Abstract:

This research examines whether a scientist can determine the time interval associated with skin when exposed to a burning substance using observation through an Environmental Scanning Electron Microscope (ESEM). The microscope barrage the surface of the skin with electrons, giving the viewer an image of the topography of the sample. Pig epithelial tissue was the medium experimented upon due to its similarity to human skin and was stored in formaldehyde to keep the tissue fresh until examination could occur. The tissues were burned chemically (with concentrated and half-diluted hydrochloric acid) as well as thermally (using a household non-industrial refrigerator/freezer unit and a toaster oven) with controlled variables allowing only exposure time and percentage of fat to be variable within the experiment. Determining the exposure time interval betters the scientific understanding of pin-pointing evidence leading to a near-definitive estimation of timed exposure. When burning the substance thermally, large bubbles and tearing were seen in the frozen tissue whereas many small bubbles were seen dotting the heated tissue. In terms of the acid, heavy pocketing occurred in the skin, eating the epithelial layer away from the sample. Thus, the results show that as the time interval of exposure increases, the observed artifacts of burning (bubbling and pocketing) also increase in number. In summary, due to the results collected in this experiment, the time interval in which the skin was exposed to a burning substance can be approximated based on the number of artifacts seen on the skin under the ESEM.

Keywords: scanning electron microscope, burn, burning, skin, epithelial tissue, freezing
Introduction

Forensic science, to be defined simply, is the utilization of science to analyze evidence within a legal framework. It is often used in criminal as well as civil investigations. In these investigations, time is usually of the essence. Due to this, scientists may want to estimate the length of time a person or body has been exposed to a certain environmental condition. Knowing that the current techniques can have estimations that are off by hours to days is always a challenge for investigators. That is why, in this study, scientists will be observing thermal and chemical burns on epithelial tissues to determine how long it was exposed to a certain burning stimulus.

To begin, there are three classifications of burns: first, second, and third degree. First degree burns only affect the epidermis, the outermost layer of the skin. Second degree burns affect the epidermis and part of the underlying dermis. Being slightly more severe than first degree, second degree burns cause pain, redness, swelling, and even blistering. On the other hand, third degree burns destroy both the dermis and epidermis. In extreme cases, third degree burns may also cause damage to the tendons and bones\(^\text{10}\). Knowing this, the research outlined in this paper will focus on determining the time interval associated with exposure to a burning substance using observation through an Environmental Scanning Electron Microscope (ESEM).

Thermal burns occur when an excess of heat or cold is applied to an area of the body. For example, this research focuses on first to second degree burns (i.e. frostbite, sunburn) on pig epithelial and muscle tissue. Cold burns are forensically relevant due to the complications of freezing a victim post-homicide. A perpetrator may need to take time after the death of the victim to devise a disposal method, thereby placing the body in the freezer in the meantime. By conducting experiments on the epithelial tissue, this research could quantify the length of time the mammalian tissue spent in the freezer.

Chemical burns occur when tissue is exposed to a corrosive substance, such as a strong acid or base. This second portion of the study also focuses on first and second degree burns by examining the reaction of the mammalian tissue depending upon the time interval wherein the acid was left on the skin before being washed off. This is especially forensically relevant in factories, public pools, or among chemists, where acid may be spilled due to a workplace accident (requiring legal action). Perhaps this acid could not be washed off right away, leaving a potential burn in its place. Being able to determine the exposure time could be presentable evidence within a courtroom.

The controlled variables in this study will be the set temperatures and lengths of time allotted per sample. The burn damage done to the epithelial tissue is dependent on these factors and will be observed using an Environmental Scanning Electron Microscope (ESEM). It is suspected that as the time interval increases the more severe the burns will become. Observing tissue under the ESEM will result in evidence of “pocketing” and “bubbling”. Pocketing emerges as a dark area on the scanned portion of the tissue, appearing farther away than the rest of the tissue. Bubbling, another indication of a burn, will appear opposite pocketing, with hills that are lighter than the surrounding area, and seem closer to the camera lens.

Upon reviewing articles, it was seen that this category of research does not have many previous peer-reviewed statistics or testing available for reference. However, an article by Schäfer exhibits extracellular extension when organ tissue is frozen over a long period. The facts presented by Schäfer mentions the tissues rising and falling during the freeze-thaw cycle\(^\text{6}\), which is what is to be expected in this experiment as well. On the other hand, no research was found
that related directly to human or pig skin being burned thermally or chemically and examined under the Scanning Electron Microscope.

Materials and Methods

To conduct the experiments detailed above, one must acquire:

- Epithelial tissue (pig specimen)
- Thermal burning mediums (non-industrial refrigerator/freezer, toaster oven)
- Chemical burning mediums (hydrochloric acid)
- Formaldehyde (37%)
- Glassware (jars for storage, beakers for acid transfer, graduated cylinders)

The tissue chosen comes from the lower leg (hock) of a pig specimen. This is because of its lower fat content, which is more like the average human body type. Typically, pig tissue is seen to be the most human-like physiologically and anatomically. Throughout the experiment, fresh pork hock was acquired and tested upon within eight hours of obtaining the fresh tissue. Squares of tissue approximately 5cm by 5cm were used for each trial, with microscope samples (0.5cm by 0.5cm) cut from the middle of the tissue to avoid any excess burning that would appear on the outer edge of the tissue. After each section of tissue was experimented upon, it was immediately stored in a solution of 37% formaldehyde to preserve the tissue until examination under the microscope.

To test the cold thermal burns, a household refrigerator/freezer unit was acquired and set to -18°C (255K) which is standard for a non-industrial freezer (Whirlpool Refrigerator/Freezer model ED2FHXKQ04). The 5cm by 5cm samples were left in for 1 hour, 2 hours, and 3-hour time intervals. For the heat burns, a standard toaster oven (Black & Decker Toaster Oven model TO1420BC) was used to mimic a sauna in terms of burning through dry heat (93°C). The heat samples were left in for 5, 10, 15, and 20-minute intervals, which were determined based on a general idea of how long the average person might spend in a sauna.

To test chemical burns, hydrochloric acid was used at both a 12.3M concentration and a 6.2M concentration. The 12.3M solution was the stock solution provided and thus the highest concentration available for this experiment. The 6.2M solution was created by diluting 50mL of the 12.3M stock solution with 100mL of distilled water. To do this dilution, the formula \(C_1V_1 = C_2V_2\) was used to determine how much water would be needed for a half-dilution. The measurements were carried out using graduated cylinders, and thus the two solutions were created and applied to the tissue. During the application process, scientists left the tissue submerged in the acid solution for 1, 3, 5, 8, and 10-minute intervals because of its fast-acting severity. Submersion was followed by a wash in distilled water for approximately 60 seconds before storage in formaldehyde.

All the above samples were stored in 37% formaldehyde for at least 24 hours before examination to ensure fixation of the tissue had occurred.

The Scanning Electron Microscope (SEM) is then used to analyze the tissues. In this experiment, the FEI Quanta 200 FEG (2005) model was used. The SEM produces images of a sample by barraging a focused beam of electrons onto the sample, allowing interaction between the electrons and the atoms within the tissue to produce a topographical image of the sample (Danilatos, 1994). Similarly, the Environmental SEM allows this same process to happen but includes a cooling and drying stage (using a gaseous environment) to collect electron micrographs of a “wet” specimen (i.e. epithelial tissue that has been stored in a preservative
chemical) in a low vacuum setting. This method was chosen for this experiment due to its high magnification capabilities, allowing the experimenter to view the epidermis at a high resolution thereby revealing surface-level details less than 5nm in size.

The relevant settings for the ESEM are as follows: minimum pressure of 800Pa, maximum of 1300Pa whilst pumping (6 cycles), the target temperature of the stage is 2°C, high voltage is set at 10kV, spot size is 3.9, and final pressure is 500Pa.

Results

Once all tissues were burned, they were analyzed under the ESEM and photographed to observe results. Photos were taken at 350, 500, 750, 1000, and 1500 magnification. However, the images taken at 1000 and 1500 are extremely blurry due to the composition of the mammalian tissue (which does not bond electrons well) and are more likely to have artifacts caused by the electron bombardment. These images are included in the results for posterity, but do not reveal any excess information that cannot be obtained at 350 to 750 magnifications.

Figure 1 depicts the control sample (with no burn damage) at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. The skin looks very smooth and porous with few bubbles (mountains), which appear lighter in colour and/or closer to the lens.
Figure 2: Control Site 2 (no damage)

Figure 2 depicts the control sample (with no burn damage) at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. The skin still appears smooth, but has more mountainous bubbling.
Figure 3: Thermal (frozen, 1 hour) Site 1

Figure 3 depicts the thermal (frozen, 1 hour) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Many large bubbles with shrunken tissue between the mountains.
Figure 4: Thermal (frozen, 1 hour) Site 2

Figure 4 depicts the thermal (frozen, 1 hour) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. Smoother area than Figure 3, but still shows artifacts in the form of small bubbles which can be seen at 750 magnifications.
Figure 5: Thermal (frozen, 2 hour) Site 1

Figure 5 depicts the thermal (frozen, 2 hours) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Tissue contains many pockets (holes) which are dark spots on the tissue and appear farther from the lens. This sample exhibits tearing.
Figure 6: Thermal (frozen, 2 hours) Site 2

Figure 6 depicts the thermal (frozen, 2 hours) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. Tissue contains bubbled areas surrounding deep pocketing.
Figure 7: Thermal (frozen, 3 hours) Site 1

Figure 7 depicts the thermal (frozen, 3 hours) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Large bubbles are seen once again. The tissue also appears extremely smooth, but small pockets are seen in the 750-magnification image.

Figure 8: Thermal (frozen, 3 hours) Site 2
Figure 8 depicts the thermal (frozen, 3 hours) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. This sample does not contain bubbling or pocketing but consists of tearing.

Figure 9: Thermal (heat, 5 minutes) Site 1

Figure 9 depicts the thermal (heat, 5 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Large bubbles seen throughout, but many more small bubbles can be seen in higher magnifications.
Figure 10: Thermal (heat, 5 minutes) Site 2

Figure 10 depicts the thermal (heat, 5 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. Bubbling becomes slightly smaller, pocketing more frequent. The 1000 magnification is not available for this sample.

Figure 11: Thermal (heat, 10 minutes) Site 1
Figure 11 depicts the thermal (heat, 10 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Small bubbling extremely common making the tissue look very rough. Some larger bubbling.

![Image of thermal sample at site 1](image1.jpg)

Figure 12: Thermal (heat, 10 minutes) Site 2

Figure 12 depicts the thermal (heat, 10 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. Many small pockets. Few small bubbles.

![Image of thermal sample at site 2](image2.jpg)
Figure 13: Thermal (heat, 15 minutes) Site 1

Figure 13 depicts the thermal (heat, 15 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Many bubbles of small to medium sizes, skin appears extremely irritated.

Figure 14: Thermal (heat, 15 minutes) Site 2
Figure 14 depicts the thermal (heat, 15 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. Irritation more severe, bubbling and pocketing rampant.

Figure 15: Thermal (heat, 20 minutes) Site 1

Figure 15 depicts the thermal (heat, 20 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Small bubbles even more common, small pockets seen as well.
Figure 16: Thermal (heat, 20 minutes) Site 2

Figure 16 depicts the thermal (heat, 20 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. More pockets than bubbles observed. Pockets are deeper than the previous figure.

Figure 17: Chemical (acid 12.3M, 1 minute) Site 1
Figure 17 depicts the chemical (acid 12.3M, 1 minute) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Many small bubbles seen, some large. Irritation.

Figure 18: Chemical (acid 12.3M, 1 minute) Site 2

Figure 18 depicts the chemical (acid 12.3M, 1 minute) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. Irritation more severe. Many small bubbles.
Figure 19: Chemical (acid 12.3M, 3 minutes) Site 1

Figure 19 depicts the chemical (acid 12.3M, 3 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Deep pocketing. Some fat layer can be seen.

Figure 20: Chemical (acid 12.3M, 3 minutes) Site 2
Figure 20 depicts the chemical (acid 12.3M, 3 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. Deep pocketing (hole right through to the fat layer which can be observed).

Figure 21: Chemical (acid 12.3M, 5 minutes) Site 1

Figure 21 depicts the chemical (acid 12.3M, 5 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Deep pocketing with small bubbles inside of the pockets.
Figure 22: Chemical (acid 12.3M, 5 minutes) Site 2

Figure 22 depicts the chemical (acid 12.3M, 5 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. Large pocket seen in upper left of 350 magnification. Small bubbles seen inside the pocket, skin appears irritated.

Figure 23: Chemical (acid 12.3M, 8 minutes) Site 1
Figure 23 depicts the chemical (acid 12.3M, 8 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Chasm from acid formed, pocket deemed enough to see the fat layer. String of epithelial tissue can be seen across the chasm.

Figure 24: Chemical (acid 12.3M, 8 minutes) Site 2

Figure 24 depicts the chemical (acid 12.3M, 8 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. Very deep crevasse caused by acid. Epithelial tissue attached to both sides. Large bubbles.
Figure 25: Chemical (acid 12.3M, 10 minutes) Site 1

Figure 25 depicts the chemical (acid 12.3M, 10 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Large bubbles seen. Tearing appears in all images.

Figure 26: Chemical (acid 12.3M, 10 minutes) Site 2
Figure 26 depicts the chemical (acid 12.3M, 10 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. Many bubbles surrounded by deep pockets. Fat layer can be seen in the 750 magnifications and above.

Figure 27: Chemical (acid 6.2M, 1 minute) Site 1

Figure 27 depicts the chemical (acid 6.2M, 1 minute) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Many large bubbles surrounded by deep pockets. The fat layer can be seen in 500 and 750 magnifications.
Figure 28: Chemical (acid 6.2M, 1 minute) Site 2

Figure 28 depicts the chemical (acid 6.2M, 1 minute) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. Tearing is seen in all images. Large bubbles also seen. Pocketing is not very deep.

Figure 29: Chemical (acid 6.2M, 3 minutes) Site 1
Figure 29 depicts the chemical (acid 6.2M, 3 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Pocketing becomes deeper. Tearing still seen.

Figure 30: Chemical (acid 6.2M, 3 minutes) Site 2

Figure 30 depicts the chemical (acid 6.2M, 3 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. Pocketing goes through tissue, created holes. Many large bubbled areas with irritated tissue in-between.
Figure 31: Chemical (acid 6.2M, 5 minutes) Site 1

Figure 31 depicts the chemical (acid 6.2M, 5 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Pocketing not as deep but more frequent. Bubbles large and tearing is seen on top of the largest bubble.

Figure 32: Chemical (acid 6.2M, 5 minutes) Site 2
Figure 32 depicts the chemical (acid 6.2M, 5 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. Tearing is seen on top of all the bubbles. Pockets do not appear any deeper than Figure 31, but fat layer can be seen.

![Image of chemical sample at different magnifications](image.png)

Figure 33: Chemical (acid 6.2M, 8 minutes) Site 1

Figure 33 depicts the chemical (acid 6.2M, 8 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Deep pocketing with extreme irritation (many small bubbles) inside.

![Image of chemical sample at different magnifications](image.png)
Figure 34: Chemical (acid 6.2M, 8 minutes) Site 2

Figure 34 depicts the chemical (acid 6.2M, 8 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. Hole-like pocketing, many large bubbles. Topography goes from very high (bottom left of 350 magnification) to very low (hole). Minimal tearing observed on bubbles.

Figure 35: Chemical (acid 6.2M, 10 minutes) Site 1
Figure 35 depicts the chemical (acid 6.2M, 10 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 1. Few large bubbles surrounded by pocketing and many small irritated bubbles. Tearing observed on all large bubbles (refer to 500 magnification). Few large bubbles surrounded by pocketing and many small irritated bubbles. Tearing observed on all large bubbles (refer to 500 magnification).

Figure 36 depicts the chemical (acid 6.2M, 10 minutes) sample at 350, 500, 750, 1000, and 1500 (left to right, top to bottom) magnification at site 2. Topography extremely bubbled, large pockets between all bubbles. Tearing seen in 350, 500, and 750 magnifications.

Through this research, it can be concluded that as the time interval increases, more artifacts are observed on the epithelial tissue. Due to this, the examiner can determine an approximate time interval of exposure. The best way to determine the period the tissue was exposed is to examine the bubbling and pocketing in the skin.

Discussion

As aforementioned, while the time of exposure increases, the number of artifacts seen on the tissue also increases. In the cold burns, it was seen that the bubbling in Figures 3-8 was very large, with only small bubbles seen occasionally. This bubbling is due to water in the fat layer which had frozen and created large mountainous lumps of crystallized water in the tissue. Once the water melted, it left these large mountains where the tissue around it had shrunk due to the cold temperature. This freeze-thaw cycle is consistent with the tissue freezing exhibited by Schäfer. Similarly, in the heat, small bubbles were seen throughout (Figures 9-16). These bubbles could also be due to water, but rather than freezing, the water boiled off leaving the artifacts seen in Figure 9. The water boiling out of the tissue left a slight blistering effect on the epidermis, leaving many small pockets and small bubbles.
Lastly, the chemical burns were observed. In these cases, many small bubbles were seen at first, as if the skin is irritated. Once the exposure time increases, deep pocketing is seen (Figures 20, 24, 29, 30, 33, 35, and 36). In Figure 24 we can see that the tissue has been cleaved in half by the acid eating away at it, leaving a crevasse in the epithelial layer. The fat layer can also be seen in these trials. In Figure 27, a “smooth” or “blurry” part of the tissue can be seen (specifically at 750 magnification) wherein the epithelial tissue has been eaten away and the fat is visible.

In the 6.2M experiment it observed that the tissue seemed to be more severely affected than the 12.3M acid experiment. This could be due to the water added to dilute the solution. In this case, the water could have created a barrier between the acid and the environment, allowing the water to evaporate first before the acid could be removed from the tissue. Due to this, the acid would be spread out for a longer period than on the 12.3M tissue.

This research contains a few variables which could have affected results. The first constraint was the pig tissue that was used for experimentation. Generally, the pig tissue is fattier than human, which is why the hock of a pig was used (to reduce fat layer differences). However, the layer of fat in this experiment was not a constant and therefore was not controlled for. This could have affected the frozen tissue, for example, by increasing the amount of water that was available in the fat layer underneath thereby increasing or decreasing the number of artifacts in the tissue. Penultimately, as mentioned earlier, the ESEM can leave artifacts when the sample is under the beam for too long. In the control sample, it was noted that if the electron beam was left on a specific area for longer than 10 minutes, the bubbles would disappear slowly, creating a pocket in the tissue. These artifacts were often seen in the 1000 and 1500 magnification due to the electron beam becoming more focused on an area for a long period of time. Lastly, the time constraint of this experiment left more time intervals to be desired. If the amount of time intervals could be extended, a more accurate representation of what the skin looks like at certain intervals could be obtained.

**Conclusion**

The results of this experiment support the hypothesis that as exposure time is increased, the artifacts on the tissue will also increase. However, due to the time constraint and funding limitations of this research, more time intervals will be needed to justify the approximation of time exposed.
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References


