Effects of Different Types of Water on the Degradation Rate of Human DNA in Bone and Tissue

Ema Graham and Michael S. Adamowicz, Ph.D.

University of New Haven - Department of Forensic Science

Henry C. Lee College of Criminal Justice and Forensic Sciences

300 Boston Post Rd. West Haven, CT 06516

Abstract

Human remains that have been subjected to aqueous environments for periods of time are often used for DNA analysis of the tissue and bone for identification purposes. This has posed a problem for investigators in the past due to the degradation and loss of DNA in the aqueous environmental conditions. The purpose of this research was to determine the quantity of viable DNA that can be obtained from human bone and tissue after a 72-hour period of immersion and whether or not a DNA profile can be made. Also, this research studied how different types of water environments such as saltwater, swamp water, or freshwater effect the amount of DNA loss and degradation over the set period of time. In this study human bone and tissue samples were placed in three aqueous environments (saltwater, swamp water, and freshwater) and allowed to incubate for 72-hours. These samples were then taken out and their DNA was extracted, quantified, amplified, and analyzed. The degradation and loss of DNA was studied for each sample of bone and tissue in comparison to a control sample that was not placed in water. It was found that there was significant DNA degradation and loss in both tissue and bone samples that were immersed in water for 72 hours. The bone samples showed on average a ~10,000-fold reduction of detectable DNA. The bone sample that was immersed in saltwater showed such extensive DNA degradation and loss that it was unable to even detect any viable DNA at all. As for the tissue there was significant DNA loss as well. For the control sample (dry sample) there was little to no DNA loss; ~341.8 ng/µL of DNA detected. The tissue samples showed much less detectable DNA than the control sample; ~7.31 ng/µL (freshwater), ~0.77 ng/µL saltwater, and ~3.66 ng/µL swamp water. These findings were consistent with the data collected in Shanae Armstrong's Master's Thesis [1], and support the theory that there is considerable DNA loss and DNA degradation after 24 hours of exposure.

Introduction

In areas along the shore or near larger bodies of water it is not uncommon for forensic investigators to find human remains that have been submerged. When remains are found submerged in water investigators rely heavily on DNA to help in the identification process. In situations such as national disasters involving water or large accidents, such as a plane crash or a boat sinking, it is vital for the remains of the victims to be identified. On March 8th, 2014 Malaysia Airlines Flight 370 went missing. It has since been theorized that the plane had crashed somewhere in the ocean but the remains of the plane and the victims have yet to be found. When the wreckage is discovered, especially considering the intensity of the crash, the bodies of the victims will be highly decomposed and battered. It will be very difficult to identify the remains of the victims by pure visual identification. Investigators will rely on different methods of identification, such as DNA analysis to try to identify the remains of the victims. Other incidents with mass victims, such as the Tsunami in Indonesia on December 26th 2004, and Hurricane Katrina in August of 2005 required the timely identification of the remains. DNA identification of victims was utilized. Many of these victims had been exposed to water environments for extended periods of time. The exposure to long periods of immersion made DNA analysis difficult.

For remains that have been submerged in bodies of water extensive DNA degradation has occurred. By determining the effect of different types of water on how quickly DNA from bone and tissue samples of human remains degrade, it will aid investigators in identifying remains that came out of aqueous environments. The soft tissue begins to detach from the bone and is either consumed by organisms living in the environment, taken away by currents, or is decomposed. Since there is such a low chance of there being viable soft tissue on remains, investigators largely rely on DNA analysis from bones.

"DNA degradation results from strand breakage, chemical modifications, and microbial attack. These degradative processes reduce the yield of high molecular mass DNA molecules and increase the chance of subsequent PCR failure" [2]. Of the many factors that lead to DNA degradation, one of the biggest factors in aqueous environments is damage due to hydrolysis. DNA has a high affinity to water and even after death DNA in dead tissues will continue to attract water molecules. When deceased bodies are submerged in large amounts of water for long periods of time, there is a high chance of damage due to hydrolysis.

Hydrolysis does not only happen in soft tissues but it also can occur in skeletal material as well. Water can enter bone through a process called *bone dissolution*. As this occurs the pores of the skeletal material "become larger and allow for hydraulic flow, leading to a greater loss of bone material. The greater the dissolution of the inorganic component of the bone, the greater the chance of DNA loss as the DNA molecules dissociate from the protection of the hydroxyapatite"[3]. Due to hydrolysis that occurs in bone and soft tissues DNA can become damaged and unable to be used for further investigation and analysis.

Experimental Setup

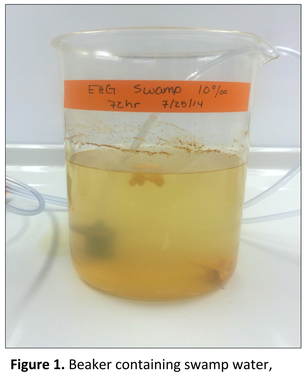

Figure 1. Beaker containing swamp water, human tissue and bone sample, and aeration system

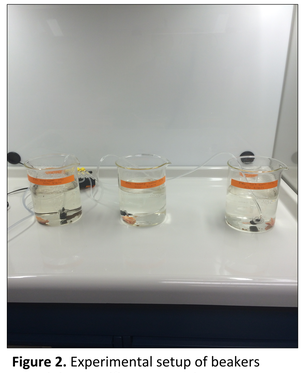

Figure 2. Experimental setup of beakers containing specific water environments (freshwater, swamp water, and salt water), human bone and tissue samples and aeration system. Experimental hood also contains beaker containing control sample

Results

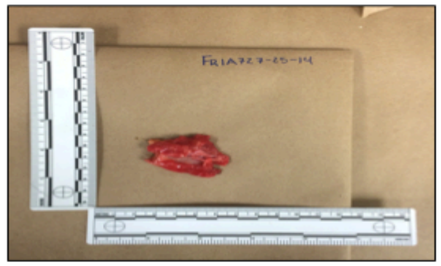

Figure 3. Human bone and tissue sample before being placed in freshwater environment for 72 hours.

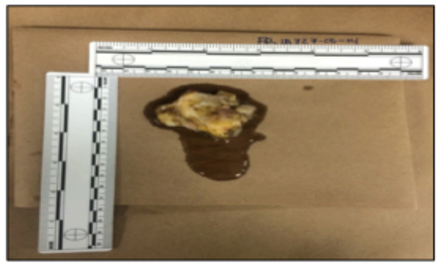

Figure 4. Human bone and tissue sample after being placed in freshwater environment for 72 hours.

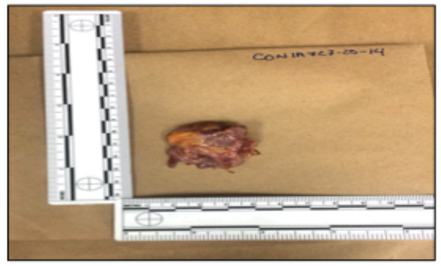

Figure 5. Control sample after 72 hours (dry environment).

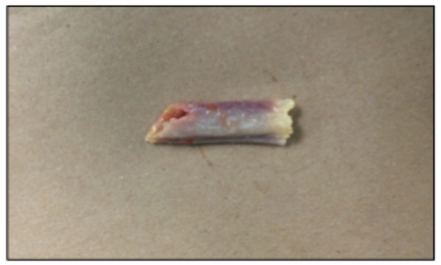

Figure 6. Defleshed bone sample after being placed in aqueous environment for 72 hours.

Quantity of DNA Detected in Human Tissue After 72-Hours

Figure 7. DNA quantification results from the human tissue samples represented in ng/µL. Freshwater, swamp water, and salt water all showed a dramatic loss of DNA over the 72-hour period. This shows that aqueous environments had a large affect on the DNA degradation in this specific time period.

Quantity of DNA Detected in Human Bone After 72-Hours

Figure 8. DNA quantification results from the human bone samples represented in ng/µL. Freshwater, swamp water, and salt water all showed a dramatic loss of DNA over the 72-hour period. This shows that aqueous environments had a large affect on the DNA degradation 72-hours.

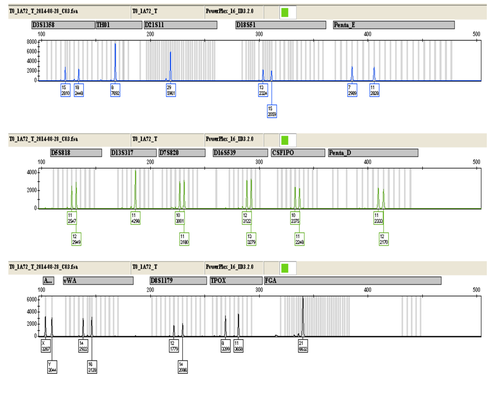

Figure 9. Electropherogram of time-zero tissue sample. This shows a DNA profile of human tissue that has been subjected to decomposition

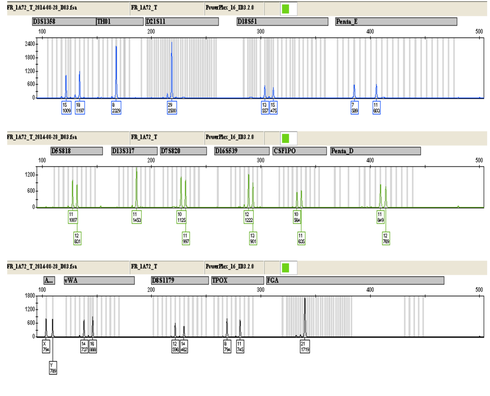

Figure 10. Electropherogram of tissue sample after being incubated in freshwater environment for 72 hours. This shows slight DNA loss in the tissue after being placed in an freshwater environment for 72 hours. Even though DNA loss is present, after 72 hours a DNA profile is still obtainable.

Materials and Methods

Human bone and tissue samples were collected from the Yale School of Medicine Department of Pathology (New Haven, CT) and stored at -20°C until needed. Experimental water was collected from the New Haven Sound and a local freshwater lake. The salinities of the water types were measured using a portable refractometer. Salinity was adjusted by either diluting with deionized water or by adding salt (Instant Ocean® Sea salt). The salinities used were 0 parts per thousand (ppt) (freshwater), 10ppt (salt fen), and 35ppt (saltwater). 1200-1400mL of water was added to three 2000mL beakers and they were labeled accordingly. Each beaker was then aerated by using a pump-system connected to an air stone. 1-2 inch sections of human rib were weighed and photographed. One rib sample was then placed in each beaker and one rib sample was placed in a 250mL beaker containing no water, to act as a control. The samples were incubated in water for 72 hours with water changes every 24 hours. Once the 72-hour period was completed the rib samples were removed, photographed, and weighed. The samples were then defleshed and tissue samples collected.

The bone was pulverized using a SPEX SamplePrep 6770 Freezer/Mill®. 0.5M EDTA at a pH of 8.0 was added to 0.3g – 0.7g of bone powder and was then lysed for 16 hours at room temperature. The bone powder was decalcified by following the procedures stated by the "Connecticut Department of Emergency Services and Public Protection in the Division of Scientific Services for the Forensic Laboratory" [4]. After decalcification DNA was extracted from the bone powder using the "Isolation of Total DNA from Bones and Teeth" protocol from the Qiagen QIAamp® DNA Investigator Handbook [5]. DNA was then extracted from the tissue using the "isolation of total DNA from tissues" protocol from the Qiagen QIAamp® DNA Investigator Handbook [6].

DNA from the bone powder and tissue was quantified using the Quantifiler™ Human DNA Quantification Kit from Applied BioSystems. DNA was amplified using the Promega PowerPlex® 16 HS Kit and the Applied BioSystems® GeneAmp PCR System 9700 thermal cycler. Amplified samples were prepared for injection by using 9.5 µL of Hi-Di™ formamide and 0.5 µL of internal lane standard (ILS600). Separation and detection of the amplified fragments was preformed on an Applied BioSystems® Prism 3130*xl* Genetic Analyzer. The data produced from the BioSystems® Prism 3130x1 Genetic Analyzer was analyzed using the Applied BioSystems® Genemapper ID v.3.2.1 software.

Discussion

Significant DNA loss was observed in the bone samples treated in all three water environments. The starting quantity of DNA in the bone (at time zero) was ~36.02 ng/µL. ~0.003 ng/µL of DNA was detected for bone samples that were incubated in freshwater for 72-hours. This was a significant loss of DNA; ~10,000 fold. ~0.02 ng/µL of DNA was detected for bone samples that were incubated in saltwater for 72-hours; ~10,000 fold. No detectable DNA was found for bone samples incubated in swamp water. The time control bone sample (incubated dry) exhibited some DNA loss, but it was not as significant as the values of the bone samples that were placed in water; ~1.12 ng/µL of DNA (~36 fold).

The tissue from the rib samples closely resembled the findings from that of the bones. The control tissue sample (dry) yielded ~341.8 ng/µL of DNA. ~7.31 ng/µL of DNA was detected for tissue samples that were incubated in freshwater for 72-hours; ~50 fold. ~3.66 ng/µL of DNA was detected for tissue samples that were incubated in swamp water for 72-hours; ~70 fold. ~0.77 ng/µL of DNA was detected for tissue samples that were incubated in saltwater for 72-hours; ~350 fold.

It was found that there were large amounts of DNA loss in both in bone and tissue from samples that were incubated in all three water environments for 72-hours. The bone samples showed much more extensive DNA loss than that of the tissue samples. There is less DNA in bone samples to begin with, resulting in proportionally larger DNA loss. The saltwater environment showed the most amount of DNA loss out of all three. This was consistent in both the bone samples and the tissue samples. From these results it is conclusive that there is a dramatic loss of DNA in human remains that have been immersed for 72 hours.

Conclusions

The 72-hour time period is very important in the timeline of DNA loss of human tissue and bone in aqueous environments. In the research previously done by Shanae Armstrong [1], it was found that there was a critical loss of DNA in between the time periods of 24 hours and 1 week. The results of the 72-hour experiment were consistent with this previous data. It was found that there was not as extensive DNA degradation but more DNA loss, especially in the saltwater samples. When compared to the control and time zero samples it is indicative that there is much more substantial DNA loss and decomposition due to the aqueous environment, proving that the types of water do in fact have an affect on the human DNA.

References

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