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TECHNICAL NOTE

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The Use of SEM/EDS Analysis to Distinguish Dental and Osseus Tissue from Other Materials

ABSTRACT: With increasing frequency, relatively small, fragmentary evidence thought to be osseous or dental tissue of human origin is submitted to the forensic laboratory for DNA analysis with the request for positive identification. Prior to performing DNA analysis, however, it is prudent to first perform a presumptive test or "screen" to determine whether the questioned material may be eliminated from further consideration. When material is shown not to be consistent with bone/teeth, DNA testing is not performed. When such determinations cannot be made from gross morphological features, elemental analysis can be indicative.

This presumptive test is made possible by applying scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDS) in conjunction with an X-ray spectral database recently developed by the FBI laboratory. This database includes spectra for many different materials including known examples of bone and tooth from many different contexts and representing the full range of taphonomic conditions. Results of SEM/EDS analysis of evidence can be compared to these standards to determine if they are consistent with bone and/or tooth and, if not, then what the material might represent. Analysis suggests that although the proportions and amounts of calcium and phosphorus are particularly important in differentiating bone and tooth from other materials, other minor differences in spectral profile can also provide significant discrimination. Analysis enables bone and tooth to be successfully distinguished from other materials in most cases. Exceptions appear to be ivory, mineral apatite, and perhaps some types of corals.

KEYWORDS: forensic science, forensic anthropology, SEM/EDS analysis, X-ray spectra, bone and tooth identification

The analysis of DNA within human osseus and dental tissue has emerged in recent years as a major advance in human identification. As a result of the increasing awareness of the potential of DNA analysis for resolving difficult forensic problems of identification, small fragmentary samples are increasingly submitted for examination. Such samples are recovered from a wide variety of contexts and frequently are significantly altered by environmental factors.

Prior to performing DNA analysis, it usually is desirable to establish whether the submitted materials may be osseus or dental tissue. The time and costs involved in DNA analysis can be reduced if prior analysis of the questioned material indicates that materials other than osseus or dental tissue are represented. This issue is important since many materials at crime scenes are morphologically similar to bone and tooth. Examination by a forensic anthropologist usually can make such determinations with confidence if the evidence is in pristine condition and sufficiently large. Morphological indicators usually allow determinations of species and other useful information. However, if the evidence is compromised by small size and/or morphological alterations by environmental conditions, even the determination of the presence of bone and/or tooth can be difficult. In such cases, analysis using SEM/EDS and comparison of results with a recently implemented database can be helpful.

SEM/EDS analysis represents a commonly used method in forensic science to elucidate the structure and elemental composition of many materials submitted as evidence. Analysis produces an X-ray spectrum, which is a compositional "fingerprint" of the material. From the spectrum, a determination of which elements are present and their concentration can be made. Pictures of structures also can be generated using various structural imaging methods. Comparative analysis of SEM/EDS spectra can facilitate materials identification (1).

In 1994, the Federal Bureau of Investigation (FBI) initiated an effort to develop a prototype database for X-ray spectra for use in comparative analysis (2). This system enabled comparison of the percentage X-ray counts of each element within a submitted sample with the database to assist in identification. Although data entry was manual and the system had other shortcomings, it demonstrated the potential of database applications of this type of analysis.

Subsequently, Spectral Library for Identification and Classification Explorer (SLICE) was developed under contract with the FBI[4]. The system was designed[5] (2) as a Windows-based application

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compatible with a modern EDS system and operational on a standard PC. Spectra resulting from analyzed specimens are converted to the standard EMSA format and archived in SLICE with associated data.

SLICE consists of two primary functions, each performed from dedicated operational pages. The first function is the collection and storage of spectra. Imported spectra are analyzed, pertinent data and images are attached, and the spectra archived. Data include preparation technique, manufacturer information, composition, analysis parameters, physical characteristics, and laboratory details.

The second function is the actual query of the archives for records containing or fitting operator-selected parameters. The comparative search capability allows queries based on individual or multiple criteria. The search may include the entire spectra or a selected portion of it. SLICE allows the spectrum of a questioned material to be compared to those of the standards in the database, providing associations based upon the similarity of the profile. Those with the best fit are those closest in composition. Numerical values of fit can provide an ordering or ranking of materials according to elemental similarity.

The FBI database was designed for general forensic analysis and therefore includes information on such diverse materials as minerals, pigments, and explosives. Each standard material is classified according to the level of confidence with which the composition is known. These classifications include certified, commercial, known, and unknown. *Certified standard* refers to materials where accurate quantitative determination of composition is available.

Commercial standard refers to materials for which composition is defined, but not known. This category includes materials that are known to be a specific product and to contain a representative and unaltered composition, but that composition is not known (e.g., Clinique Rose Bronze Lipstick). Commercial standard can also apply to non-manufactured materials (biological materials, minerals).

Known refers to materials from a known source, but either the composition cannot be considered representative or only generic information is available (e.g., "auto glass").

Unknown indicates items such as submitted evidence of unknown composition (e.g., glass fragments of any type taken from clothing). Unknowns are generally under current study and are retained only temporarily.

The FBI database was designed as a tool for both inter- and intra-laboratory materials comparisons and presumptive identifications. Absolute identification of materials based upon elemental composition is not considered possible by this method. Specific identification based upon elemental analysis would be possible only if study indicates that a particular composition is both unique and that the differences are readily demonstrable by SEM/EDS. Therefore this presumptive testing method is useful in applications assessing the association of a questioned material with materials that are (generally) compositionally similar or the exclusion of materials that are compositionally different. This method may be augmented with other more specific methods in order to reach more definitive conclusions regarding identification.

Methods

To address the problems of identifying osseus and dental fragments outlined above, the existing FBI database was augmented with examples of known bone and tooth fragments in both pristine and taphonomically altered conditions. Available collections at the Smithsonian Institution were surveyed for variation in preservation and morphological alteration due to apparent environmental factors. Bone and tooth samples from human and non-human animals were made available for SEM/EDS analysis representing specimens in pristine condition from modern contexts as well as those displaying evidence of exposure to heat and varying degrees of deterioration. Specimens were provided from archeological contexts in Ecuador dating as old as approximately 8,000 years. Human bone samples were also provided from two archeological shell-mound contexts in Brazil, Sambaqui da Beirada, dating to about 4,160 years ago and Sambaqui do Moa, dating to about 3,960 years ago. The former samples were poorly preserved with compromised microstructure, while the latter were comparatively well preserved.

In the specific case of bones and teeth, fresh unaltered samples are considered to be commercial standards. Although their compositions have not been determined, the spectral profile is considered representative of material. Samples from archeological contexts, however, are considered to be "knowns" since, although it is known that they are bone or even human bone, the composition may have been altered by environmental factors.

Presently, of the approximately 1800 entries in the database, 48 represent "bone" (34 human, 14 non-human), 44 "tooth" (22 human, 22 non-human), and 23 other biological materials. Of the total 115 biological entries, 55 are either "commercial" or "certified."

The dominant elemental signature of bone and tooth is the relative proportion of calcium and phosphorus. As shown in Fig. 1, these elements are more common than others and generally occur in predictable proportions. As noted by Braz (3), the Ca/P ratio in powdered cortical bone (weight) shows slight variation in modern samples with a mean of 1.88, standard deviation of 0.15, and range of 1.61 to 2.02. Older samples from archeological contexts show higher values. Those from the Brazilian site of Beirada, Layer II, discussed above, show a mean of 2.03, standard deviation of 0.03, and range from 2.01 to 2.06. Values from the Brazilian Moa site, Layer II, also discussed above, show a mean of 2.27, standard deviation of 0.21, and range from 1.94 to 2.58. Since forensic samples originate from both fresh and archeological contexts, this range is important.

Expressions of the Ca/P ratio using the percentage of atoms analysis show a very similar range (3:75). The control sample showed a mean value of 1.46 with a standard deviation of 0.12 and a range of 1.24 to 1.56. The Beirada sample presented a mean of 1.57, standard deviation of 0.02, and range of 1.56 to 1.59. The Moa sample produced a mean of 1.72, standard deviation of 0.16, and range of 1.50 to 1.99.

Since the exoskeleton of the sea horse technically is considered to be a type of bone, it is not surprising that even its elemental analysis (Fig. 1) is similar to that found in mammal bone. Analysis of three samples from a Brazilian seahorse, *Hippocampus reidi* from Ceará, Brazil, provided by Ricardo Zaluar Guimarães, Laboratório de Gestão da Biodiveridade, Federal University of Rio de Janeiro, Brazil, revealed Ca/P ratios by percentage weight of 1.48, 1.97, and 1.88. The values of percentage of atoms was 1.14, 1.53, and 1.45.

The Ca/P ratios and supportive information allow bones and teeth to be distinguished from most other materials in the database with great confidence. Other materials in the database that produce similar elemental profiles include synthetic hydroxyapatite, mineral apatite, octocorals (certain species of corals), and ivory.

Gorgonian octoral (Coelenterata) was also selected for database inclusion, as it contains a carbonate hydroxylapatite. Samples were extracted from the core of *Leptogorgia setacea* by a sodium hypochlorite wash (4).

Samples of synthetic hydroxyapatite were made available by Gloria de Almeida Soares of the Metallurgy and Materials Engi-

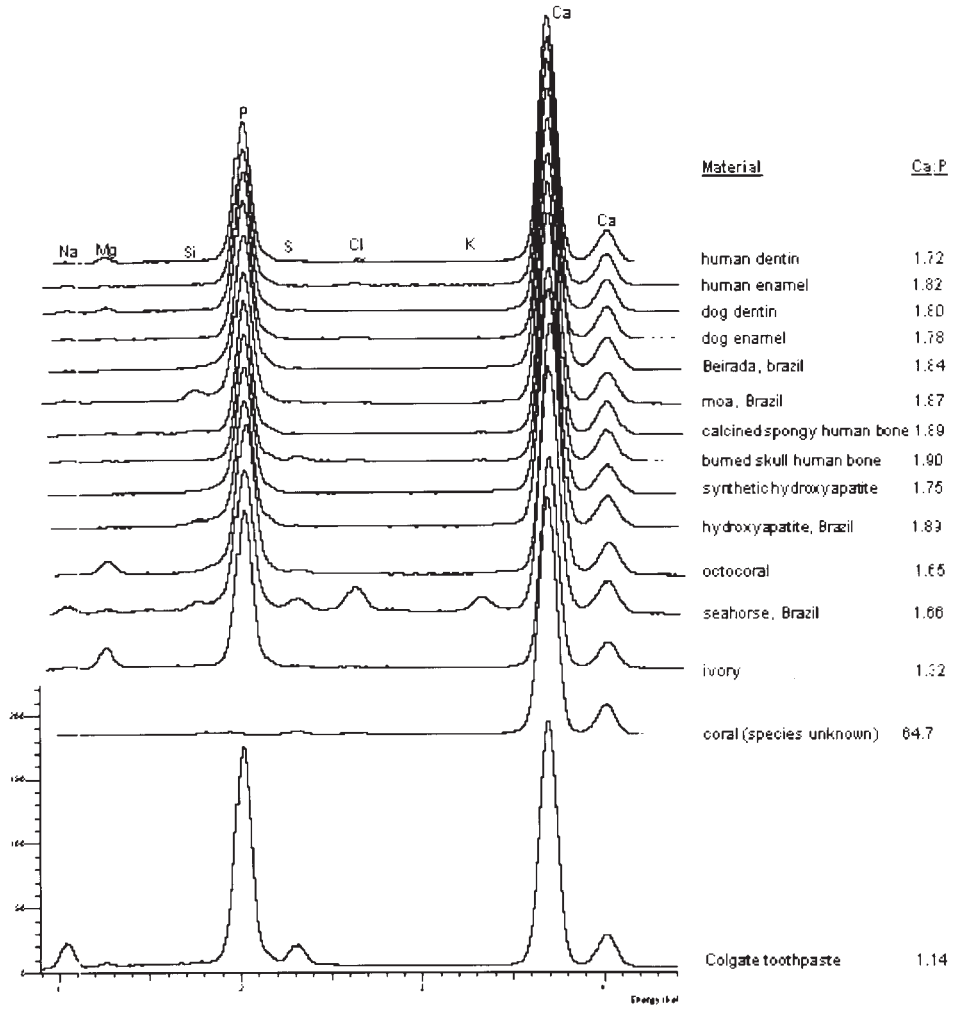

FIG. 1—*Spectral profiles of the materials referred to in article. Ca:P ratios as measured from the absolute peak heights of each element in the respective spectra are included in the column to the right. The differences between spectra can be quantitatively evaluated with a fitting algorithm as in SLICE and observed visually by a display method such as this.*

neering Department of Federal University of Rio de Janeiro in Brazil. Since hydroxyapatite is the major structural component of bone, it is expected that elemental analysis of this material would produce similar results. As seen in Fig. 1, the elemental profile is very similar. Ca/P ratios in two analyses of particles from the synthetic sample were found to be 1.95 and 1.82 using the percentage weight expression and 1.50 and 1.41 using the percentage of atoms approach. The values are close to the human bone samples reported above.

Although usually morphologically distinct from bone, mineral apatite produces a generally similar elemental profile (Fig. 1). Analysis of a specimen from Brazil provided Ca/P ratios of 3.80 and 4.42 by percentage weight and 2.94 and 3.42 by percentage atoms. These values are substantially greater than those found in the human values reported above.

An X-ray spectrum for each of the standard materials mentioned above was produced by SEM/EDS analysis. Standard analysis conditions include a beam voltage of 25 kV, spectral resolution of 10 eV per channel, and a dead time of 30% with a mid-value pulse processor time constant. From each of the spectra, the peak height of the principal peak of Ca and P was measured and ratioed: Beirada 1.84, moa 1.87, seahorse, 1.66, synthetic hydroxyapatite 1.75, hydroxyapatite 1.89, octocoral 1.65, and ivory 1.32 (Fig. 1).

Whereas the Ca:P between enamel and dentin/cementum is similar, enamel contains a small amount of Cl not detected in the dentin/cementum. This difference presents a difference in the fitting results. When a query is performed, all enamel entries are grouped, followed by dentin and cementum entries.

Classification Using the Database

Although the architecture of SLICE presents numerous options for query, the "By Best Fit" function is generally most useful. With it, the spectrum of an unknown (questioned) material is mathematically compared to other database spectra for differences. The results are returned with a numerical (modified Chi square) and ranked accordingly. The search may be confined to a specific materials category or may include the entire database.

Consider, for example a small, fractured piece of human tooth enamel. If it is treated as an unknown material, it may be searched against the entire database. Within the manufactured products, Colgate Fluoride toothpaste is found also to contain Ca and P; how-

ever, there are significant differences in S, Na concentration, as well as differences in the Ca/P ratio. Therefore the "fitting" factor is low, and it is considered to be compositionally different. Within the natural products category, the spectrum of the tooth fits very closely with bone and tooth of several species.

Use of the database indicates bone and tooth can be distinguished from all manufactured products analyzed to date except the synthetic hydroxyapatite. This system cannot distinguish dental samples from bone, and species cannot be distinguished using either tissue. Dental and osseus samples cannot be distinguished from mineral apatite by spectral profile, although some apatite specimens have distinct morphological characteristics. To date, the biological materials most compositionally similar to bone and tooth are ivory and octocoral. Some differences, however, are evident.

Conclusions

SEM/EDS analysis utilizing the database and classification system described here appears to represent a powerful new tool for distinguishing dental and/or osseus samples from other materials that appear similar morphologically. Efforts continue to enlarge the database and to explore alternative approaches to distinguish the materials most likely confused with bone and tooth, namely ivory, mineral apatite, and the octocorals. The existing database is adequate to distinguish most materials that are compositionally different from bone and teeth. Such determinations are invaluable considering the increasing number of evidence submissions involving small, taphonomically compromised fragments from forensic contexts that are morphologically consistent with, but not diagnostic of, bone and tooth.

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