H103 Scanning Electron Microscopy (SEM) in the Identification of Fly Artifacts (FAs): A Preliminary Qualitative Study

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Learning Overview: After attending this presentation, attendees will understand that SEM is a useful and promising tool to assist in the differential diagnosis between FAs and unaltered bloodstains.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by discussing a key aspect of bloodstain pattern analysis, since many techniques have been applied for the differential diagnosis between bloodstains and FAs, but most of them rely on the experience and opinion of the analyst rather than on standardized and reproducible methods. Morphological analysis through SEM may provide reliable objective parameters to daily forensic casework.

Bloodstain Pattern Analysis (BPA) has a key role in crime scene investigation. After deposition, bloodstains can be altered by diverse forces, some of which are related with the crime, while others may act as confounding factors, such as the stains produced by insect activity, which are commonly referred to as FAs. Flies can produce FAs in different manners: by contact, by regurgitation, and by defecation. Differential diagnosis between FAs and bloodstains is sometimes cumbersome, especially in cases of small stains (0.1cm–0.3cm). Several techniques and methods of analysis have been used to differentiate between unaltered bloodstains and FA, such as visual macro-microscopic and contextual analysis, heme-based presumptive tests and immunological confirmatory tests. However, these techniques mostly rely on the experience and opinion of the analyst rather than on standardized and reproducible methodology.

The present survey aimed at testing the potential utility of SEM for distinguishing bloodstains from FA produced by *Sarcophaga carnaria* under experimental conditions.

One hundred adults of *Sarcophaga carnaria* were placed in a scaled-down room analog with free access to 50ml of fresh human blood and were free to deposit fly artifacts on five different surfaces (two porous and three non-porous types of paper) for 48 hours. Experimentally produced bloodstains were used as controls. FAs and controls were compared through visual analysis (color, shape, tail, edges) and SEM analysis (surface, deposits, presence of red blood cells) analysis.

Visual analysis allowed the identification of two types of FAs. Type 1FAs (FAs1) showed brownish color with no or short tails and resembled the controls, while Type 2FAs (FAs2) showed yellow-brownish color with longer and curved tails, being easily distinguishable from controls and FAs1. SEM analysis allowed the distinguishing between controls and FAs through the identification of red blood cells on the surface of the controls, which were absent in both types of FAs. Other distinctive morphological features were observed: (1) FAs1 showed luminescent deposits on the surface, which were absent on controls and FAs2; and (2) FAs2 showed irregular/glomerular surfaces, while controls and FAs1 showed flat or cratered surfaces.

Based on these features, the detection through SEM of red blood cells on the surface of the controls was the key element for the differentiation between FAs and bloodstains, since red blood cells were present in all controls and absent in all FAs. Other features can be considered to confirm the differential diagnosis, such as the presence of luminescent deposits and the morphological features of the surface of the spot.

Therefore, SEM analysis demonstrated to be a useful tool for the distinction between FAs and unaltered bloodstains, since it allowed identification of some objective morphological features that do not rely on subjective evaluation. In the future, this technique could be used for the differentiation of FAs and bloodstains deposited by different species on other types of surfaces to assess the potentialities of this technique in real forensic scenarios.

Fly Artifacts, Bloodstain, Scanning Electron Microscopy (SEM)