



General forensics

Bloodstain pattern dynamics in microgravity: Observations of a pilot study in the next frontier of forensic science

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ABSTRACT

As humanity advances into a space-faring species, the risk of injury by multiple means and intentions will follow. Expanding understanding of how forensic science adapts to extraterrestrial environments is a novel and inevitable expansion into the next forensic frontier. This study considers the unique challenges of bloodstain pattern analysis in microgravity environments. Specifically, observation in novel experimentation aboard a parabolic flight research airplane which yielded fluid dynamic behaviors in a microgravity environment that provides practical understanding of Earth-based and off-world bloodstain applications.

Introduction

As humanity continues to develop into a space exploration species, we can assume that our risk of injury by multiple means and intentions will follow. Therefore, expanding the understanding of how contemporary forensic sciences translate into extraterrestrial environments is a novel and inevitable discipline expansion into the next forensic frontier. This investigation considers the discipline of bloodstain pattern analysis (BPA) in a gravity-altered (reduced) environment or an environment that exhibits gravity at a degree other than Earth-normal. Contemporary BPA reconstruction relies on the measurements of individual bloodstains to determine the flight path of the blood droplet. Within this reconstruction, the analyst is determining three key metrics: the angle at which the blood drop struck the surface (known as the angle of impact), the two-dimensional area in which the stains from an overall pattern intersect (the area of convergence), and the volume in space at which the stains intersect (the area of origin). These three metrics assist in determining where liquid blood originated, an essential aspect of event reconstruction. By removing the influence of gravity, we can isolate the principal equation of the angle of impact estimation and therefore better understand the independent forces acting on bloodstains in terrestrial environments.

Space medicine literature and research illustrate the extreme reactions the body and physical systems undergo when subjected to an

altered gravity environment. Bone density loss, fluid shift within the body, and muscle atrophy are just a few examples of the changes experienced in the loss of gravitational forces [1]. Consider the example of an astronaut bleeding in microgravity. Due to the lower venous blood pressure and the absence of any gravitational force, blood will form a liquid dome around the injury site, with surface tension driving the blood's adherence to the surrounding tissue [1]. However, if the injury causes an arterial breach, there would be sufficient pressure to eject the liquid into a stream of droplets [2]. This fluid dome of venous blood will remain adhered to the surrounding wound tissues until there is motion to overcome the cohesive forces, or until an external force interacts with the fluid dome and causes shearing. In the absence of gravity, this liquid dome can grow larger than would be possible on Earth, with the motion of the mass being slower in directional movement [3]. This fluid dynamic action occurs independent of the blood's chemistry, hematocrit, or any alteration of the body's homeostasis. While blood's hematocrit and density characteristics fluctuate based on microgravity exposure duration, previous literature demonstrates that hematocrit remains within normal ranges observed on Earth (40–45% in men and 36–48% in women) [4,5]. This makes the experimental comparison between the environments even less complex.

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Bloodstain pattern analysis in space

Where the inception of BPA research was conceived from 19th-century forensic medicine, the future application of BPA in extraterrestrial environments will evolve from the research of 21st-century space medicine. Early astroforensic research within BPA can aid in at least three applications: (1) the investigation of eventual violent criminal acts that occur outside of Earth's environment, (2) accident reconstruction onboard space station/starship platforms, and more immediately applicable by (3) providing a better understanding of blood flight and deposition action terrestrially in the reduction or absence of gravitational forces. [Note: Moon = 0.166 g, Mars = 0.378 g, Earth orbit = 10^{-6} g].

The foundational mathematical relationship in BPA is the ratio between the stain's width and length as a function of the angle at which the drop struck the surface. This angle is a crucial step in any reconstruction of impact stain patterns and is reflected in the angle of impact equation (Eq. (1)), [6–8]. However, this core calculation fails to account for any environmental variable, including gravity or air resistance.

$$\begin{aligned} \text{Angle of Impact} &= \arcsin(\text{width} / \text{length}) \text{ Or expressed as } : \theta \\ &= \sin^{-1}\left(\frac{\text{width}}{\text{length}}\right) \end{aligned} \quad (1)$$

Therefore, a microgravity environment is an ideal testing ground for validating the equation's trigonometric function. The study of fluid dynamics within space environments is heavily tasked in engineering and biomedical development. Consequently, research data and observations exist that can be qualitatively examined for forensic application, such as the spherical shape of fluids in free flight or the compelling role of surface tension [2,9]. However, of previous research, the studies have been limited to the examination of blood within the body or attached to the body during hemorrhage. No research has been published relating to the behavior of blood in free flight or direct relevance to BPA. Therefore, the research question is, will blood behave in a predictable manner that permits accurate flight path reconstruction in a microgravity environment? This analysis proposes an answer to that question through physical data collection observations. At the core of the present pilot study, we hypothesize that if gravity is removed as an acting force on a blood drop in flight, then the calculated angle of impact will be more accurate.

Materials and methods

Equipment

Due to the limited flight time in microgravity and cargo space for materials, the experiment had to be as simplified as possible. To accomplish this, the experimental question was simplified: "How do angles of impact manifest in a microgravity setting?" This only required a blood analog, a target, and a method of setting the fluid into flight. Due to health safety concerns in the flight laboratory, this study utilized a synthetic blood analog with a solution derived from previous space hemorrhage medicine research conducted by Hayden et al. [10,11]. The blood analog consisted of a mixture of 40% glycerin and 60% red food coloring, simulating the relative density 1.06 g/mL and viscosity (3.4 cP at 115 s^{-1} , $23 \text{ }^\circ\text{C}$) of human blood. The fluid was delivered from a 1cc syringe that was manually depressed, with a 16.5 cm \times 16.5 cm target of 50 lb. white paper affixed to a foam board backing.

The experiments took place within a glovebox, adapted from the design of a standard pediatric incubator (47.0 cm \times 58.4 cm \times 54.6 cm) specifically for flight research. The researcher accessed the inside of the canopy through two pairs of arm access ports with attached sleeves to perform all in-flight activities. Foot straps to restrain the investigator were fastened to the baseplate of the glovebox and bolted to the aircraft

cabin's deck. In addition to the test chamber, target samples, blood analog, and syringe, an accelerometer, clock, thermometer, video camera, and absorbent material pads to absorb spills were secured inside the glove box.

Procedure

In research, microgravity environments can be achieved through drop towers, parabolic aircraft flights, and research stations in Earth's orbit, posing between 1 and 1/1000,000th% of Earth's gravity [1]. This research utilized the laboratory space of the parabolic aircraft flight, which is well established in the biomedical and fluid dynamics fields [12]. Experiments were conducted during a parabolic flight experiment series. The experiments were conducted aboard the Zero Gravity Corporation modified Boeing 727 aircraft capable of approximating microgravity by creating periods of reduced gravity between 0.00 and 0.05 g. The flight departed and landed at the Fort Lauderdale International Airport, FL, in coordination with the NASA Flight Opportunities program. The flight consisted of multiple parabolas flown in sets of five with periods of level flight between parabolic sets. Each parabolic arc resulted in approximately 15–20 s of microgravity. Our procedures were conducted during microgravity trajectories. Air drag is still a present force within the airplane cabin atmosphere, just as it would be on Earth or a spacecraft cabin; consequently, gravity is the only altered variable.

During the first and second experiments, a 1-cc syringe containing the blood analog was used to project the fluid in a stream on a flight path of approximately 20 cm length that would intercept with a 16.5 cm \times 16.5 cm target of the 50 lb. paper affixed to a foam board backing. While the angle of impact is not typically a primary consideration in projected patterns, for this experiment, we used the hydraulic pressure of a syringe to propel blood droplets toward a target, thereby creating an angle of impact for analysis. The syringe was only used to set the fluid into motion, with the variation in the amount of pressure conveyed into the syringe between data sets being negligible and having no direct influence on the scope of this study. This study focuses less on the cause of the bloodstains and more on how individual bloodstreams interact with a target to form measurable stains. From these stains, we can then reconstruct the angle of impact. At the start of each microgravity maneuver, the target was held at a chosen relative angle to the syringe, and the syringe plunger was then manually depressed. The first experimental set was conducted at a relative angle of 90 degrees between the target and the syringe. The second experimental set was projected at the relative angles of 45 and 55 degrees during two ejections between the target and syringe.

The experiments were recorded using a Nikon Key Mission 170 4 K action video camera mounted in the top of the glovebox. These video recordings were analyzed to qualitatively assess the fluid behavior on contact with the target during the period of microgravity. Still images were captured from the video to permit the measurement of the simulated bloodstains. Stains were measured in this manner due to the alteration of the stain's shape and size upon re-entry into a gravity-influenced environment. These images were then corrected for perspective distortion in Adobe Photoshop using the Lens Correction Filter under the Smart Objects tools. The images were manually corrected using the target as the known reference for vertical and horizontal correction. After Photoshop processing, the adjusted images were uploaded into FARO Zone 3D Expert (FARO Inc., Lake Mary, Florida) to conduct measurements of the stains. FARO Zone 3D Expert is a computer program used in crime scene analysis that allows the user to, among many other features, measure bloodstains and estimate the spatial reconstruction of bloodstain patterns. Measurements of the stain's width and length were collected using the program's BPA tool, scaling the image using the known dimension of the target sides (16.5 cm \times 16.5 cm) and then using the ellipse measuring tool to select individual stains. The width and length measurements were recorded into a Microsoft Excel spreadsheet where Equation 1 was used to

determine the calculated angle of impact.

Terrestrial one gravity (1 g) comparison data was collected using a similar methodology. Equine blood was measured in single drops onto the 50 lb paper target at 45° and 90° angles. Equine blood is an established substitute in BPA research and exhibits similar hematocrit ranges [13,14]. It is acknowledged that two different fluids were utilized within this study, and both are shown to be favorable human blood replacements for BPA research. Therefore, as this study examines observations of simulated blood behavior manifesting shape and sizes in microgravity, the limitation is noted but does not have a significant influence on the comparisons drawn between environments. This data was collected within the Roswell (Georgia) Police Department's Forensic Science Laboratory. The target surface was positioned at the desired angle on an adjustable surface, with the angle confirmed by a digital Leica Disto inclinometer. A laboratory bench stand clamp held a BrandZig 3 mL syringe with a 1" 23-gauge needle one meter above the target surface. The bloodstock, heated in a water bath to 37° Celsius, was drawn into the syringe, and then sets of ten drops were deposited onto sheets of the paper target surface. The needle was wiped between drops, and the syringe was replaced between angle sets to prevent the buildup of blood material that may alter the initial drop size. After deposition onto the target, the stains were photographed using a Fuji XT1 DLSR camera and a metric scale. The individual stains were then measured from the digital photographs using the FARO Zone 3D Expert BPA module as previously described.

Results and discussion

The microgravity environment presents unique challenges to the analysis of bloodstain patterns compared to a traditional 1 g environment. Without the force of gravity, surface tension and inertia are the predominant forces driving blood in flight and deposition. Fig. 1 charts the calculated angle of impact for stain within the microgravity data sets. With twenty-one stains being measured, a limitation of this study was the sample size. Due to operational costs associated with microgravity research, this was unavoidable. Stains with an angle of impact of 90° exhibited no deviation in the calculated versus the actual angle. In contrast, the stains with acute angles of impact reflect large ranges in the angle of impact estimation. This relationship between orthogonal and acute angles is observed in the 1 g data set (Fig. 2) but at a lesser range. In the 1 g data set, there was also no variation at 90°, but the 45° drop set reflected a calculated mean of 46.28° and a range of 5.4°. Table 1

provides a comparison of the 90° and 45° data sets from both environments.

Pre-Impact Observations - Qualitative analysis of the video evidence provides another viewpoint of the fluid actions taking place. In the initial phase of drop development, the researcher begins to apply pressure to the syringe's plunger; a mass of analog blood forms at the syringe tip and is then ejected toward the target (Fig. 3a). Mid-air breakup of the fluid mass occurs prior to target contact (Fig. 3b). However, surface tension and cohesion continue to connect parts of the volume to the syringe (Fig. 3c). The effects of intermolecular attraction (cohesion) between the liquid molecules become more apparent in the microgravity environment. As a result, droplets of liquid cling together to form larger structures, such as liquid bridges or clusters. This connection detaches from the syringe and breaks up in flight (Fig. 3d-f). This entire process occurs over the course of 0.79 s (19 frames, at a rate of 24 frames per second).

Fluid flight characteristics exhibit similar behaviors of oscillation of the droplet, as observed terrestrially. The flight paths of freed droplets demonstrate straight vectors, which remain unchanged until contact is made with a target. During the second ejection of the 90° angle set, the liquid strikes the preexisting stain on the target, causing multiple droplets to be ejected. These droplets follow a straight vector outward from the original stain (Fig. 4).

Contact and Collapse Observations - When a blood droplet hits a surface on Earth it transitions through fluid dynamic stages of collapse that end with a bloodstain. Upon initial contact with a target surface, the blood drop begins to collapse at the bottom of the droplet, moving upwards, with surface tension retaining the round shape of parts of the drop not in contact with the surface. As the drop collapses, the blood at the contact point is pushed outwards, forming a rim around the droplet, propelling a wave-like action influenced by the surface's characteristics. This rim movement causes the blood to spread outward and, depending on the amount of energy transferred by the drop, results in secondary drop detachment or spine-like characteristics emanating from the parent stain. As the droplet's inertia fades, surface tension starts the retraction phase, pulling fluid back from the stain's edges and leveling the fluid [15]. Within the microgravity environment, stain deposition follows the same stages of collapse and dispersion as observed terrestrially. However, the wave-like action and dispersion appear inhibited. Examination of the stains found that they retained a microfluid dome instead of completely spreading and settling. This fluid dome is the manifestation of surface tension and the main characteristic that results in the broad

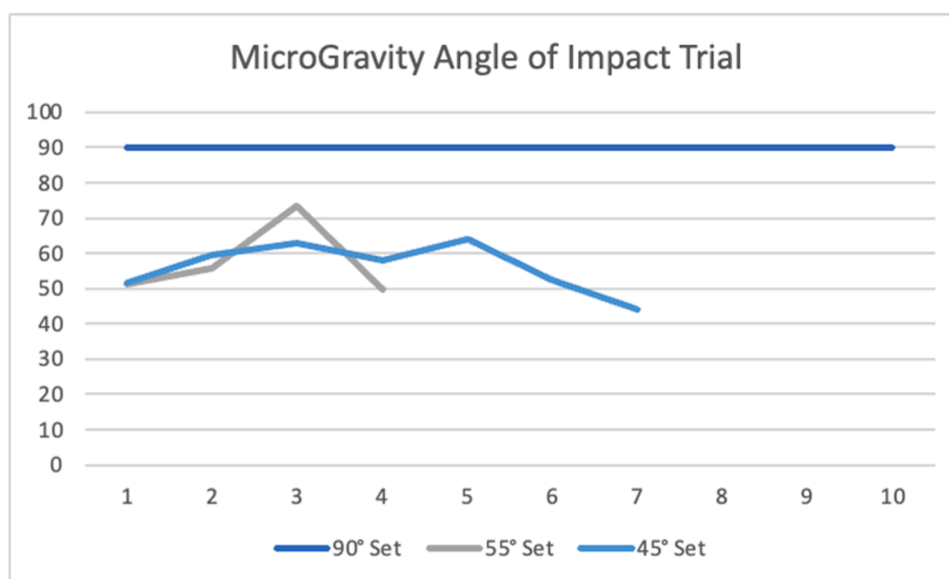


Fig. 1. Line chart depicting the calculated angle of impact for simulated blood drops in microgravity at 45°, 55°, and 90°.

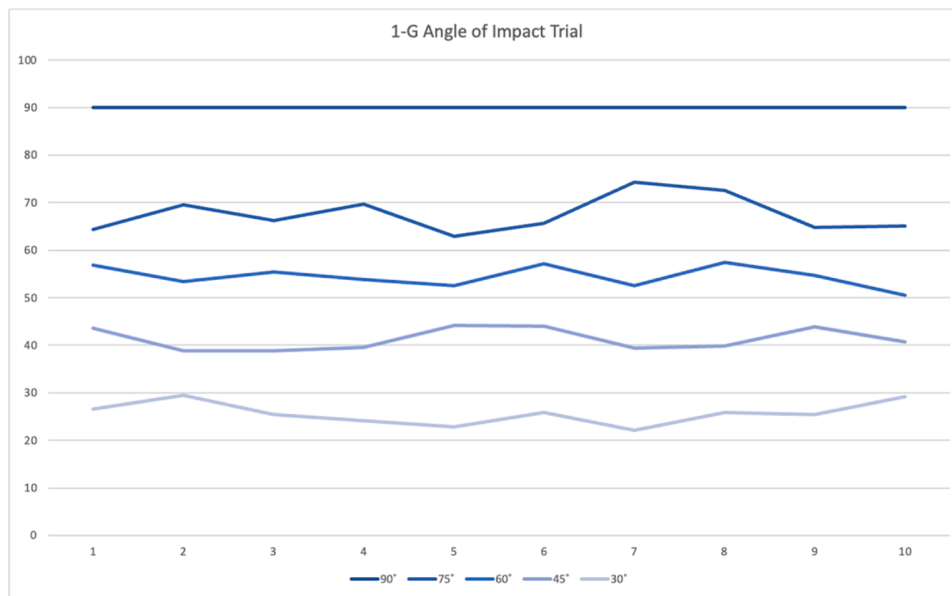


Fig. 2. Calculated angle of impact for simulated blood drops in microgravity at 45°, 55°, and 90°.

Table 1
Comparison of select Microgravity and 1 g data sets.

	Microgravity Data Set		1 g Data Set	
	90	45	90	45
Actual Angle of Impact	90	45	90	45
Calculated Mean	90	56.0191192	90	46.287
Difference	0	-11.019119	0	-1.287
Standard Deviation	0	7.09695812	0	3.42335784

range of calculated angles of impact for acute flight paths.

In microgravity, surface tension shapes liquid droplets to be spherical or nearly spherical when in free flight [16]. Without gravity pulling the liquid downwards, surface tension assumes the dominant force that influences droplet shape, size, and flow behavior. The observed inhibition of inertial spreading results in incomplete stain width and length development. This is why the 90° stains retain their actual width-to-length ratio; surface tension is evenly pulling from all sides, causing the stain to retain the circular shape. In comparison, angled stains result



Fig. 3. 3a (top-left), 3b (top-middle), 3c (top-right), 3d (bottom-left), 3e (bottom-middle), 3f (bottom-right). Fluid volume ejection and stain creation in microgravity.

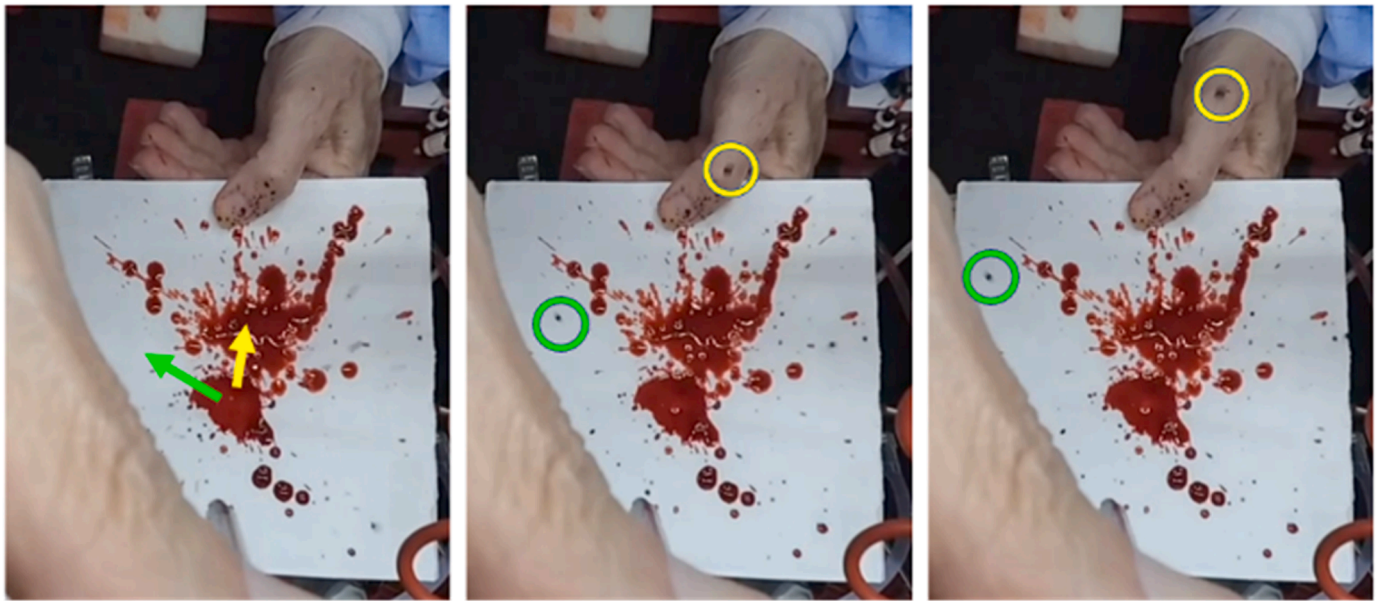


Fig. 4. Depicting the straight vector of droplets in microgravity.

in an incorrect angle of impact calculation because the inhibited spread has led to a repressed length and width development.

Conclusion

The singular question remains at the core of this study: does a blood drop, in the absence of gravity, interact with a surface so that the shape alone exhibits physical characteristics that can relate to the flight path and origin of that droplet? Despite surface tension and cohesion altering the stain's shape and size, stains can retain shape characteristics that indicate vector directionality. The fluid dynamics and behavior of liquid droplets in microgravity is an essential study in contemporary scientific and engineering fields such as space medicine, fluid/fuel management systems, and rocket design and performance. The properties of surface tension and cohesion are crucial to understanding and controlling the behavior of droplets in these disciplines, as well as relevant to the forensic application of BPA in the unique microgravity environment. Studying bloodstain patterns can be a crucial aspect of forensic science, as they provide valuable reconstructive information. However, little is known about how liquid blood behaves in flight or during the deposition in an altered gravity environment. This is an area of study that, while novel, has implications for forensic investigations in space or non-terrestrial environments with reduced gravity while simultaneously assuring accurate metric estimations on Earth. This suggests that analysts must consider environmental effects when analyzing bloodstain patterns in any setting. With the rate of technological evolution in space exploration advancing humanity's pioneering into the extraterrestrial frontier, the need for reliable forensic science techniques will become increasingly important. Although the effects of microgravity present unique challenges for investigators, understanding the variables and physical forces at play can lead to a more reliable interpretation of bloodstain patterns both on and off Earth.

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CRedit authorship contribution statement

Pantalos George: Writing – review & editing, Writing – original

draft, Resources, Project administration, Investigation, Conceptualization. **Kowalske Zack:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Williams Graham:** Writing – review & editing, Supervision, Conceptualization. **Olewi Abdulrahman:** Writing – review & editing, Supervision.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT4 (OpenAI, San Francisco, California) and Grammarly (Grammarly, San Francisco, California) in order to improve readability and language syntax of the initial authored draft. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of Competing Interest

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

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Prior Presentations

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*Methodology was discussed, but data results were not presented.

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*Methodology discusses, data results not presented.

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