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Forensic Quest For Age Determination Of Blood

[1]MUNIGETI RAVI,

[1]Student,

[1]Department of Forensic Science ,

[1]Parul University , Vadodara, India

Abstract: Estimating the age of a bloodstain is a complex forensic task influenced by various environmental factors. Forensic scientists examine color changes, clotting, and DNA degradation to estimate when blood was dropped in a crime scene. Bloodstains serve as valuable forensic evidence, aiding in pattern identification and DNA analysis to establish suspect involvement. It remains challenging to say exactly when a crime was committed. An accurate timeline is critical in criminal investigations because it permits reconstruction. This research tests the chemical and organic reactions happening as a bloodstain ages, including enzymatic activity, bacterial degradation, and environmental effects. It also defines strategies for acquiring, preserving, and transporting bloodstains to minimize infection, degradation, and decay. Various methods of estimating the age of bloodstains, including biochemical, microbiological, radiometric, spectroscopy, and PCR methods, are elaborated. Familiarity with these methods in a forensic setting, along with other evidence, is essential for proper interpretation while considering possible mistakes. In addition, the research emphasizes limitations and challenges of estimating bloodstain ages, like the effect of trauma, contamination, and environmental factors. Advancing research in this field could significantly enhance forensic investigations, improving crime scene reconstruction and criminal case timeline accuracy.

Keywords: Bloodstain decomposition, Bloodstain age, Age determination, Contamination and Degradation.

Introduction

Bloodstains are critical evidence in forensic investigations, used for sample evaluation and DNA profiling. However, determining the precise time of a crime based on bloodstains remains a challenge. Despite extensive research, no standardized forensic technique for bloodstain age estimation has been widely adopted in forensic casework [1]. DNA can identify an individual but doesn't provide much about the sample's date of deposit. Forensic science could benefit from establishing the age of a biological sample, as it could indicate the crime's date and rule out the human source as a suspect [2].

The innovative application of the aspartic acid racemization method for age determination in bloodstain dating has been explored. This method, which estimates d-aspartic acid ratio in slow turnover tissues, could be used to estimate the age of bloodstains. Standard kinetic experiments showed a stronger correlation between elapsed time and d-aspartic acid content in bloodstains. Further research is needed to develop this innovative application [4].

The use of hyperspectral imaging in bloodstain age assessment reveals the age of blood stains up to 200 days old. Researchers used a Push broom device to record visible reflectance spectra of blood stains, comparing hemoglobin derivative fractions with a reference dataset. The results showed that even without a reliable reference dataset, the sequence of blood stains could be ascertained, making this a significant step towards practical application in forensic casework [3]. RNA analysis is crucial in forensic laboratories for determining biological evidence and determining the age of stains found at crime scenes. A qPCR experiment revealed bloodstains could be accurately aged using the 5′–3′ qPCR assay. The assay measured

the 90 bp amplicons generated from the 5′ and 3′ ends, revealing blood stain ages between two and four weeks old for stains under six months old and six to one year old for older stains [5].

The importance of approaching the crime scene scientifically in the light of all the evidence is emphasized. Both the theory and experimental data pertaining to bloodstain pattern analysis are taken into consideration to demonstrate and understand the limits and bounds of that evidence [6]. Blood stains are crucial in reconstructing crime events. No reliable methods are currently available to establish the age of a blood stain on the crime scene. Determining the fractions of three hemoglobin derivatives in a blood stain at various ages enables relating these time varying fractions to the age of the blood stain. Application of light transport theory allows addressing the spectroscopic changes in ageing blood stains to changes in chemical composition, i.e., the transition of oxy-hemoglobin into met-hemoglobin and hemichrome. The chemistry of blood and the biomolecular changes that occur as a bloodstain ages are both inherently intricate, but can be monitored using specific analytical techniques. Studies have shown that bloodstains are a rich source of information, which can be used to help in solving crimes. Particularly, the time since deposition (TSD) can be estimated by analyzing bloodstains and extracting information related to the natural chemical processes that occur as bloodstains age [7].

Optical reflection spectroscopies have been used for the age estimation of blood stains on white cotton. To make this method suitable for forensic practice, it was adapted to correct the influence of the optical properties of the various substrates where blood may be found by extending a one-dimensional light-transport model to a two-layered model. Relative amounts of Oxyhemoglobin, MetHemoglobin, and Hemichrome in blood stains on colored surfaces, based on their reflectance spectra were calculated. A statistical method to calculate the 95% confidence interval around the calculated age was described [8].

According to the age of bloodstains could be calculated simply on the basis of the relative levels of miRNAs. Although it was difficult to utilize the concentrations of ethanol, methamphetamine, and amphetamine sulfate for estimating the age of the bloodstain, these data could provide evidence of the victim or assailant having used these substances at the scene of an accident or crime [9].

The feasibility to apply near infrared (NIR) spectroscopy was evaluated for blood stain identity and age estimation on dark backgrounds. Using NIR reflectance spectroscopy, blood stains were distinguished from different substances with 100% sensitivity and 100% specificity. In addition, Partial Least Squares Regression analysis was applied to estimate the age of blood stains on colored backgrounds. The age of blood stains up to 1 month old was estimated successfully with a root mean squared error of prediction of 8.9%. These findings are an important step toward the practical implementation of blood stain identification and age estimation in forensic casework, where a large variety of backgrounds could be encountered [10].

To determine the age of bloodstains, use of oxygen electrodes, electron paramagnetic resonance (EPR), high-performance liquid chromatography (HPLC) and RNA degradation had been used. Unfortunately, these approaches were not robust, limiting their forensic application. Several novel techniques were explored to determine the age of bloodstains, including fluorescence lifetime measurements, atomic force microscopy and the use of smartphones for quantifiable colour change correlations. In this review, attenuated total reflection (ATR)-Fourier transform infrared (FTIR) technique combined with advanced chemometric methods was utilized to determine the age of indoor and outdoor bloodstains [7].

The interpretation of bloodstain patterns furnishes clues including the character of the offence, the possible series of events, any disturbance to the scene that may have occurred, and even the location of people and items at some stage in the incident. It proves useful in refuting or corroborating eyewitness accounts. The appearance of a bloodstain depends on a variety of factors, including the rate at which it is traveling, distance traveled, the amount of blood, the angle of effect, and the type of target onto which it lands. The types of bloodstains include: single drop, impact spatter, cast off stain, transfer bloodstains, projected pattern/arterial damage stain, pool stains, insect stains and expiration stains [11].

Materials and Methods

Data Sources:

This evaluation focuses on interpreting bloodstain age estimates in a forensic investigation, taking into account different evidence and sources of errors, as well as understanding the limitations and problems of bloodstain age estimation, along with the impact of trauma, contamination, and environmental factors. This study focused on papers published from the year 1997 to 2019. The review only included papers that were published in peer-reviewed publications in English language. Research sites such as PubMed, Google Scholar, Research Gate etc. were used to obtain information about several papers.

Study Selection:

The data was taken from many studies, including experiments, observations and different cases, that showed the techniques and results of the mentioned methods. Any articles that didn't meet the criteria or repeated data from other included articles were not included.

Data Extraction and Analysis:

The information from the articles was carefully reviewed and manually arranged in a standard form. The search method had three steps:

1. Checking through the titles,
2. Evaluation of the abstracts,
3. Selection of articles by examination and reading of their full context.

METHOD:

- **Visual Examination**: The initial step at a crime scene is the visible examination of bloodstains. This includes examining the coloration, texture, and consistency of the blood. Dried blood is matte and deeper in color, whereas liquid blood is glossy and crimson. The presence of coagulation or clotting also indicates the age of the stain.
- **Sample Collection**: Bloodstain samples are collected using sterile swabs. To avoid infection or deterioration, the samples are clearly labeled and preserved.
- **Luminescence Spectroscopy**: Luminescence spectroscopy is a powerful analytical method that measures light emitted by materials following excitation by an external power supply. In the context of forensic technology, it is particularly useful for differentiating between clean and aged stains, such as bloodstains, by studying changes in their fluorescent properties over time. Using this method, the amount of light absorbed by the blood as it dries is measured. The tempo at which blood dries is influenced by a variety of factors, including surface features, temperature, and humidity. The age of the stain can be accurately calculated using the determined absorbance spectrum of the dried blood.
- **Reference Standards**: Reference standards are utilized to verify that age determination is accurate. These are known-age samples prepared in a controlled laboratory setting. Matching the absorbance spectra of unknown stains to reference standards allows for precise age assessment.
- **Interpretation**: When assessing luminescence spectroscopy results, it is critical to consider additional evidence from the crime scene. For example, the presence of additional sources of blood in the vicinity that are known to be older or younger than the stains being examined might offer context and confirm or reject the age determination results.
- **Reporting**: The age determination findings must be reported in a clear and exact way, using appropriate scientific language and measures. Any limitations or ambiguities in the study are stated in the report, as well as any recommendations for more research or testing.

SAMPLE PREPARATION:

Fresh whole-blood samples (without anticoagulants) were received from healthy volunteers and deposited directly onto glass slides to form bloodstains. Time factors were set. For every time factor, bloodstain samples per donor were prepared, of which few samples were saved in indoor surroundings and few in outdoor surroundings. It should be emphasized that the indoor situation could not be specifically controlled and the samples were exposed to dim sunlight throughout the day and no light at night. Bloodstain samples located outside were exposed to light, heat, and humidity but not rain. The test groups were employed to validate the constructed chemometric models [12].

SPECTRA COLLECTION AND DATA PROCESSING:

Spectral collection was accomplished using a Nicolet iS 50 FTIR Spectrometer (Thermo Fisher Scientific, Waltham, WA, USA) equipped with an ATR attachment (Thermo Fisher Scientific, Waltham, WA, USA) with a diamond crystal face of around 2 mm in diameter. Bloodstain samples were amassed in an Eppendorf tube and evenly mixed with 10 μL of regular saline before every dimension. Subsequently, 1 μL of pattern was deposited at the ATR crystal face and dried with an air dryer for about 4 min. The spectra was recorded within the range of 900–1800 cm−1 at a resolution of 4 cm−1 with 32 scans. The background spectrum was automatically subtracted from the sample spectra. For every sample, replicate spectra were accrued and then averaged to form a single spectrum. The spectra was recorded with OMNIC software version 9.2 (Thermo Fisher Scientific, Waltham, WA, USA). Next, baseline correction, unit vector normalization and multiplicative scatter correction (MSC) offsets, removed artifacts associated with the analytical strategies and samples under study, and reduced the effects of light scattering. The data preprocessing was performed. PLS regression analysis was performed [12–16].

Results and Discussion

Environmental Factors Affecting Bloodstain Age Estimation:

- **Temperature**: High temperatures cause blood to dry and coagulate more quickly, resulting in faster breakdown of DNA and proteins. Low temperatures, on the other hand, slow decomposition and allow bloodstains to be preserved for extended durations of time. Freezing temperatures can cause hemolysis (the dying of red blood cells), which affects analysis.
- **Humidity**: High humidity promotes bacterial and fungal development, resulting in faster degradation of biological materials. Low humidity causes rapid drying, which retains bloodstains but changes their chemical composition.
- **Sunlight (UV Exposure)**: Ultraviolet (UV) light degrades hemoglobin and other blood components, resulting in color shift and faster degradation. Long-term exposure makes bloodstains more difficult to detect and investigate.
- **Rainfall and Moisture**: Water dilutes and cleanses bloodstains, making detection more difficult. Rain also increases bacterial activity, resulting in faster breakdown.
- **Wind and Air Quality**: Strong winds spread or dissolve dried bloodstains, while pollutants and chemicals in the air change their composition.

Applications of Bloodstain Age Estimation in Forensic Investigations:

- **Narrowing down the time frame of the crime**: Forensic scientists provide investigators a more accurate timeline for the crime scene by estimating the age of the bloodstains. This data assists in reducing the number of potential scenarios and concentrating the inquiry on possible culprits.
- **Identifying the perpetrator**: Age determination yields vital information that helps identify the offender. If the bloodstains, for instance, are discovered to be several days old, it implies that the person was slain somewhere else and brought to the crime site, which provides the investigators with new leads.
- **Exonerating innocent suspects**: In different conditions, determining a suspect's age clears innocent individuals who were previously thought to be related to the crime. For instance, it implies

that the suspect was not at the crime scene when the victim was killed if the bloodstains are discovered to be many weeks old.

- **Providing evidence in court**: The assessment of age can also be used as evidence in court to bolster or contradict the testimony of witnesses or other arguments put out by the prosecution or defense. For instance, age determination can be used to ascertain whether the bloodstains are consistent with a witness's account of seeing a suspect at the crime site at a particular time.
- **Advancing scientific knowledge**: Lastly, the forensic search for the age of blood lines can further scientific understanding and improve forensic technology. Forensic scientists create more unique and trustworthy strategies for figuring out a person's age by examining the modifications and drying processes of blood, which also have wider applications in other domains. The forensic look for the age of blood stains has bright future potential so long as technology keeps growing. Using Raman spectroscopy is one area of studies that has numerous promises. By the use of this method, chemical substances are identified depending on how they scatter light. Forensic scientists can identify the type of blood and whether it was human or animal by examining the Raman spectra of bloodstains. They can also ascertain the stain's age. The application of machine learning methods to the analysis of huge datasets of bloodstain age determination data is another interesting discovery. More accurate and reliable results are acquired by the usage of those algorithms to identify patterns and developments that human analysts might not see right away.

Significance

Forensic science uses radiometric, microbiological, and biochemical methods to estimate the age of bloodstains, examining chemical and biological alterations over time. This evidence can disprove witness accounts, validate counterarguments, and provide a fuller picture of crime events, aiding in the exoneration of innocent people.

Conclusion

Age estimation in forensic engineering is greatly aided by blood stain analysis. Analyzing blood patterns reveals important information about when an incident occurred. It supports an inquiry to ascertain the age of a specific occurrence. Timing events are ascertained with great benefit by analyzing stain characteristics, degradation, and clotting patterns. Even though it is not a precise science, the meticulous collection of blood evidence greatly improves age estimation accuracy overall, aids forensic investigators in reconstructing timelines, and helps them unravel the riddles surrounding incidents.

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