of bloodstains on common indoor surfaces

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Received: 6 April 2012 / Accepted: 13 June 2012 / Published online: 30 June 2012

Abstract When blood reaches an extracorporeal surface, a drying process is initiated. Properties of this drying process may be crucial for the correct assessment of case-specific time lapses, however, there is a lack of systematic studies concerning the drying times of blood. We present a study on drying properties of small blood droplets with a standardized size of 25 μl (resembling droplets originating from pointed and sharp objects, e.g. the tip of a knife) under different environmental conditions to elucidate the effect of different ambient temperatures, indoor surfaces and anticoagulant treatment. As a rule of thumb, wiping a typical small blood droplet will not lead to a macroscopically visible smear after a time period of approximately 60 min ($time_{min}$=45 min; $time_{max}$=75 min) at an average room temperature of 20 °C. Alteration of the ambient temperature has a remarkable effect, as the time needed for the drying process leading to wipe resistance of the droplets decreases to 30 min ($time_{min}$) at an ambient temperature of 24 °C, and is prolonged up to >120 min ($time_{max}$) at an ambient temperature of 15 °C. As for the surface materials in our study, significant differences in drying periods were only found between wood and linoleum (80th percentile 45 vs. 75 min). Treatment with anticoagulants did not influence extracorporeal drying times. In synopsis, the present study shows that ambient temperature is a major determinant of the drying process of blood droplets and should always be documented accurately and continuously on a crime scene. In certain situations, an estimation of the time elapsed since bloodstain origination may be of importance to answer questions related to the time course of actions. However, further systematic studies are needed to clarify the effect of other properties such as droplet size, humidity, or evaporation.

Keywords Forensic science · Bloodstain pattern analysis · Drying properties · Wipe characteristics · Anticoagulant treatment

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Introduction

Bloodstain pattern analysis, which may be associated with the distribution, form, shape, and size of single bloodstains and the pattern itself, may substantially contribute to the reconstruction of events in the forensic setting. Although

reviews of renowned experts in the field [1–8], case reports [9–12], and intensive research on the detection, molecular identification and estimation of deposition time of human traces [13–20] have considerably improved bloodstain pattern analysis, systematic research and knowledge still remain scarce concerning the physical properties of extracorporeal blood droplets [20–26].

The wipe pattern of bloodstains at a crime scene may be used to determine whether the stains were wet or dry at the time of manipulation by either victim or suspect. In some cases, the time period in which pre-existing blood stains can be altered by wiping is crucial for reconstruction purposes, e.g., to determine when a suspect had contact with a stain and then establish or eliminate an alibi. Therefore, detailed knowledge of bloodstain physical properties is of high importance.

The drying process of fluids is a function of volume and surface area and mainly depends on temperature, humidity, air circulation and vapor pressure [27, 28]. In addition, drying properties of viscous fluids such as blood are also affected by the target surface on which the fluid falls. Furthermore, droplet volumes may vary due to varying kinetic energy states and/or velocities and depend on the shape of the object from which the droplet originates [1, 3, 23, 24, 28].

In crime scenes with a complex bloodstain pattern, incompletely dried droplets are frequently wiped secondarily, either in the later course of the chain of action or due to actions by law enforcement or paramedics. Wiped blood droplets are well known to show a skeletonized ring marking their initial position, which consecutively dries from the outside to the inner parts of the droplet increasing in width (in this study referred to as thickness) over drying time [2, 29].

In the present study, we investigated blood droplet drying properties based on morphological assessments of wipe effects on different common indoor surfaces under varying ambient temperatures. We examined wipe resistancy, skeletonization, skeletonization ring thickness, length and width of a wipe tail. We analyzed a possible correlation of these parameters with the time elapsed since droplet origination. In addition, we examined the consequences of anticoagulant treatment for bloodstain drying behavior, as we were confronted with this very same question in several bloodstain pattern analysis cases.

Material and methods

Wipe characteristics of blood droplets were analyzed on five different indoor surfaces: linoleum, wood, glass, tile, and mirror. Separate surface units were used for data collection over 13 different time points (Table 1). The selected specific points in time, at which wipe characteristics of droplets were examined, were chosen on the basis of previous experiments indicating a very limited time period for the evaluation of skeletonization (1, 2, 3 min) and tail characteristics (5, 10, 15 min). Since, due to practical observations, droplets were expected to become wipe-resistant within 2 h, we chose intervals of 15 min up to a total period of 120 min for the

Table 1 Time table showing the wiping times and the respective time periods

Droplets wiped after (min)	Respective time period (min)
1	≤1
2	>1≤2
3	>2≤3
5	>3≤5
10	>5≤10
15	>10≤15
30	>15≤30
45	>30≤45
60	>45≤60
75	>60≤75
90	>75≤90
105	>90≤105
120	>105≤120

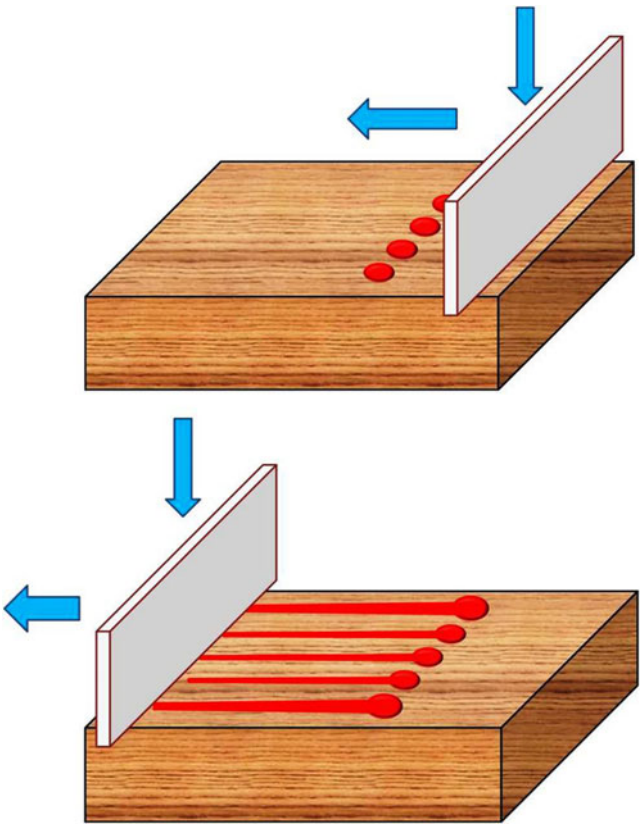

Fig.1 Schematic view illustrating the wiping process. The edge of a plastic bar wipes the deposited blood droplets with a total contact pressure of approximately 15 g/mm^2 (applied weight 3000 g)

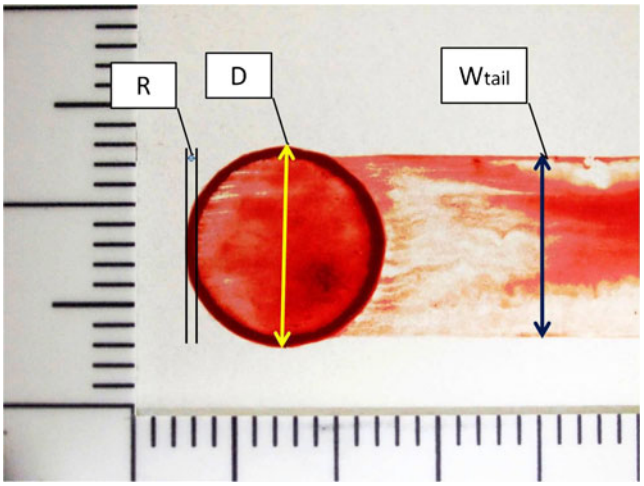

Fig. 2 Representative bloodstains (linoleum after 10 min) illustrating the morphological features recorded in the study (*D* droplet diameter; *R* ring thickness; W_{length} smear tail length; W_{tail} smear tail width)

further evaluation of wipe parameters. Venous blood samples were continuously taken from healthy aesculapian donors, allowing for a storage time of blood samples of less than one minute. Blood samples were analyzed for standard blood parameters (standard hemogram), which showed no alterations, prior to experimentation. To simulate drops from a relevant height, e.g., an upper limb in a neutral position, blood droplets were then dropped on the respective surfaces from a height of 0.65 m using a butterfly cannula fixed to a bracket to ensure a standardized droplet size of approximately 25 µl (resembling a droplet originating, e.g., from a sharp or pointed object). To yield a statistically relevant quantity of data, a total of 1,978 blood droplets were dropped on the respective surface units and wiped manually (contact pressure approximately 15 g/mm^2, applied weight 3,000 g) after the respective time periods using the straight edge of a plastic bar (Fig. 1). For each experimental sample, five independent examiners re-enacted activities at a crime scene, such as bloody surface contact with parts of objects, limbs, or clothing. These experiments yielded contact pressures during the wiping process ranging from approximately 10-20 g/mm^2. Applying minimum and maximum pressures within this range showed no discernable effect on the examined properties as compared to the standardized contact pressure. To guarantee a reproducibility of the applied forces, the wiping examiners were trained using a weighing scale until they were able to apply a constant and reproducible total contact pressure of 3,000 g±10 %.

Continuous monitoring of room temperature (surface, air at a height of 0.1 and 2 m) was carried out using an iButton temperature logging system (Maxim Integrated Products, Sunnyvale, CA, USA). Furthermore, humidity was analyzed with a humidity-testing instrument (Testo 615, Testo AG, Lenzkirch, Germany). Experiments were conducted in an enclosed working area without relevant air flow.

We examined drying time periods at three different ambient temperatures (15, 20, and 24 °C) resembling unheated, moderately heated, and rather warm room temperatures. To elucidate a possible influence of anticoagulant treatment on drying properties, three healthy individuals (physicians involved in the experiments) treated themselves with acetylsalicylic acid (Aspirin® 500 mg), heparin (Fragmin P ®), and clopidogrel (Plavix 75 mg) respectively, for at least 3 days, ensuring significant anticoagulation [30, 31].

Morphological features (droplet diameter, skeletonization, skeletonization ring thickness, maximum wipe tail width) were measured using a caliper (Fig. 2) and documented with a digital camera system (DSLR E-510, Olympus Europa GmbH, Hamburg, Germany). In a second step, the same parameters were measured digitally by two independent observers based on the digital image documentation.

In this study, the "drying" time was defined as the point in time, at which a wiping attempt did not lead to a macroscopically visible smear, albeit the physical drying process may or may not be finished at that time. Although the "drying" time is given as a specific point in time (e.g., 60 min.), it resembles the time period elapsed since the last wiping attempt (e.g., 46–60 min).

Statistics

Statistical analyses of the data were carried out using the software program Statistical Package for the Social Sciences (SPSS ® V.18.0).

Analysis of variance was used to detect significant differences in bloodstain drying times on different surfaces. In

Table 2 Descriptive statistics of blood droplets on different surfaces at ambient temperature of 20 °C

Surface	Mean [mm]	*n*	Std. deviation	Minimum [mm]	Maximum [mm]
Glass	12.96	123	.936	10.15	14.55
Linoleum	11.94	120	.855	9.82	13.48
Mirror	12.33	123	.980	9.45	14.59
Tile	13.72	124	.824	10.50	15.62
Wood	12.71	124	1.014	9.65	14.50
Total	12.74	614	1.101	9.45	15.62

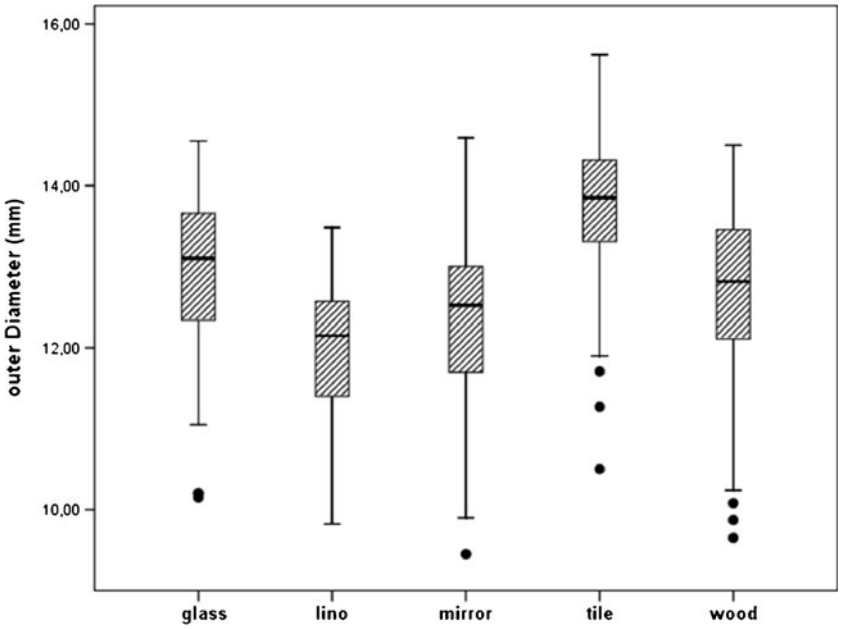

Fig. 3 The *box-and-whisker plots* (Fig. 2) depict a graphical statistical summary of the different surface materials, margin upper and lower quartiles, and the outliers of droplet diameter. Droplet diameters vary between upper and lower quartile range from 11.8 to 14.2 mm. After ensuring the normal distribution of data via Kolmogorov–Smirnov test variance analysis was performed (F=64.8, p<0.0001)

addition to the maximum and minimum drying times (t_{max} defined as the 100th percentile; t_{min} defined as the point in time when a wipe of at least one drop was no longer visible), 80th- and 90th-percentiles of "dried" droplets were also computed for each surface.

Multiple linear regression analyses were carried out between time (t), thickness of wiped droplet skeletonization (ring), and blood wipe tail width and length.

Assessment of interobserver differences of the metric measurements was based on repeated measurement conducted by two observers on the digital image documents for all metric parameters.

Results

In the present experimental setup, altogether 1,978 blood droplets were evaluated. Measurements at an ambient temperature of 20 °C (n=614) yielded droplet diameters ranging from 9.45 to 15.62 mm and exhibited low standard deviations between 0.82 and 1.10 (Table 2). The distribution of droplet diameters was similar for all surface materials (Fig. 3). Normal distribution of the data was confirmed via Kolmogorov–Smirnov test. Variance analysis showed no statistically significant differences between the different surfaces (F=64.8, p<0.0001).

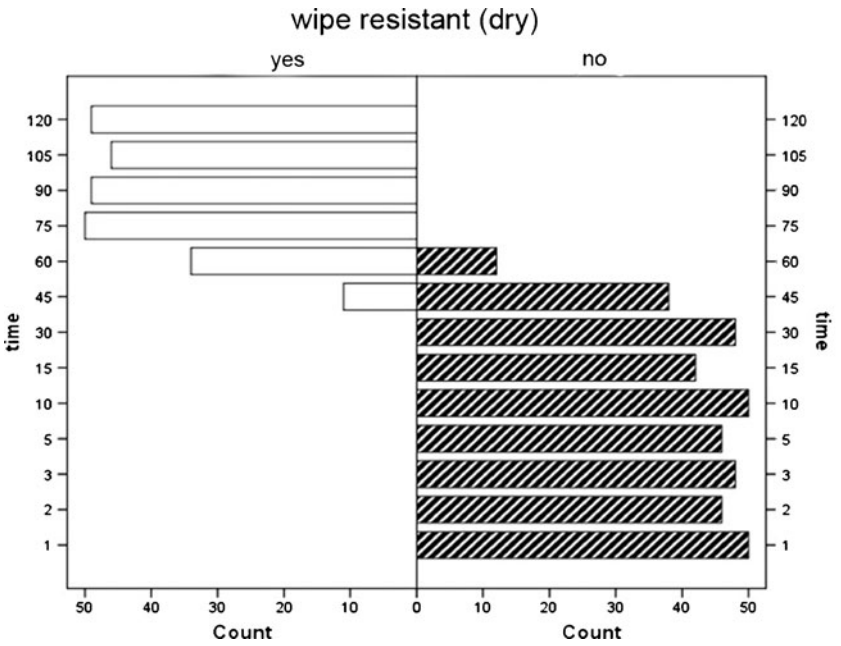

Fig. 4 Note the change of the rate of wipe-resistant (=dry) droplets between 45 and 60 min after droplet deposition (at ambient temperature of 20 °C). *Count* number of droplets

Table 3 Percentiles (80th, 90th, and 100th) showing the percentage of "dried" (smear resistant) droplets on different indoor surfaces at ambient temperature of 20 °C

	Percentage of "dried" droplets		
	80 % (min)	90 % (min)	100 % (min)
Linoleum	75	75	75
Wood	45	60	60
Glass	60	60	75
Mirror	60	75	75
Tile	60	60	60

At an ambient temperature of 20 °C, more than 75 % of all droplets on lineoleum were "dry" within 60 min and nearly 100 % within 75 min after deposition (Fig. 4). All other surfaces showed wipe resistance in more than 95 % of the droplets after 60 min already. As far as the effect of surface materials is concerned, comparison of the 80th, 90th, and 100th percentiles of "dried" (wipe-resistant) blood droplets resulted in significant differences only between linoleum and wood (Table 3).

An increase of the ambient temperature to 24 °C caused a reduction of the minimal drying time to 30 min for all surface materials, whereas a decrease of the ambient temperature to 15 °C lead to a minimum drying time of 75 min (wood) to 90 min. Furthermore, maximum drying times showed values of up to 45 to 60 min at 24 °C and >120 min at 15 °C, respectively (Table 4).

With respect to the morphological features, skeletonization ring thickness increased over time, whereas wipe length initially did not show a strong correlation with time (t). Beginning after approximately 10 min, the wipe length decreased over time. Moreover, wipe tail width decreased over time but was broader than the droplet's inner diameter within the first 30 min (Fig. 5). Independent from surface or ambient temperature skeletonization of droplets was discernible after no later than 5 min. At an ambient temperature of 24 °C, 90 % of droplets showed skeletonization after 1 min on all surface materials. Skeletonization ring thickness and time showed a strong correlation, however, with an increasing variability over time. This variability became particularly pronounced when the skeletonization ring covered more than 50 % of the total droplet area (Fig. 6).

Table 4 Wipe times frames (ω) on five different surfaces at three different ambient temperatures

	Ambient temperature	15 °C ω_{15} (min)	20 °C ω_{20} (min)	24 °C ω_{24} (min)
Linoleum	Min	90	45	30
	Max	>120	75	60
Wood	Min	75	30	30
	Max	>120	60	45
Glass	Min	90	45	30
	Max	>120	75	60
Mirror	Min	90	45	30
	Max	>120	75	75
Tile	Min	90	45	30
	Max	>120	60	45

After a stepwise analysis of the significance of a parameters' influence on the estimation of time elapsed since formation, the parameter wipe tail length was excluded, whereas skeletonization ring thickness and wipe tail width were included. Based on an ambient temperature of 20 °C linear regression equations were calculated for the respective surfaces (Table 5).

Anticoagulant treatment with acetylsalicylic acid (Aspirin®), heparin (Fragmin P®), or clopidogrel (Plavix®) over a period of 3 days did not cause any changes of minimum and maximum drying times (Table 6).

Discussion

In the present study, drying properties and wipe characteristics of small blood droplets were analyzed on five different indoor surfaces and in three different ambient temperatures. Parameters such as skeletonization ring thickness, wipe length, and wipe width were examined after standardized wiping. The main goal of the study was to examine the time period in which typical small droplets of 25 μl "dry" to the degree, that wiping will not lead to a visible smear. Furthermore, the experiments were conducted to clarify more precisely, whether morphological or metric features of wiped droplets would aid in calculation of the time period since bloodstain origination.

At an ambient temperature of 20 °C, analysis yielded comparable results for drying times on various indoor surfaces with drying times ranging from 45 to 75 min. The influence of ambient temperature was more distinct than expected. A decrease of 5 °C led to an increase in maximum drying time of up to 70 %, which underlines the importance of accurate and continuous temperature recording at a crime scene.

Comparison of five different surfaces revealed a significant difference only between linoleum and wood surfaces ($p<0.02$), which may be explained by the greater absorptive capacity of wood. Blood droplet size was independent from surfaces as demonstrated by applying variance analysis ($F=64.8$, $p<0.0001$).

The results of this study prove that a reciprocal estimation of the time interval between bloodstain origination and manipulation is possible using a regression formula, if ambient temperature data and metric parameters of wiped

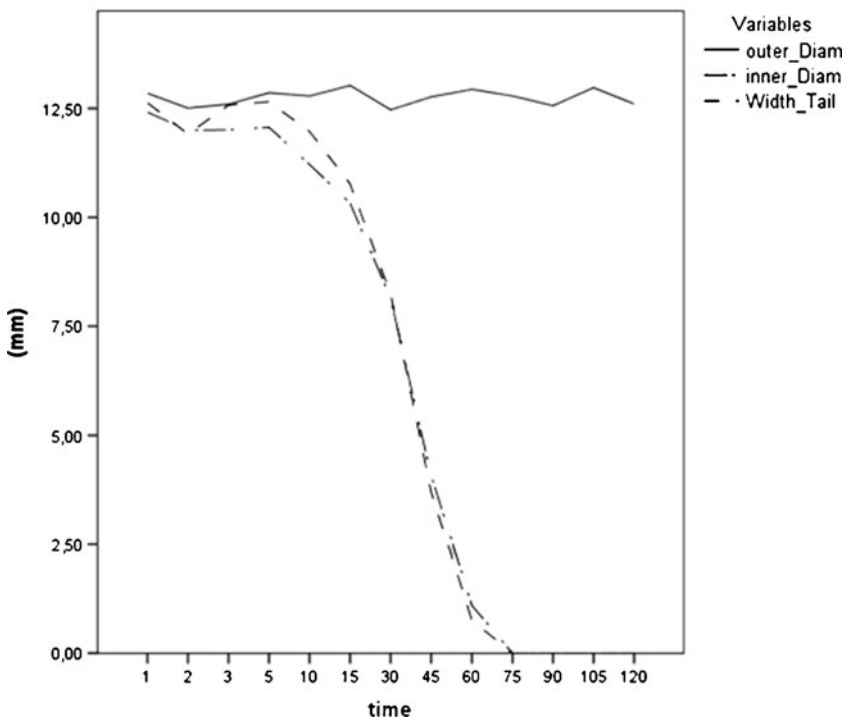

Fig. 5 Correlation diagram showing the influence of time on different wipe characteristics (on the lino surface). Note that wipe tail width is broader than the inner droplet diameter between 2 and 30 min after deposition

stains as well as information about surface materials are available. Nevertheless, error ranges of a residual standard deviation of approximately ±15 min demonstrate the limited value of a formula-based approach. Furthermore relevant changes of skeletonization ring thickness as well as tail characteristics occur in the short time frame between approximately 30 and 75 min after bloodstain origination.

The following two examples based on measurements originating from this study (linoleum surface at 20 °C ambient temperature) may demonstrate the possibilities and limitations of a regression approach:

A bloodstain wiped 30 min after its formation showing a ring thickness of 4.7 mm and a tail width of 7.9 mm has an estimated age of 32 min (20.2–

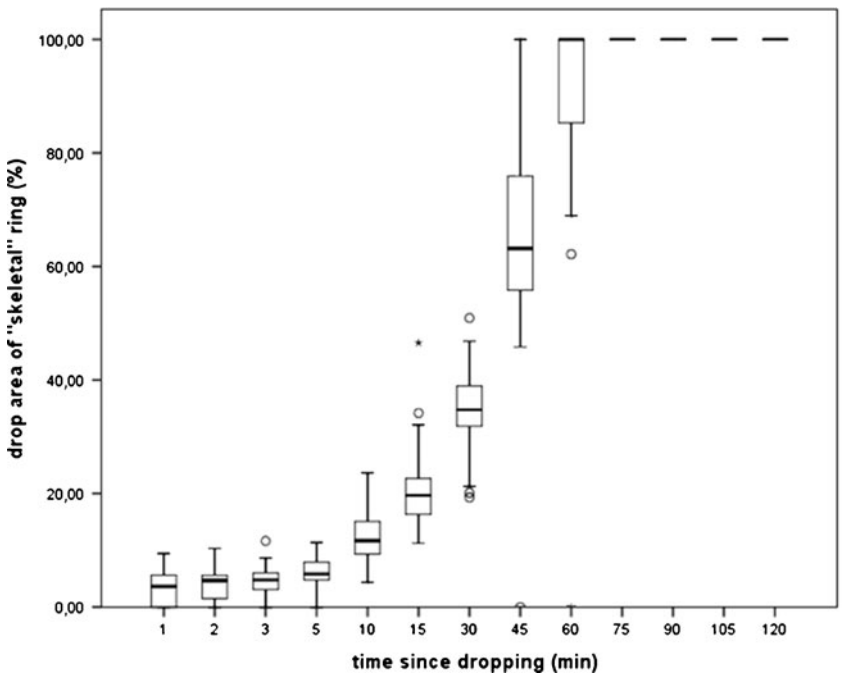

Fig. 6 *Box plots* illustrating strong correlation between skeletonization ring thickness and time. Note the increasing spread of drying areas after 30–45 min or when the percentaged drying ring zone exceeds approximately 30 %

Table 5 Multiple regression equations for each surface

material	Coefficient W_{tail}	coefficient R	*b*	Residual SD	*p*
Glass	−5.05	1.43	69.94	15.01	<0.001
Wood	−2.44	4.08	31.08	17.43	<0.001
Mirror	−3.16	4.33	37.86	13.51	<0.001
Tile	2.00	8.43	−30.76	13.98	<0.001
Linoleum	−4.85	3.00	56.48	12.72	<0.001

W_{tail} width of the wipe tail, *R* droplet ring thickness, *SD* standard deviation. Example $Time_{glass}=-0.505\times W_{tail}+1.43\times R+69.94$

44.3 min),which resembles the real time frame. In the second example, a 3-min-old blood droplet is wiped without a visible skeletonization ring (R=0 mm) and with a tail width of 9.4 mm. The calculated age is 10.8 min. The calculated time frame (0.0–22.9 min) does include the real age but at least the upper side of the calculated time-range does not appear plausible, since a skeletonization ring will always have formed within the first 5 min.

As a consequence, the respective findings should be interpreted with caution, when an estimation of drying times is required in a specific forensic case of bloodstain pattern analysis. Nevertheless, the results of this study may enable the forensic expert to assess time frames, if wiped bloodstains attributed to the initial course of action are present on a crime scene.

On the other hand, these results may also be helpful to clarify whether wiped or still wipeable stains may be included in or excluded from bloodstain pattern analysis (e.g., transport artifacts on stairs).

Taking into account that anticoagulant treatment had no selective effect on the drying times, ambient temperature, which regulates water evaporation, seems to be the main determinant of the drying process of similar blood droplets [28]. Even though our results clearly demonstrate that ambient temperatures influence drying times, it remains to be clarified what effect varying droplet sizes, amount of blood or the influence of individual blood parameters (e.g., hemoglobin, hematocrit) that might differ from normal physiological value ranges, might have on drying behavior.

In synopsis, the results of the present study confirm expectations resulting from practical casework and provide a scientific basis for future case assessments regarding wipe characteristics, and thus drying times, which underline the necessity for systematic experimental examinations in the field of bloodstain pattern analysis.

Table 6 Data of different parameters demonstrates the independence of bloodstain drying properties from anticoagulant treatment

	Untreated blood	Aspirin®	Fragmin P®	Plavix®
20 °C, linoleum				
Diameter (mean; mm)	11.94	12.34	13.36	13.01
ω_{min} (min)	45	45	45	45
ω_{max} (min)	75	75	75	75
20 °C, wood				
Diameter (mean; mm)	12.71	12.59	12.52	12.02
ω_{min} (min)	30	30	30	30
ω_{max} (min)	60	60	60	60
20 °C, glass				
Diameter (mean; mm)	12.96	12.41	14.55	2.44
ω_{min} (min)	45	45	45	45
ω_{max} (min)	75	75	75	75
20 °C, mirror				
Diameter (mean; mm)	12.33	13.62	13.08	11.29
ω_{min} (min)	45	45	45	45
ω_{max} (min)	75	75	75	75
20 °C, tile				
Diameter (mean; mm)	13.72	12.67	12.45	14.34
ω_{min} (min)	45	45	45	45
ω_{max} (min)	60	60	60	60

ω time until droplets became wipe-resistant

Disclosure/conflict of interest We have no conflict of interest to declare.

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