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EXECUTIVE SUMMARY

The State of the Problem

Human skeletal remains are useful specimens for forensic DNA analysis in a variety of situations when other tissue types are not available, such as when analyzing buried or decomposed skeletal remains (Alonso *et al*., 2005; Budowle *et al*., 2005; Holland *et al.,* 2003; Lawler, 2001). Prior to isolating DNA from bone samples, the cleaning of the bones is required to remove the potential of having co-mingled remains and contamination by physical contact (Sampietro *et al.,* 2006; von Wurmb-Schwark *et al*., 2008; Zehner, 2007). Additionally, polymerase chain reaction (PCR) inhibitors and bacterial contamination that interfere with forensic DNA analysis must be removed during the cleaning process (Calacal *et al.,* 2005). The outer surface of the bone fragment usually is cleaned using manual mechanical cleaning methods, such as sanding the outer surface of the sample.

However, the bone dust generated by sanding can potentially increase the risk for cross-contamination between samples (Kitayama *et al*., 2010). Especially when the bone surface is porous and fragile, surface cleaning using a mechanical method is not possible. Moreover, the sanding method cannot easily be used to process multiple samples simultaneously, and it is difficult to adapt for automation. Therefore, the processing of bone specimens is more labor-intensive and time-consuming than other types of specimens, such as bodily fluids and soft tissues. Thus, developing a high-throughput bone processing method, a "gap" in forensic DNA testing, is highly desired.

The Purpose of the Study

To address these issues, an enzymatic cleaning method using trypsin solution was evaluated. Trypsin, secreted in the digestive system, is a proteolytic enzyme which degrades various types of proteins (Buck *et al*., 1962; Walsh, 1970). In our previous study, the trypsin technique was characterized and adapted to the sample cleaning method prior to DNA isolation from fresh bone samples (Li *et al*., 209; Li & Liriano, 2011). Our data suggest that this method can be potentially used in the initial sample preparation for cleaning the outer surface of human bone samples, which results in a powder-less method and can process multiple samples at the same time. In this study, the application of the trypsin cleaning method for DNA isolation was studied in samples that are more typically encountered in actual forensic cases. The yield and the quality of DNA extracted from challenged bones was compared between the mechanical (sanding) and enzymatic (trypsin) method side-by-side.

Our *goal* of this proposed study is to develop a high throughput, low cost, less time-consuming and labor-intensive method for bone processing than existing methods. Additionally, this improved method should avoid cross-contamination and have low health risks. Thus, the method should improve the effectiveness of processing of skeletal remains from mass fatality incidents and missing-person casework for the forensic community and law enforcement agencies. Our *objective* is to make an improvement to existing methods of processing bone samples by developing a simple

trypsin method for processing challenged bone specimens prior to DNA isolation. In particular, the study consisted of two parts: first, to characterize the effect of trypsin treatment on the yield and the quality of DNA isolated and, second, to characterize the effect of trypsin treatment on the quality of DNA profiling.

Method and Research Design

Sample Preparation. In this study, challenged human bone specimens were used. Specimens (a similar sample size as in Loreille *et al*., 2007) were selected and represented a variety of bone quality for this study. For instance, aged bones (including buried bones) were included. Bones ranging in age from 50 to over 100 years post-mortem were selected for this study. Different types of bones were included (such as cranium, rib, and tibia). Additionally, bones exposed to potential insults (such as exposed to high heat, humidity, potential bleaching or boiling) were included.

A pair of bone fragments (approximately 1 g each) was dissected from each bone specimen. A pair of bone fragments was then processed using the sanding and trypsin method separately for pair-wise comparisons. A paired-sample t-test (two-tail) was conducted to compare the data from the sanding and trypsin methods in this study.

The Characterization of the Effects of Trypsin Treatment on the Yield of DNA Isolated. To characterize the effect of trypsin treatment on the yield and the quality of DNA isolated, DNA extracts were compared side-by-side: 1) DNA extracts isolated using the sanding method, and 2) DNA extracts isolated with the trypsin treatment. To find out if this trypsin method will achieve sufficient DNA yield for forensic DNA profiling, total DNA, was isolated and quantified. PCR inhibitors cause inhibitions in PCR-based forensic DNA analysis. To evaluate the capabilities of the cleaning effect of the bone processing method, the presence of environment-born inhibitors in the DNA extracts was measured by monitoring the amplification of the internal positive control (*IPC*).

The Characterization of the Effects of Trypsin Treatment on the Quality of STR Analysis. To find out if this processing method causes any adverse effect on DNA, such as DNA degradation, the quality of DNA profiles was evaluated. DNA samples were examined using short tandem repeat (STR) analysis. In highly degraded specimens, STR analysis is often difficult due to the high degree of DNA fragmentation. To increase success in STR analysis, we utilized AmpFℓSTR® MiniFiler™ amplification kit (Applied Biosystems) with primer pairs that produce shorter amplicons.

To evaluate the integrity of the DNA isolated, the number of allele calls was compared. The integrity of the DNA samples was also examined by comparing the average peak height values of each allele at each locus between sanded and trypsin-treated samples.

The Characterization of the Effects of the Trypsin Treatment on the Amplification of Mitochondrial DNA (mtDNA). The characterization of the effects of the trypsin method on the quality of mtDNA analysis results was carried out. To amplify the