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A Preliminary Assessment of the Correlation of Drying Time and the Peripheral Rim Thickness of Perimeter Bloodstains

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Abstract

Bloodstain patterns can be used to attempt to sequence events that occurred during a bloodshed event at a crime scene. Perimeter bloodstains may be useful for sequencing as they can indicate movement or the presence of some interacting object between the time that the blood was initially deposited onto its target surface and when it dried completely. The purpose of this series of experiments was to assess the reliability of perimeter bloodstains as an estimation tool for bloodstain drying time as well as to compare the properties of perimeter bloodstains on different target surfaces. After measuring over 600 bloodstains, it was determined that there was a significant difference in the peripheral rim widths of perimeter bloodstains of different drying times under the conditions of this experiment. It was also determined that similar peripheral rim width characteristics are seen on both cardboard and glass surfaces, however, the diameter of the same volume deposited on both surfaces is smaller on cardboard than on glass.

Keywords: Forensic science; Perimeter stain; Peripheral rim; Drying time; Substrate; Bloodstain patterns

Introduction

Blood is a common body fluid that is often found at the scenes of violent crimes [1]. The analysis of blood and the patterns that it creates is a powerful investigative tool in forensic science and often provides information pertinent to crime scene reconstruction [2-4]. The presence of perimeter bloodstains, the focus of this study, can provide important information about the possible sequence and chronology of events that occurred during the commission of a crime [5].

There are many factors that can affect the drying of blood. Temperature, humidity, and airflow are among these factors [6]. As temperature increases, a bloodstain will dry faster. As humidity increases, a bloodstain will dry slower. Increased airflow can speed up the drying time of blood. As a bloodstain increases in size or volume of blood, the stain will take longer to dry completely [6]. The target surface and its porosity can also affect drying time. Numerous studies have been conducted to assess how these and other factors affect the drying of blood to better understand the behavior of blood at crime scenes [7-12]. Most of the studies focus on whole bloodstains, though, with only one article directly addressing perimeter bloodstains [13].

Blood begins to dry approximately 50 seconds after it has been deposited [13]. The center of a blood droplet or pool is the last part to dry as blood droplets dry from the exterior region to the interior or central region. A perimeter bloodstain, or skeletonized bloodstain, occurs when the wet central area of the bloodstain has been removed leaving behind the dried outer edges of the stain by an action such as a wiping motion. Blood droplets that exhibit flaking in the central region with an intact outer edge are also considered perimeter bloodstains. The flaking of dried blood is most commonly seen on a smooth surface or a surface that has a greasy film [5]. If a perimeter stain is present, then some sort of disruptive activity had to have occurred between its initial deposition and the completion of the drying process of the bloodstain. The amount of skeletonization, or the thickness of the edge characteristics of the stain's perimeter, can possibly corroborate or refute a statement given to the police. For example, multiple stains were found at a crime scene, some of which showed skeletonization. The questioned individual claimed that he came into first contact with the blood upon discovering the bodies. However, the level of skeletonization indicated that contact more than likely occurred at an earlier time, closer to the time of bloodstain deposition. This information refuted his story and served as valuable evidence in the case. Understanding the characteristics of perimeter bloodstains, the relationship between the peripheral rim width and drying time, is important in being able to form correct conclusions about the stain and the events that affected it.

The dried edges of a perimeter stain are often referred to as its peripheral rim [5]. A relative time frame can be established because the thickness of the dried portion of the stain can be correlated with time elapsed since the blood was initially deposited. This research proposes to assess the reliability of using the dried peripheral rim width as an indicator for time since deposition as well as assess the effect of the target surface on the peripheral rim width of perimeter bloodstains.

Materials and Methods

The dimensions of perimeter bloodstains were analyzed over 8 different time periods. The time periods refer to the time between the deposition of the blood on the surface and the wiping of the bloodstain. The time periods were 2, 3, 4, 5, 10, 15, 20, and 25 minutes and were chosen based on preliminary testing which showed measurable edge characteristics starting at approximately 2 minutes on a glass surface at room temperature. Human venous blood samples were collected from a volunteer by a licensed phlebotomist after

University of New Haven IRB approval and informed consent. The samples were stored in BD Vacutainer™ Glass Blood Collection Tubes with K3 EDTA in a refrigerator at 4°C until ready for use. Blood samples were allowed to come to room temperature before testing began.

A Finnipipette by Thermo Lab Systems, 20-200 µL range, was used to deposit approximately 25 µL of blood 15 cm on a level, glass surface. The volume of blood was chosen to resemble a droplet originating from a pointed object such as a knife [13]. A total of 50 drops were created for each time period. When dispensing each drop of blood, the micropipette was clicked to the first stop and the button was depressed at a similar speed to maintain a relatively constant velocity of the blood droplet in its fall to the glass surface. The pipette tip was also replaced after every single drop was deposited in an effort to avoid the accumulation of blood on the tip between drops and maintain a more constant volume of blood in each drop.

Once the respective time elapsed after deposition, the droplet was wiped using a Kimwipe™ to create a perimeter bloodstain. Preliminary testing demonstrated that the dynamic, wiping motion did not significantly disrupt the peripheral rim of the perimeter bloodstain compared to a more static, non-wiping method. The wiping method was chosen because it more closely resembled a typical dynamic case scenario and made the peripheral rim more visible than the static method.

The temperature and humidity were monitored regularly using an Oasis® Digital Thermometer and Hygrometer to monitor these factors and their possible influence on the drying of the bloodstains. The experiment was also conducted in a laboratory hood to eliminate ambient room air current as a possible factor affecting drying time.

The dimensions of each blood droplet were measured using a Peak Scale Loupe 10X. Preliminary testing compared the CP8806-T Carrera Precision 6 inch Titanium Electronic Digital Caliper and scale loupe for measuring the peripheral rim of perimeter bloodstains. While both instruments produced similar results, the scale loupe was more practical because of its magnification feature and more defined scale. The overall diameter of the drop was measured as well as the peripheral rim width in 4 different locations on the drop. The measurements taken from each droplet are demonstrated in Figure 1. There were a total of 400 blood droplets that were analyzed across all time periods.

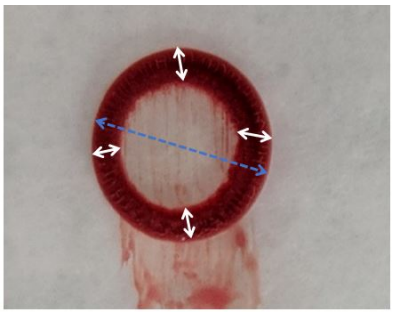

Figure 1: The measurements taken of each perimeter bloodstain are illustrated. The solid arrows represents the four measurements of the peripheral rim width and the dotted arrow represents the diameter measurement.

The characteristics of the skeletonized bloodstains were also analysed on different target surfaces. The substrates that were compared were glass, cardboard, and cotton t-shirt material. Approximately 25 µL of blood was deposited in the same manner as before onto the specific substrate from a height of 15 cm. The drops were wiped with a Kimwipe™ 10 minutes after the blood was deposited. The same characteristics of each blood droplet were measured as in the previous testing. The overall stain diameter and 4 different widths of the peripheral rim of each drop were measured using the scale loupe. There were 100 drops analyzed on each different target surface, a total of 200 bloodstains.

Results and Discussion

Comparison of bloodstain drying time

The edge characteristics of the perimeter bloodstains of different drying times were observed. A visualization of the perimeter bloodstains of the different time periods can be seen in Figure 2. The peripheral rim of each blood droplet that was wiped 2 minutes after deposition was measured in four different places; the compass points of the blood droplet. The same measurements were taken for all the time periods after 2 minutes as well. An average was taken from all 4 measurements of each of the 50 drops for each time period. The bloodstains that were wiped after 2, 3, and 4 minutes have the same minimum width measurement, 0.1 mm. The bloodstains that were wiped after 3 and 4 minutes shared a maximum measurement of 0.5 mm. The bloodstains wiped after 20 and 25 minutes also had the same maximum width measurement of 2.8 mm.

QuickCalcs by GraphPad Software, Inc. was used to conduct unpaired t-tests to compare the mean of the peripheral rim width of the bloodstains from each time period. The average width for 2 minutes was compared to the average for 3 minutes; the average for 3 minutes was compared to the average for 4 minutes, and so on for all time periods. A p-value less than 0.05 indicated a significant result. A comparison between the time periods all gave a p-value less than 0.0001, which demonstrates that there is a statistically significant difference between the peripheral rim widths of the perimeter bloodstains for all time periods.

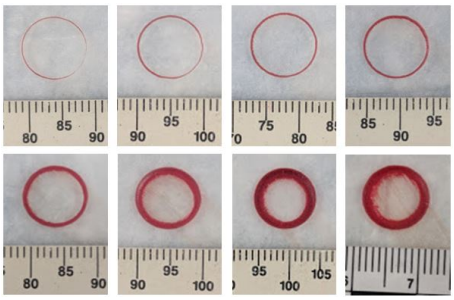

Figure 2: Photographs representative of the appearance of the perimeter bloodstains from each time period (top row from left: 2, 3, 4, and 5 minutes, bottom row from left: 10, 15, 20, and 25 minutes). The time period represents the time elapsed between the deposition of the blood droplet and when it was wiped. These photos were taken with a Google Pixel™ smartphone camera.

Figure 3 shows that as the time between deposition and wiping increased, the peripheral rim also increased, as is expected. The R^2 value of 0.9728 indicates that the trend between time and peripheral rim width is relatively linear. This trend is expected because as the bloodstain dries over time, the thickness of the dried edge of the bloodstain will become larger until eventually the entire stain is dry.

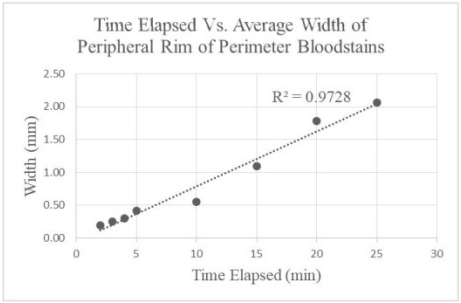

Figure 3: Results of the analysis of the peripheral rim of perimeter bloodstains that were wiped after drying for 2, 3, 4, 5, 10, 15, 20 and 25 minutes. An average was taken of the width measurements for each blood droplet examined. The R2 value is shown.

The standard deviation values for the diameters of the bloodstains within each time period were low, indicating that the diameters varied little between the data points within each time trial. The minimum average diameter of the bloodstains was 8.4 mm and the maximum was 10.3 mm, with a difference of 1.9 mm. QuickCalcs by GraphPad Software, Inc. was used to conduct unpaired t-tests to compare the mean diameter for each time period. A p-value less than 0.05 indicated a statistically significant difference. The results of the t-tests are shown in Table 1. There was a significant statistical difference between the diameters of the bloodstains for almost all the time periods. No significant statistical difference was found between the diameters of the bloodstains that were wiped after 10, 15, 20 and 25 minutes since their p-values were greater than 0.05.

	Time Elapsed (min)						
	3	**4**	**5**	**10**	**15**	**20**	**25**
2	0.0261	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
3		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
4			<0.0001	0.0004	0.0014	<0.0001	0.0014
5				<0.0001	<0.0001	<0.0001	<0.0001
10					0.8769	0.3931	0.0993
15						0.543	0.1206
20							0.0084

Table 1: The resulting p-values for the t-tests of the average peripheral rim widths of perimeter bloodstains wiped after 2, 3, 4, 5, 10, 15, 20, and 25 minutes, when compared against each other. A p-value less than 0.05 indicates a statistically significant result.

Theoretically, the diameter of all bloodstains in this experiment should have been the same. The volume of blood was kept constant at 25 µL, the height from which the blood drop was deposited was kept constant at 15 cm, and for the time period analysis the target surface was always glass. These factors have been shown to affect the diameter of bloodstains in previous research. In this experiment, the diameters of the bloodstains for the time analysis were found to be significantly different statistically by t-testing. The difference in diameter may be explained by the variability in the blood itself. For example, hematocrit was not measured which could have affected the behaviour of the blood as it interacted with the target surface. Another factor that may have caused the differences in diameter may be the environmental conditions such as ambient temperature and humidity. Temperature and humidity were monitored, but not controlled very stringently during experimentation. It has been shown that temperature variations as small as 4°C will cause discernible changes in droplet drying time [13]. More studies are needed to assess the effect of additional factors such as humidity on the drying properties of bloodstains. It is unclear whether diameter of the bloodstain has an effect on the edge characteristics of perimeter bloodstains. It may be possible that bloodstains of the same volume with different diameters have different drying times as the volume to surface area ratio of the blood is changed.

The variation in the diameters of the bloodstains further demonstrates the limitations to using measurements of the bloodstain to predict the drying time. Even under controlled conditions, the diameter was not constant. Since the conditions of crime scenes as well as the conditions of the blood itself are so varied, trends in diameter or peripheral rim width according to drying time of the stain are very hard to define.

Figure 4 shows box-and-whisker plots generated by BoxplotR, an open-source application. It includes the average peripheral width data from all time periods studied. The graphical summary shows that the widths for time periods 2-10 minutes are similar in their distribution. The widths for time periods 15, 20, and 25 minutes have a larger spread.

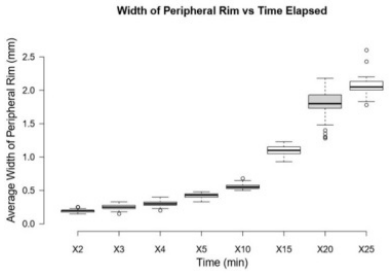

Figure 4: Graphical summary of the spread of peripheral rim measurements of perimeter bloodstains for each time period including upper and lower quartiles and mean.

Although the curve seems to be exponential, it is important to note the time increments are not the same all the way through. However, there does appear to be a larger difference between the measured peripheral rim width from 15-20 minutes and 10-15 minutes, as compared to 20 and 25 minutes and the smaller time periods. There is overlap between the peripheral rim width ranges for time periods 2-5 minutes. The difference in the data between 15 and 20 minutes is greater than the difference between 20 and 25 minutes. The data for the 15-minute time period appears to be more evenly distributed,

while the distribution for 20 and 25 minutes is more irregular with some outliers (Figure 4).

The peripheral rim width may be a potential indicator for bloodstain drying time, however, not with a high level of accuracy. The overlap seen in the data of the average width of the peripheral rim of bloodstains that were wiped 2, 3, 4, and 5 minutes after deposition demonstrates that the deviation of the average of the widths is too great to be able to differentiate between these time periods. In other words, if the time between deposition and wiping of a bloodstain were approximated, the approximation would be in a 2 to 5 minute range. Even though the t-tests showed that there was a significant difference between the average widths of the different times, the values are still too close to be differentiated. In some instances, there were individual outliers observed which appeared to overlap different time ranges. As the time elapsed increased, there appeared to be more of a sharp difference in the average of the peripheral rim widths (Figure 4). This propagation of measurement results is similar to what is observed when measuring the width and length characteristics of a spatter droplet for angle of impact calculations; those stains in the approximate 90° to 60° range appear to have a minimally observable difference when attempting to record a diameter measurement. It is only when the stain becomes more elongated (smaller calculated angle of impact) does both the accuracy and precision of the measurement improve. The analogous scenario can be seen here in Figure 4 in the 2 to 10 minutes range. There is an apparent large amount of overlap in the calculated ranges. However, from 10 to 20 minutes there is no appreciable overlap allowing for an increased confidence in using peripheral rim width as a correlating "marker" to establish drying time.

Comparison of target surfaces

The edge characteristics of the perimeter bloodstains were observed on glass, cardboard, and cotton target surfaces. The cotton surface was not conducive to the development of perimeter bloodstains, so data was not collected from the cotton substrate. When the blood was deposited onto the t-shirt material, the blood absorbed into the substrate within seconds and the wiping motion had no effect on the bloodstain. The reason why a perimeter stain is not able to form on a cotton surface is likely due to the space between the fibres of the material and absorbency of the fabric. Once the blood is absorbed, there is no longer the necessary liquid portion that can be disrupted to form a perimeter stain. Alternatively, perimeter stains are formed on glass and cardboard surfaces because of their non-porosity/porosity, respectively. With a portion of the blood droplet volume remaining on top of their surface, there is material able to retain the stain shape, as well as providing a volume to be displaced after drying begins. The components that make up glass and cardboard are in a much more compacted and rigid structure, providing these surfaces with varying levels of absorbency. Figure 5 shows the appearance of the bloodstain on the cotton material compared to the glass and cardboard substrates.

A summary of the peripheral rim widths of the stains on the glass and cardboard surfaces as shown in Table 2. A summary of the diameters of the bloodstains on both surfaces is also shown in Table 2. An unpaired t-test was performed between the two data sets and the p-value was found to be 0.095 indicating the difference in the means of the peripheral rim widths of the bloodstains on glass and on cardboard were not statistically significant. A graphical representation of the data is shown in Figure 6. The box-and-whisker plot shows the similarity in the peripheral rim widths observed on both surfaces under the specific conditions of the experiment.

In comparing the characteristics of perimeter bloodstains that were deposited on the glass and cardboard target surfaces under the conditions of this experiment, it can be determined that the characteristics are similar. The box-and-whisker plot (Figure 6) shows the similarity between the data sets and t-tests also demonstrated that there was not a statistically significant difference in the peripheral rim widths of the perimeter bloodstains deposited onto these two surfaces. Further research is needed to identify the effects of other target surfaces on the edge characteristics of perimeter bloodstains.

Surface	Average Width of Peripheral Rim (mm)	SD of Widths (mm)	Min Width (mm)	Max Width (mm)
Glass	0.87	0.109	0.6	1.2
Cardboard	0.85	0.101	0.6	1.1

Table 2: A summary of the edge characteristics of the perimeter bloodstains on glass and cardboard substrates wiped after 10 minutes. The average width of the peripheral rim refers to the average of all measurements on that surface (4 measurements for each drop).

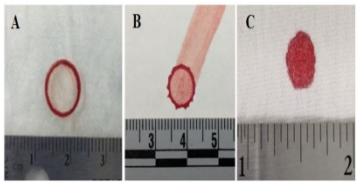

Figure 5: 25 µL of human blood deposited from 15 cm onto (A) glass, (B) cardboard and (C) cotton target surfaces and wiped after 10 minutes. These photos were taken with a Google Pixel™ smartphone camera.

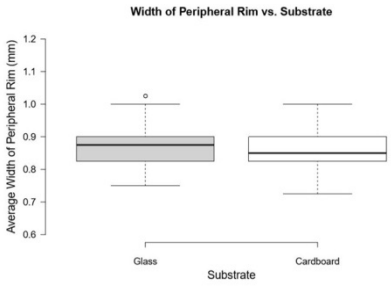

Figure 6: Box-and-whisker plots of peripheral rim measurements of perimeter bloodstains on each target surface showing a graphical summary of the data, upper quartile, lower quartile and outliers.

The average diameters of the bloodstains on the two target surfaces are shown in Table 3. The difference between the average diameter of the bloodstains deposited on the glass surface and the average of the diameters of the bloodstains deposited on the cardboard surface is 0.9 mm. An unpaired t-test was performed on the two sets of data and the p-value was less than 0.0001, which indicates that there is a statistically

significant difference between the diameters on the glass and cardboard surfaces. An explanation for the difference may be the difference in the composition of the materials. Cardboard is porous while glass is not. Therefore, some of the blood may have absorbed into the cardboard surface instead of spreading out on top of it. On the glass, the blood is not readily absorbed, so the volume spreads primarily on top of the contact surface. The larger the volume of blood on the contact surface, the larger the resulting stain's diameter.

Surface	Average Diameter of Drop (mm)	SD of Diameter (mm)
Glass	9.9	0.53
Cardboard	9	0.33

Table 3: A summary of the diameters of the perimeter bloodstains on each substrate and the standard deviation value of the diameter.

Conclusion

The results of this research demonstrate the limitations of estimating drying time of perimeter bloodstains using the width of the peripheral rim. Under controlled, specific conditions, the data showed that there were distinct ranges of peripheral rim widths of perimeter bloodstains with different drying times. The distinction between the 10, 15, and 20 minutes times was especially prominent. Time estimates for the origin of perimeter bloodstains, even under such controlled conditions, have to be made with great caution. However, since crime scene conditions vary greatly and represent very dynamic scenarios, it is very likely that this approach cannot be used reliably.

Further research is warranted to assess the effects of blood volume and environmental factors such as humidity, as well as other target surfaces, on the peripheral rim width. Also to be considered in possible future experiments would be factors associated with the blood source itself; properties such as hematocrit, diluted blood, and where the sample comes from, in particular blood containing EDTA (purple top tubes) versus those without coagulant (red top tubes). The results above clearly establish that even under controlled conditions attempting to use peripheral rim thickness as an accurate drying time indicator at the crime scene is not recommended.

Under the conditions of this study (25 μL volume of blood, 15 cm drop height, air temperature of 20-25°C) the peripheral rim widths of perimeter bloodstains observed on the glass and cardboard target surfaces were very similar (Table 2). Alternatively, the diameter of the perimeter bloodstains on both surfaces was significantly different (Table 3). The diameter of the perimeter bloodstain on the cardboard surface was smaller than that on the glass surface, which can be explained by the absorbency of the cardboard. Since the glass surface provides no absorbency, the blood volume is primarily displaced in a lateral direction, thereby increasing its overall stain diameter. The differences in stain diameter seen on the different substrates did not seem to have an effect on the width of the forming peripheral rim as time elapsed; drying time was similar for the bloodstains on both surfaces. Further research is needed to assess the effect of different substrates on the formation of perimeter bloodstains.

Disclaimer

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