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Scanning Electron Microscopic Analysis of Skin Resolution as an Aid in Identifying Trauma in Forensic Investigations*

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ABSTRACT: The forensic investigator is frequently confronted with cases that present with wounds and blunt force trauma. Presently, the forensic investigator depends upon previous experience and further investigative deduction of the crime scene to analyze these injuries. Although not readily apparent to the naked eye, many skin tissue injuries can be visualized with scanning electron microscopy (SEM). This study was designed to establish skin trauma resolution using SEM in various skin preparations. Tissue trauma was induced on leather, preserved skin, fresh skin, and living skin using dies of varying thread size. Calibrated pressure forces in pounds per square inch (psi) were applied and impressions made using vinyl polysiloxane. Positive replicas of the tissues were prepared for SEM using isocyanate resin. After sputter coating the cast with 35 nm of gold-palladium, electron micrographs were generated using a Jeol JSM-5310LV scanning electron microscope. To establish resolution, thread widths of 52, 104, and 208 threads per inch (tpi) and trauma forces of 150, 200, and 250 psi were used to produce the impressions. Microgrooves that were identified on the die threads were analyzed. The optimum pressure for resolution studies was 150 psi using the 52 tpi die on the leather sample (4.67 $\pm$ 0.88 μm, $p = 0.046$ and 0.025, respectively, by ANOVA). The resolution was compared to that of leather using preserved, fresh, and living skin. The resolution in preserved and fresh skin was less than for leather (9.00 $\pm$ 1.73 and 10.5 $\pm$ 4.5 versus 4.67 $\pm$ 0.88 μm, $p = 0.09$ and $p = 0.20$, respectively). Living skin resolution was 3 μm at 52 tpi and 100 psi. Various implements of blunt force trauma were also examined using the leather sample. Time after trauma resolution was examined at 0 (3 μm), 5 (6 μm), 10 (8 μm), and 20 (9 μm) min in living tissue. A comparison between the microgrooves on the die replicas and the tissue trauma impressions revealed striking agreement for both linearity and resolution. Analysis of the microgrooves suggests that discrete morphological characteristics are seen in skin tissue traumas. This method could expand the tools available for the forensic investigation of blunt force trauma.

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In forensic pathology, a primary concern is to identify the implement that induced trauma as part of a criminal investigation. Typically, blunt force trauma is induced by large implements. However, smaller implements of trauma are often used which are harder to identify. Many tissue traumas are not identifiable with the naked eye and must be magnified, typically through photographic processes. There is an inherent limitation in the use of photography since the photograph fails to capture the third dimension or depth of a wound. Consequently, the scanning electron microscope (SEM) has become recognized as an established tool for the forensic investigator (1). The value of SEM analysis is the ability to capture the third dimension seen in trauma, as well as the higher magnification that can be achieved (2,3) Many studies have qualitatively used SEM to identify individual characteristics, but the quantitative standards of trauma registered in skin have not been established (4). Indeed, a literature search using MEDLINE failed to identify published standards for the resolution of trauma in skin. The purpose of this study is to quantify the visual resolution of skin to register trauma using scanning electron microscopic analysis.

Methods

Harvesting Tissue

Several different tissue media were used, including: leather, preserved human cadaver skin, fresh skin obtained from organ donors, and living skin. Fresh skin was harvested from organ donors using round plastic rings 3 to 4 in. in diameter placed upon the desired tissue sample, allowing for adequate margins (5). Prolene 2.0 sutures were placed through the skin and around the ring using a continuous suture to secure the tissue in place. The skin sample was then undermined using a scalpel in the hypodermis layer for removal. The plastic ring maintained the tissue in the same condition as found with minimal distortion.

Simulating Trauma

To simulate trauma, several different dies were machined from a 0.3125-in. aluminum rod on a Logan lathe. These dies had thread patterns of 52 threads per inch (tpi), 104 tpi, and 208 tpi, resembling the threads of a common hardware bolt (Fig. 1). Modified mechanical vise grips were used to produce the trauma force. The modification consisted of a plastic plate to hold the tissue sample on one jaw, and the aluminum die on the other jaw

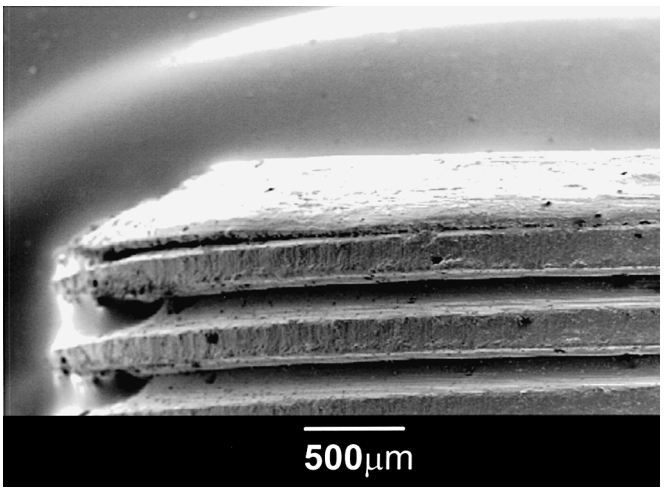

FIG. 1—*Aluminum dies machined from rods on a Logan lathe to produce 52, 104, and 208 tpi.*

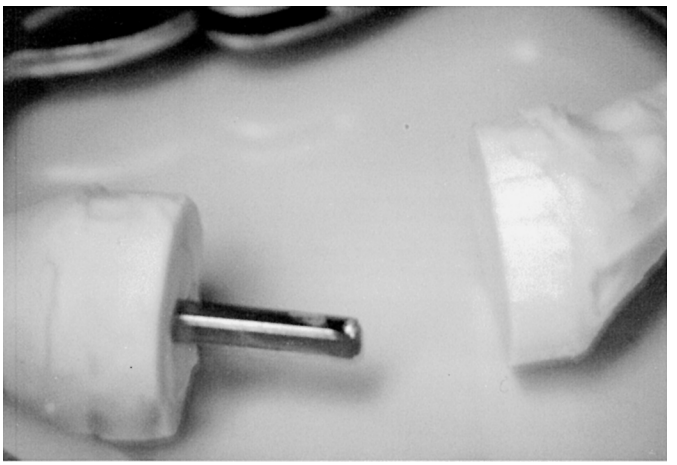

FIG. 2—*Trauma induction implement used in the study. Instrument consisted of a modified mechanical vise grip with the tissue sample held in a plastic plate on one jaw, and the aluminum die on the other jaw.*

(Fig. 2). A pressure transducer was placed between the tissue sample and the plastic plate. This transducer was connected to a Pentium computer with Metscan software that measures the pressure in grids, which was graphed on screen or saved for later replay. The trauma force can be adjusted on the vise grips by turning an adjustment screw. The aluminum dies were then pressed into the tissue samples using the modified vise grips to simulate a traumatic injury. Trauma forces of 150 psi, 200 psi, and 250 psi were used with the various size dies in each of the different tissues. The forces generated were analyzed to ensure that consistent pressures were applied throughout the experiment.

Producing Replicas

Impression material was then applied to the tissue at the site of the injury leaving approximately 1 cm margins around the trauma (Fig. 3). Aquasil vinyl polysiloxane was used for ultrafine grain impressions, because it sets quickly and produces an accurate mold. Pour-A-Kast™ isocyanate resin was then mixed with a catalyst and poured into the impression mold to produce a replica of the tissue sample (6). The resin took about 20 min to set up firmly, after which the replicas were shaped on a grinding wheel and mounted on brass stubs using carbon paint. The replica was then coated with approximately 35 nm of gold-palladium using a vacuum of 60 to 80 millitorr with argon process gas and 15 mA current in an Anatech Hummer 6.2 sputter system for 2.5 min. The gold-coated replicas were then placed on a eucentric stage of a Jeol JSM-5310LV scanning electron microscope. Electrons are then accelerated using high vacuum in the column with variable accelerating voltages of 5 to 25 kV, and a magnification of ×35 to ×500.

SEM Photomicrographs

Once the desired tissue trauma was identified using the electron microscope, photomicrographs were produced. The image was scanned digitally through an interface to a Pentium computer. Each digital image included a legend that indicated the accelerating voltage, the magnification, and a micrometer scale. The image could then be saved onto the computer. Polaroid photomicrographs were also produced. This provided a hardcopy image that included the same legend and scale to determine the resolution of the trauma

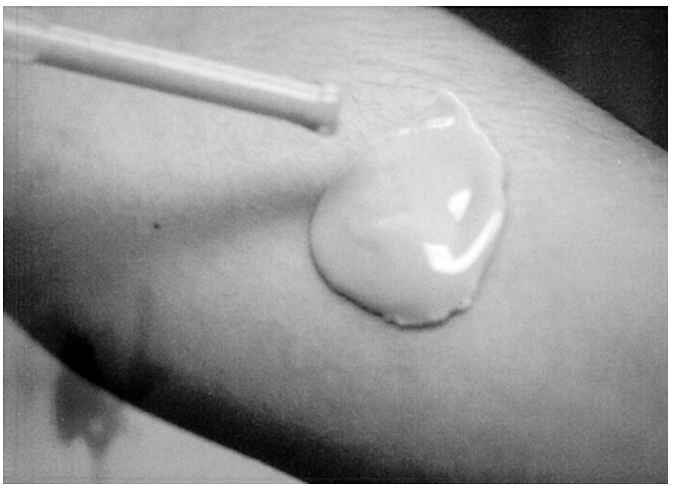

FIG. 3—*Example of the application of the impression material. Impression materials applied to the injury site extend approximately 1 cm around the trauma.*

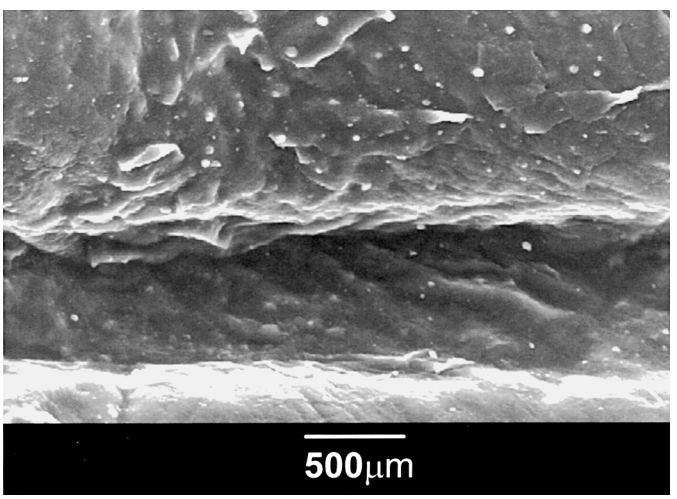

FIG. 4—*Resolution of thread impressions on living skin. Measurement performed on Polaroid photomicrographs of the digital images produced by the electron micrograph.*

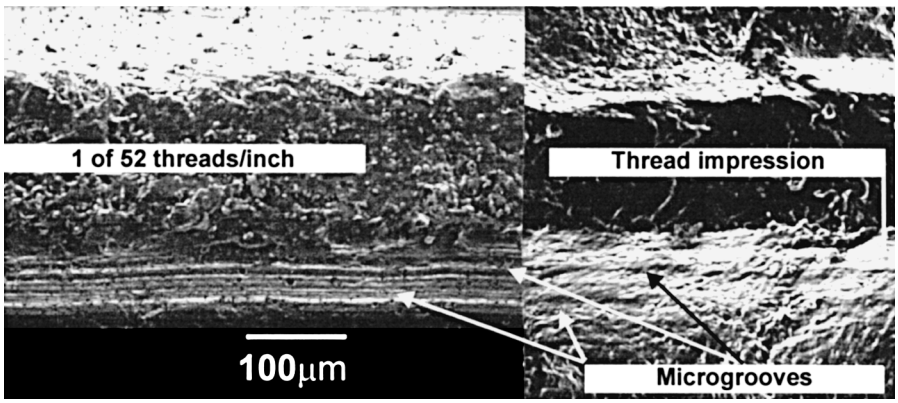

FIG. 5—*Comparison of aluminum die* (left) *with machine marks made on side of threads, and corresponding thread impressions in the tissue* (right). *Also indicated is the structure of the microgrooves.*

pattern. Measurement of the distance between the threads yielded the trauma resolution in tissue (Fig. 4).

Each micrograph is measured to determine the resolution, the optimum trauma force, and the thread size used. This was repeated with each tissue type, and then statistically analyzed using the analysis of variation (ANOVA) technique. A few of the micrographs were scanned with a Hewlett Packard flat bed scanner at 600 dpi. Using Photoshop software the thread pattern image was then superimposed side-by-side with the traumatized tissue image. With this technique, the tissue can be compared with the implement of trauma (Fig. 5).

Results

The leather tissue sample was used first to develop a technique. Using the leather sample the trauma resolution was determined to be approximately 100 μm (Fig. 6). Initially, this was the resolution limit of the thread patterns. However, after making a replica of the dies and analyzing the threads using SEM, distinct machine marks were identified on the side of each thread. These machine marks were termed microgrooves since they appeared approximately 2 to 20 μm apart in between the major threads (Fig. 7). Subsequently, the corresponding tissue trauma was sought in the leather using three die sizes to examine the microgrooves (52 tpi, 104 tpi, 208 tpi) and three different trauma forces (150 psi, 200 psi, 250 psi).

With the technique refined, each tissue was subsequently analyzed after trauma and measurements were recorded. The optimum pressure and thread size for resolution studies in leather was 150 psi using the 52 tpi die, yielding a resolution of 4.67 μm ± 0.88 μm, $p = 0.046$ and 0.025, respectively, by ANOVA (Fig. 8). The preserved skin resolution was 9.00 μm ± 1.73 μm, $p = 0.09$ using the trauma force of 150 psi and 52 tpi die size. The fresh skin resolution was determined to be 10.5 μm ± 4.5 μm, $p = 0.20$ using 150 psi and 52 tpi. Living skin resolution was 3.0 μm at 100 psi and 52 tpi (Fig. 8). The living (fresh) skin measurements required the use of reduced trauma forces since the pressures were painful to the volunteers. This was the last tissue measured; consequently, the difference in the trauma forces was not foreseen. These trauma forces, however, were still consistent with biting force pressures determined using the force transducer with the Metscan software.

The resolution of living skin was also examined for time after trauma measurements. Using a trauma force of 100 psi and the 52 tpi die size, living skin was traumatized with the die and impressions taken with the vinyl polysiloxane material at 0 min (3 μm), 5

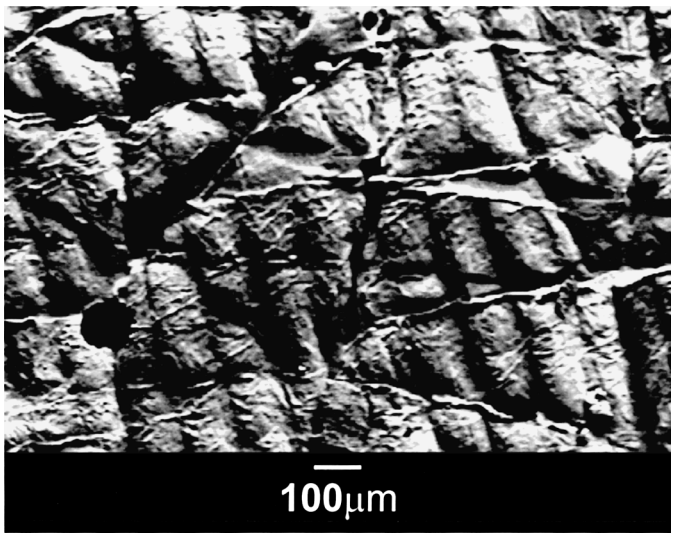

FIG. 6—*Photomicrograph of leather sample after die was impressed to simulate trauma. Major groove impressions are approximately 100* μm.

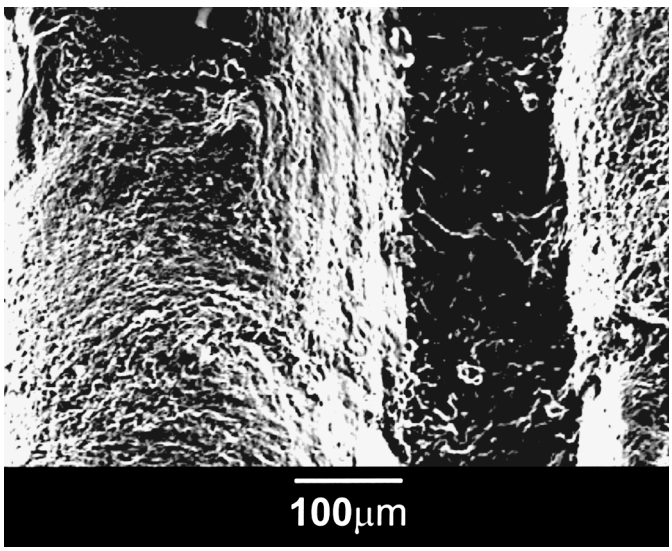

FIG. 7—*Leather sample of simulated trauma using die impression into leather. Microgrooves can be seen on the side of major groove impression.*

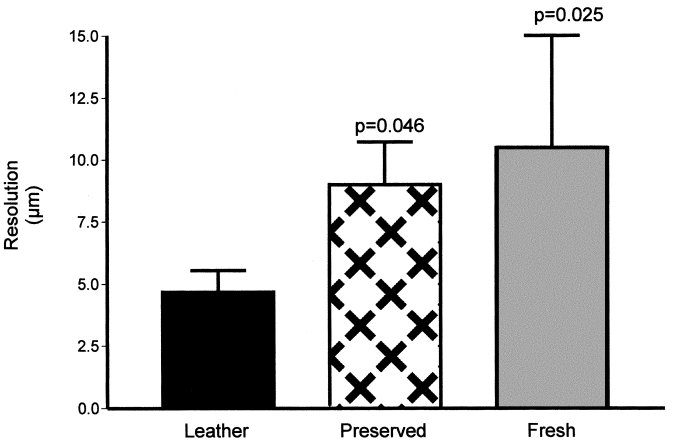

FIG. 8—*Comparison of microgroove resolution at 150 psi using the 52 tpi die. Statistical analysis done by one-way ANOVA; significance equals* $p < 0.05$.

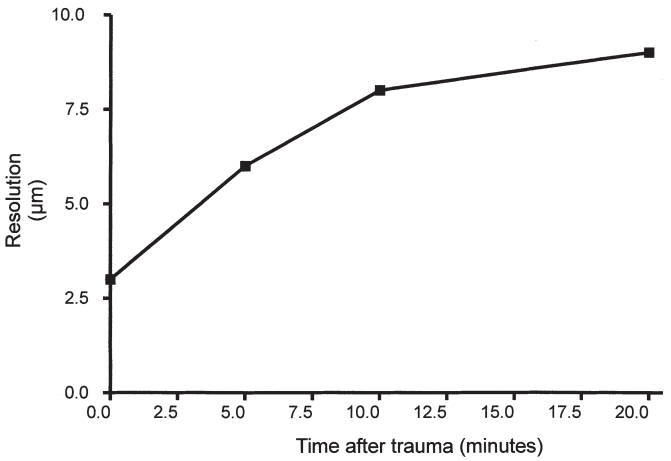

FIG. 9—*Relationship between time after trauma in living tissue and SEM microgroove resolution.*

min (6 μm), 10 min (8 μm), and 20 min (9 μm) after trauma (Fig. 9). The results showed a gradual deterioration of resolution over time; however, this was still similar to the preserved (9.0 μm) or fresh skin (10.5 μm) resolution.

Various implements of trauma were also examined including a knife (Fig. 10*a*), a saw blade (Fig. 10*b*), and a screwdriver (Fig. 10*c*) to induce trauma in the tissue. These implements left distinctive patterns in the tissue that are consistent with the type of tool making the impression (7).

Discussion

Using leather as the first tissue sampled provided the SEM analyst with valuable information about the trauma pattern that was beneficial in the subsequent recognition of patterned injury in the other tissues. Similarly, making a replica of the implement of trauma and noting characteristics using SEM provided the analyst with specific information to aid in the identification of matching features in the traumatized tissue. While both of these steps required a longer time to produce replicas, they significantly reduced

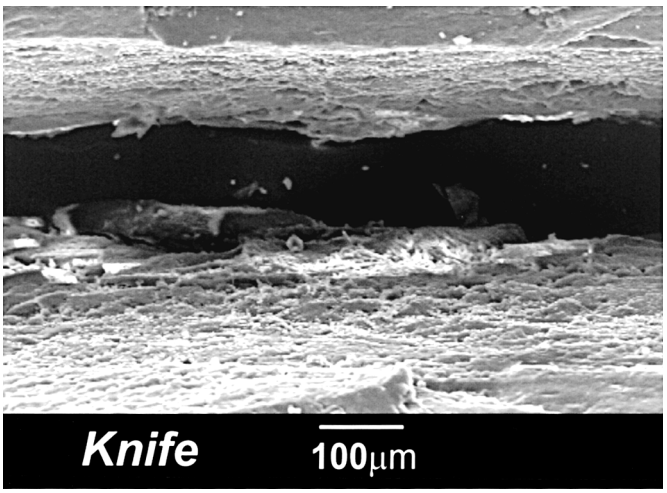

FIG. 10*a*—*SEM photomicrograph of blunt force trauma produced by a knife on fresh skin.*

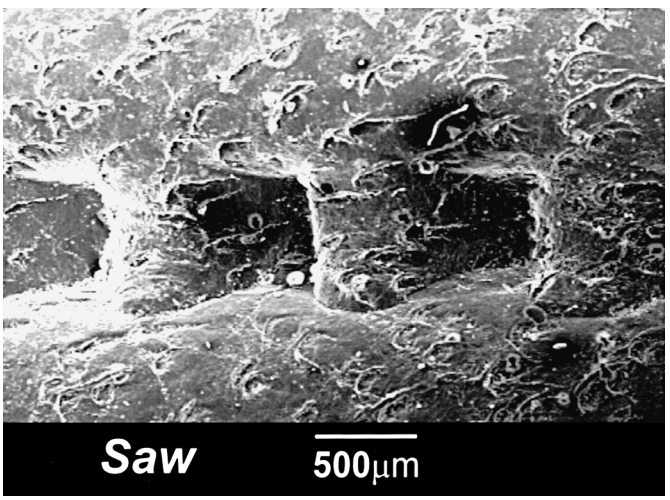

FIG. 10*b*—*SEM photomicrograph of blunt force trauma produced by a saw on fresh skin.*

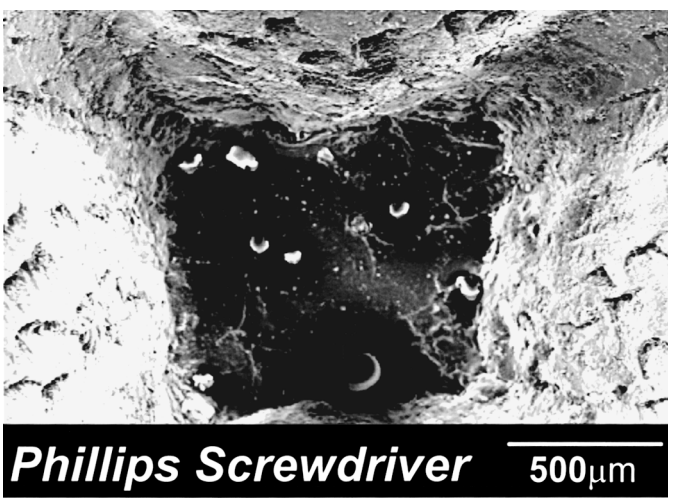

FIG. 10*c*—*SEM photomicrograph of blunt force trauma produced by a Phillips screwdriver.*

the time spent using the SEM searching for characteristic features. Since SEM time is expensive (ranging from $90 to $150 per hour) the time and money saved by these steps can be quite significant. It should also be noted that many times the criminal evidence does not include an implement of trauma. However, through application of impression material at the wound site to prepare a replica of the traumatized skin, SEM analysis can still be used, although with a corresponding increase in time consumed by analysis. This type of analysis is quite specific and perhaps beyond the scope of an SEM technician. A case in point: matching a bite mark wound to human dentition requires the use of a consulting forensic specialist. Ideally, the consultant is present during SEM analysis to aid in the identification of matching features.

Further study is needed to examine the time after trauma resolution. This study examined only 0 to 20 min after trauma, which indicated a decrease in resolution over time. Other factors that influence the deterioration of resolution over time in a homicide victim include autolysis of the tissues, environmental factors including weather, fire, water, insect, and rodent activity. Recognizing these factors, a control study should be undertaken to establish resolution over time in a controlled environment. This may provide further data to substantiate the time of death in forensic investigations.

The analysis of the fresh skin samples showed a resolution of 0.5 $\mu m \pm 4.5$ μm. The preserved skin had a resolution of 9.00 $\mu m \pm 1.73$ μm. There are several factors that might account for this variation in the fresh and preserved skin resolution compared to the living skin resolution that was 3.0 μm. When these tissues were harvested they were placed in either a saline solution (fresh skin) or a formalin solution (preserved skin). With the increase in moisture content of the tissue, there was a loss of resolution. This was apparent using SEM, since it was more difficult to identify the tissue trauma amidst the background edematous dermal papillae. The statistical analysis seems to bear this out. Another factor could very well be the location of the tissue being harvested. It is recognized that human skin varies from site to site (4). In addition, there is individual variation between donors, including actinic keratosis from ultraviolet light exposure, the amount of subcutaneous adipose tissue, and the relative state of hydration of each donor (4,8–10). The living skin samples were measured using the ventral forearms as the trauma site. The preserved and fresh skin samples were harvested from the abdomen of organ donors. Controlling for these variables could increase the agreement in resolution between the samples.

Accordingly, with the use of high-resolution impression material, replicas of blunt force trauma can be made from the victim's wounds. This study shows that skin is capable of registering trauma in the 3 to 10 μm range, which is beyond standard photographic or dissecting microscope capability. The resolution of skin is ideally suited for analysis by scanning electron microscopy, especially since the added third dimension found in trauma is exploited using this instrument. Using these techniques the weapon may be recognized as consistent with the causative agent of skin trauma; further, the techniques were designed to meet the requirements for admissibility in a court of law.

The skin resolution standards developed in this study are intended to meet the Frye test for admissibility as evidence in state and Federal courts of law. The Frye test has three requirements: the principle must be demonstrable, it must be sufficiently established, and it must gain the general acceptance of experts working in the particular scientific fields to which the evidence belongs (5). Scanning electron microscope technology was introduced in the late sixties. Its applications to anatomy, pathology, and forensic science have since been noted. SEM comparisons have been found to be admissible in court, as well as the replica production techniques previously described (2,3,5,6). This study provides a significant contribution to the field of forensic pathology in that it elaborates the resolution of skin after trauma, and further aids in the recognition of the blunt force implements that are consistent with that trauma.

Acknowledgments

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