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

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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 affect 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. The 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 a previous study, 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. The exposure to long periods of immersion made DNA analysis difficult.

When remains are exposed to aqueous conditions for periods of time 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 that have been submerged in water for long periods of time investigators largely rely on DNA analysis from skeletal remains.

After DNA is extracted a forensic DNA analyst will perform an amplification process on the DNA called *Polymerase Chain Reactions*, also commonly known as PCR. PCR essentially acts as a highly efficient copy machine for DNA to make multiple copies of DNA so that it can undergo further testing and analysis.

If there is not enough viable DNA in a sample then PCR cannot be performed and DNA analysis is not viable. When bodies are subjected to water the amount of DNA in skeletal and soft tissues such as skin that is available for PCR is decreased overtime due to many different factors.

"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" [1]. Of these many factors that lead to DNA degradation, one of the biggest factors in aqueous environments is damage due to hydrolysis, or the breakage of chemical bonds through the addition of water [1]. When hydrolysis occurs it can result in damage to the DNA, which is referred to as *deamination* (when there is a loss of a amine group), *depurination* (when there is a loss of an adenine and guanine group), and or *depyrimidination* (when there is a loss of thymine and cytosine) [1]. Deamination, depurination, and depyrimidination will result in damage to the DNA and inhibit the PCR process. 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

are employed by the Connecticut Department of Emergency Services and Public Protection in the Division of Scientific Services for the Forensic Laboratory [4]. The DNA from the decalcified bone powder was then extracted using the "isolation of total DNA from tissues" protocol outlined in the Qiagen QIAamp® DNA Investigator Handbook [3].

The DNA extracts from the bone and tissue samples were then quantified using the Quantifilier™ Human DNA Quantification Kit from Applied Biosystems [5]. The following series of dilutions of known human DNA were used to produce a standard sizing curve for quantification: 50 ng/µL, 16.67 ng/µL, 5.56 ng/µL, 1.85 ng/µL, 0.62 ng/µL, 0.21 ng/µL, 0.068 ng/µL, and 0.023 ng/µL. A master mix containing 10.5 µL per reaction of Primer mix and 12.5 µL per reaction of Reaction mix was made. 2 µL of each standard, sample, and negative control (DNAase free H_2O) were pipetted out into individual wells in a 96-well plate. 23 µL of the master mix was then added to each well containing sample, standard or a control. The well plate was then sealed using optically clear plate tape and then centrifuged for a minute to eliminate any bubbles that were present at the bottom of the wells. The plate was then placed in an Applied Biosystems® 7500 Real-time PCR System (Foster City, CA). The samples were then quantified and the results were analyzed using the 7500 System SDS Software (Foster City, CA). The settings that were used for analysis were a 0.2000 Threshold, Manual Ct and Autobase line for all reactions. The slope of the standard curve was checked and had to be close to -3.32 with a R^2 value greater than 0.98 or the run was rejected and the quantification process was preformed again.

From the results obtained from quantification, dilutions for the DNA extracts were calculated so that the mass of DNA was 1 ng. The appropriate dilutions for each sample were pipetted out, including the negative control. Each sample was then amplified using the Promega PowerPlex® 16HS Kit (Madison, WI). A master mix was made that contained 5 µL per reaction of 5X master mix and 2.5 µL per reaction of primer pair mix. PCR tubes were obtained and 7.5 µL of master mix and the appropriate volume of sample and water were pipetted into the PCR tube to achieve a total volume of 25 µL. The samples were then amplified in the Applied Biosystems® GeneAmp PCR System 9700 thermal cycler (Foster City, CA). All samples, including the negative control, were run for 30 cycles and the recommendations for amplification from the manufacturer of the PowerPlex® 16HS Kit were used [6].

To prepare the amplified samples for injection, 9.5 µL of Hi-Di™ formamide and 0.5 µL of internal lane standard (ILS600) were pipetted into individual wells of a clean 96-well plate. For each module, 1 µL of allelic ladder was placed into two of the wells. Then in the remaining wells 0.5 µL to 1 µL of amplified sample products was added. Any of the remaining wells that were not being used (did not contain allelic ladder nor amplified sample) were filled with 10 µL of Hi-Di™ formamide. A rubber septum was placed on the well plate in order to seal it, and then the well plate was centrifuged to bring all samples to the bottom of each well. The 96-well plate was then placed in the Applied Biosystems® GeneAmp PCR System 9700 thermal cycler (Foster City, CA) for 6 minutes to denature the samples. After denaturation the 96-well plate was then placed in the Applied Biosystems® Prism 3130xl Genetic Analyzer (Foster City, CA) to separate and detect each sample. Each injection was run for five seconds at 3kV following the manufacturer's recommended settings.

The data from the samples that were separated using the Applied Biosystems® GeneAmp PCR System 9700 thermal cycler (Foster City, CA) was analyzed and edited using the Applied Biosystems® Genemapper ID v.3.2.1. software (Foster City, CA). All the electropharagrams were assessed and edited to eliminate allelic drop out, allelic drop in, and artifacts. The parameters for analysis were set at a minimum peak height of 50 relative fluorescent units (RFU) for the blue, green, yellow, and red channels. The sizing algorithm used was the Local Southern method. "The data from the electropharagrams was assessed based on the number of correct alleles present, the number of loci that has a 70% or more peak height balance and how the average peak height between the smallest locus (D3S1358) and the largest locus (FGA) differed."[7].

Results

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 1d ((7b)62u(n)12lu)-</61 22i34h3(s >() tu/t5p)12lut0 P 0Ñ= @

Figure 4. Electropherogram of tissue sample after being incubated in freshwater environment for 72 hours. Even though the sample showed a large loss in

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