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TECHNICAL NOTE

Roshale Gaytmenn,[1] M.Sc. and David Sweet,[2] D.M.D., Ph.D.

Quantification of Forensic DNA from Various Regions of Human Teeth*

ABSTRACT: When the use of traditional forensic identification methods such as fingerprints or dental radiographs is difficult or impossible, identification by DNA analysis has proven valuable. In situations such as explosions or airplane crashes, identification is even more difficult because human remains are often fragmented and may be commingled. Teeth are a useful source of DNA and can often survive extreme environmental conditions. However, teeth may be fragmented into several identifiable regions. Therefore it is important to determine if DNA is present in forensically significant yields in all regions of the tooth. The main objectives of this study were to determine which region(s) of the tooth contains quantifiable DNA, if all regions contain similar yields of DNA and whether there is enough DNA in all regions to justify DNA extraction from a found tooth fragment. Results demonstrate that there is sufficient quantity of DNA in the crown body, root body, and root tip to support DNA extraction. Additionally, the root body is the region with the highest yield of DNA. This information will aid forensic DNA analysts in producing a useful DNA profile in a timely and cost-effective manner.

KEYWORDS: forensic science, teeth, DNA, human identification, fragmented

The identification of found human remains may be critical for an investigation, insurance purposes or estate settlement, as well as providing closure for loved ones. In situations such as explosions or airplane crashes, human remains are frequently fragmented and may even be commingled. Therefore, identification by traditional forensic methods such as fingerprints or comparison between antemortem and postmortem dental radiographs is often difficult or impossible. As a result, forensic scientists often turn to DNA analysis. Teeth have been recognized as a valuable source of DNA because the tongue, jaws and dental enamel often protect them. Since teeth may be fragmented during explosions or airplane crashes, it is important to determine if DNA is present in forensically significant yields in all regions of the tooth. This information will aid forensic DNA analysts in producing a usable DNA profile in a timely and cost efficient manner.

This study was undertaken to determine if all regions (crown tip, crown body, root body and root tip) contain similar yields of DNA and whether there is enough DNA in all four regions to justify DNA extraction from a found tooth fragment.

[1] Present address: Florida Department of Law Enforcement, 4211 N. Lois Avenue, Tampa, FL.

[2] Bureau of Legal Dentistry, #146 - 2355 East Mall, Vancouver, BC, Canada, V6T 1Z4.

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Methods

Two hundred fifty recently extracted permanent human teeth were collected from the offices of oral and maxillofacial surgeons and general dental practitioners in British Columbia, under the conditions set out by the Clinical Research Ethics Board of the University of British Columbia. One of the University's requirements for experiments that deal with human subjects, including samples recovered from human subjects (extracted teeth), is to gain prior approval for the research protocol from the Clinical Research Ethics Board. An application was submitted to the Board outlining the purposes and objectives; the subject recruitment and selection process; exclusionary protocols; methodology and procedures; facilities required and available; risks and benefits to the subjects; discomfort or known side effects; who has access to the specific data, etc. An application with the detailed experimental protocol was submitted on July 17, 1998 and the Board issued a certificate of approval on September 8, 1998.

To ensure identical treatment of the teeth, all dental personnel were instructed to air-dry the teeth only, and not to clean them with any chemicals. The dental staff provided the following information for this study: date of extraction, age of patient, gender of patient and Federation Dentaire Internationale (FDI) tooth number. After the teeth were dry, they were stored at $-20°C$ until grinding. Any dried soft tissue or bone adhering to the tooth was removed. The weight of each tooth was recorded and a radiograph of each tooth was exposed.

Previous studies examining the effect of age on tooth morphology grouped individuals into the following age categories: 11 to 24 years, 25 to 39 years, 40 to 55 years and 55+ years (1). Using this classification format as a guide, adult chronological age has been

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divided into the following groups for convenience for this study: Group A (under 26 years), Group B (26 to 40 years), Group C (41 to 55 years) and Group D (over 55 years).

Fifty-six teeth were eliminated from the study either because the requested information was not provided by the dental staff, or the teeth contained caries or open apices. One hundred ninety-four teeth were decontaminated, sectioned into four regions, cryogenically ground as previously described (2), subjected to DNA extraction by organic extraction (2) followed by concentration in Microcon-100 concentrators (Millipore Canada, Toronto, ON), and quantified using the AluQuant™ Human DNA Quantitation System (Promega Corporation, Madison, WI) (3) on a TD-20/20 Luminometer (Turner Designs, Inc., Sunnyvale, CA) following the manufacturers' protocol. Non-parametric statistical analysis was performed.

Decontamination

The teeth were individually decontaminated in 5.25% sodium hypochlorite for 20 min at RT to remove any contaminating DNA, degraders of DNA or PCR inhibitors such as heme. The sodium hypochlorite was decanted and the individual teeth were soaked in filtered autoclaved water for 20 min at RT to remove any residual bleach. A second decontamination step, rinsing the individual teeth with 95% ethanol for 1 min at RT, was performed. During the third decontamination step, each tooth was dried under a 256 nm ultraviolet light source (Philips TUV 30 W, Microzone Corp., Nepean, Ontario) for 20 min at RT.

Decontamination of tooth fragments should be altered as follows to avoid degrading any DNA exposed to the chemicals during soaking or to the ultraviolet light source. The fragments should be wiped with a paper wipe wetted with sodium hypochlorite, followed by filtered autoclaved water and 95% ethanol. Exposure of the fragment(s) to ultraviolet light should be eliminated.

Sectioning

Using a saw with a diamond blade (Model 1680 16-in. scroll saw, Dremel®, Racine, WI), the teeth were sectioned into four anatomical regions according to Fig. 1. The crown and root were separated at the cemento-enamel junction. The crown was separated into two halves and the root was sectioned into an apical one-third and cervical two-thirds. The sections were termed: i) crown tip, ii) crown body, iii) root body, and iv) root tip. The weight of each tooth section was recorded.

Quantification

DNA quantification was performed utilizing the AluQuant™ Human DNA Quantitation System (sensitive to 20 pg of DNA) according to the manufacturer's protocol. Measurements were taken for 10×, 100× and 1000× dilutions to ensure reliability of the measurement. Using the appropriate multiplication factor, an average DNA yield was calculated for each tooth section.

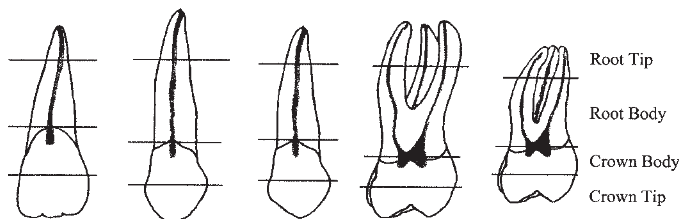

FIG. 1—*Sectioning into anatomical regions of incisors, canines, premolars, first/second molars and third molars, respectively (left to right). Shading illustrates the relative position of the dental pulp.*

Non-parametric Statistical Analysis

SPSS statistical software was utilized to perform the statistical analysis. Since the assumption of normality used in analysis of variance (ANOVA) was violated, the Kruskal-Wallis test (a non-parametric equivalent of the one-way ANOVA test) was utilized to analyze the data. The Kruskal-Wallis test, which relies on rating-scale data, ranked each mean value (DNA yield or concentration) from 1 (lowest) to n (highest). These rankings were then substituted for the raw measured data to get a mean average rank within each group. The Kruskal-Wallis test then compared the mean ranks of the groups and indicated when there was a difference between the groups (4,5). Because the Kruskal-Wallis test did not indicate which of the teeth means (i.e. crown tip versus crown body, crown body versus root body etc. for all group comparisons) were different on DNA yield or concentration, Mann-Whitney tests, as recommended in the SPSS manual, comparing each of the teeth mean ranks for each group to all other groups were implemented (4,5). One drawback to the Mann-Whitney test is that it does not take into account the number of comparisons that must be made (4,5). Thus, utilizing a 1% significance level, 1% of the time a significant result may be observed by chance.

Results

Yield for Each Region by Age Group

The mean DNA yields (ng of DNA) and mean ranks calculated by the Kruskal-Wallis test for each tooth region are shown in Table 1. The root body contained the greatest yield of DNA for all age groups ($p < 0.001$). The crown body resulted in a higher DNA

TABLE 1—*Mean DNA yield and mean ranking for each region within each age group. (The region in each class with the greatest ranking in DNA yield is shown in italic.) The Kruskal-Wallis test compared the mean ranks (not the mean DNA yield) between the regions to determine when there was a difference in DNA yield between the regions. The Mann-Whitney test then determined if the difference was statistically significant.*

Region	Mean (ng DNA)	*n*	Std Deviation (ng DNA)	Mean Rank
		Group A		
Crown tip	70	43	123	29
Crown body	1,213	43	1,453	91
Root body	7,289	43	8,490	*136*
Root tip	1,766	43	4,412	89
		Group B		
Crown tip	93	45	1,135	32
Crown body	1,048	45	1,135	101
Root body	8,195	45	16,610	*148*
Root tip	625	45	1901	79
		Group C		
Crown tip	48	61	216	53
Crown body	658	61	934	134
Root body	4,346	61	9,170	*191*
Root tip	406	61	689	116
		Group D		
Crown tip	522	45	3,346	41
Crown body	344	45	513	85
Root body	8,058	45	28,006	*142*
Root tip	382	45	816	89

TABLE 2—*Mean DNA concentration and mean ranking for each tooth region in each age group. (The region within an age group with the highest ranking in DNA concentration is shown in italic.) The Kruskal-Wallis test compared the mean ranks (not the mean DNA concentration) between the regions to determine when there was a difference in DNA concentration between the regions. The Mann-Whitney test then determined if the difference was statistically significant.*

Region	Mean (ng DNA/g tooth)	*n*	Std Deviation (ng DNA/g tooth)	Mean Rank
		Group A		
Crown tip	214	43	1,570	168
Crown body	6,381	43	1,130	434
Root body	22,178	43	16,612	*633*
Root tip	48,025	43	2,344	388
		Group B		
Crown tip	423	45	1,655	31
Crown body	4,593	45	6,340	93
Root body	17,406	45	26,370	*135*
Root tip	6,537	45	12,113	102
		Group C		
Crown tip	254	61	1,263	47
Crown body	3,700	61	7,047	129
Root body	9,384	61	16,088	*171*
Root tip	5,990	61	9,480	146
		Group D		
Crown tip	784	45	4,673	38
Crown body	1,906	45	2,954	81
Root body	12,094	45	26,820	*129*
Root tip	4,764	45	6,578	109

yield than the crown tip for all age groups ($p < 0.001$). The crown body contained a greater yield of DNA than the root tip in Group B ($p = 0.001$). There was no statistically significant difference between the crown body and root tip for the other age groups. Finally, the root tip contained a higher yield of DNA than the crown tip for all age groups ($p < 0.001$).

Analysis of the data (not shown) showed that 49 out of 194 crown tip samples did not yield quantifiable DNA. A random sampling of 12 of these 49 crown tip samples was further analyzed at 1× dilution to confirm that there was inadequate quantity of DNA. Results confirmed that 11 of the 12 samples contained less than 20 pg of DNA. Using Mainland's method (6) to calculate a confidence interval, approximately 28–42% of the time there is less than 20 pg of DNA recovered from crown tips using the techniques applied in this study.

Concentration for Each Region by Age Group

To control for the size of the teeth, the mean DNA concentrations (ng of DNA per gram of tooth region) were calculated. The mean DNA concentrations and mean ranks calculated by the Kruskal-Wallis test for each tooth region are shown for each age group in Table 2.

Results from the root body showed a greater concentration of DNA than the crown tip, crown body and root tip for all age groups ($p < 0.001$). The crown tip contained the lowest concentration of DNA in all age groups ($p < 0.001$). There was no statistically significant difference in DNA concentration between the crown body and root tip.

Discussion

The results of this study show that the root body is the region with the greatest yield of DNA, followed by the crown body, the root tip and finally the crown tip. Furthermore, the root body contains the highest concentration of DNA. The root tip and crown body contain the next highest concentration of DNA, followed by the crown tip. The results of this study indicate a broad person-to-person variation in DNA yield from the different tooth regions, suggesting that the number of DNA containing cells in teeth, and the different regions, differs significantly between people. This person-to-person variation has also been seen in studies involving the transfer of epithelial cells to various surfaces (7,8).

The various sections were chosen because the separations between these sections are common locations where teeth fracture producing the fragments that are often found at crime scenes. The separation of the crown tip and crown body is approximately the boundary of the coronal pulp. Therefore, the crown tip consists of enamel and dentin, and the crown body consists mainly of coronal pulp, dentin and enamel. Since the root body is largely comprised of radicular dental pulp and dentin, it contains the greatest yield of DNA. At the junction between the pulp tissue and the periodontal ligament (9), the accessory canals potentially contain sources of DNA similar to the pulp tissue. Additional sources of DNA include nuclear remnants of the odontoblasts within the dentin (10) and cementocytes in the cellular cementum (9) covering the apical region of the root. This clarifies why the root tip is a source of DNA. Enamel does not contain living cells or cell remnants and therefore is not a viable source of DNA. Although the crown body contains dental pulp, it is not as good a source of DNA as the root body since enamel is a large component of the crown. Results demonstrate that there is sufficient quantity of DNA in the crown body, root body and root tip to support DNA extraction. Approximately 28–42% of the time there is less than 20 pg of DNA recovered from crown tips with the techniques used in this study. These results should be beneficial to DNA analysts who are asked to analyze found tooth fragments.

Further studies with a larger sample size should be undertaken to determine whether or not there is a statistical difference in DNA yield of these regions in all classes of teeth (incisors, canines, premolars and molars) for all age groups. Additionally, future studies should also focus on the effect of environmental insults on recoverable DNA from tooth fragments.

Although the teeth used in this study (unrestored) were obtained and maintained under optimum conditions, these results are important to forensic DNA analysts faced with the identification of remains in which identification by fingerprints or dental records is not possible. Under environmental conditions that compromise the amount of DNA, it is essential that the tooth with the greatest likelihood of yielding the highest quantity of DNA be utilized. Since studies have previously investigated the effect of environmental conditions on whole teeth, that information can be combined with the results from this study to provide the forensic DNA analyst with the information necessary to choose the optimum tooth fragment for analysis.

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Additional information and reprint requests:
Roshale Gaytmenn
Crime Laboratory Analyst
Florida Department of Law Enforcement
4211 N. Lois Avenue
Tampa, FL 33614