H68 Chemical Differentiation of Osseous Samples From Non-Osseous Materials Using Scanning Electron Microscopy-Energy Dispersive X-Ray Spectrometry (SEM/EDX) and Multi-Step Statistical Analysis

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After attending this presentation, attendees will have a better understanding of the benefits of utilizing Scanning Electron Microscopy-Energy Dispersive X-Ray Spectrometry (SEM/EDX) and multi-step statistical analysis to distinguish fragmentary osseous samples from non-osseous materials of similar appearance and chemical composition.

This presentation will impact the forensic science community by demonstrating that, when combined with a multi-step statistical analysis process, SEM/EDX is a practical method for distinguishing between fragmentary osseous samples and non-osseous materials of similar appearance and chemical composition in a forensic laboratory setting. This method involves simple sample collection and analysis and utilizes instrumentation currently possessed by many forensic laboratories.

While identification of osseous materials is generally established on gross anatomical qualities, highly fragmented or taphonomically altered materials are often problematic and alternative methods, such as histological or chemical analysis, must be utilized. Recently, chemical methods have been proposed to sort unknown materials according to their Ca/P ratios. Ubelaker et al. proposed using SEM/EDX to achieve this distinction and Christensen et al. have validated XRF spectrometry for this application.1,2 An alternative method of analysis involves performing Principal Component Analysis (PCA) on element spectra to classify unknown materials based on their trace element composition. Zimmerman et al. proposed the validity of this method with data obtained using XRF.3 Subsequently, performing PCA on elemental data obtained using SEM/EDX demonstrates potential for sorting capabilities.

Samples were prepared by extracting a small amount of material from each location of analysis using a Dremel® tool. The resulting powder was adhered to the SEM stub using a carbon dot, and a small piece of copper tape was placed on each stub to use in calibration. Twenty-six samples were analyzed, with five spectra taken at each site, resulting in a total of 130 spectra. Samples consisted of human and non-human osseous materials (bone, antler, ivory, and shell), non-biological specimens (rock apatite and synthetic hydroxyapatite), and other biological specimens (sand dollar, shell, and two coral species). Data was collected using a LEO 1450VP Scanning Electron Microscope and an Oxford® Energy Dispersive Spectrometer. After all five spectra were collected for a sample, the detected elements were assessed. Carbon was removed due to suspected contamination from the stub as well as elements appearing in two or less spectra per sample. The remaining elements were normalized. Weight percent data was then processed in R, version 3.0.1, by the R Foundation for Statistical Computing using PCA, Linear Discriminant Analysis (LDA), and Quadratic Discriminant Analysis (QDA) based on principal components representing 99% of the variation within the data.

A three-tiered analysis was undertaken to improve discrimination between sample groups. The first tier involved distinguishing between marine and non-marine samples. This resulted in 100% classification using LDA and QDA and was successful in discriminating octocoral, which has proven difficult when using Ca/P ratio-based analysis.[1,2] Tier two analyzed only the non-marine samples indicated in tier one, dividing them into osseous and non-osseous materials. Classification was 100% using LDA and 97% using QDA with three rock apatite spectra classifying as osseous. Finally, tier three assessed osseous materials to determine if human and non-human samples could be distinguished. Results indicated 58% average classification between human and non-human osseous materials using LDA, with 80% of human samples misclassifying as non-human and 19% of non-human samples misclassifying as human, and 70% average classification using QDA, with 40% of human samples and 25% of non-human samples misclassifying. Misclassification may be a result of the large variety of species with limited representative samples and discrimination is anticipated to improve with a larger data set. Additionally, alternative statistical methods are being considered to improve classification.

Multi-tiered statistical analysis of elemental composition acquired using SEM/EDX demonstrate the ability to discriminate between osseous and non-osseous materials and show potential for differentiating between human and non-human samples. Further research will expand the data set by adding dental materials and additional samples chemically similar to bone in an effort to increase the accuracy of human and non-human differentiation and create a standard reference set to allow for use of this method in the forensic laboratory setting.

References:

1. Ubelaker DH, Ward DC, Braz VS, Stewart J. The use of SEM/EDS analysis to distinguish dental and osseus tissue from other materials. J Forensic Sci 2002;47(5):1-4.
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3. Zimmerman HA, Schultz JJ, Rinke C, Sigman ME.. Preliminary validation of handheld X-Ray fluorescence (HHXRF) spectrometry for distinguishing osseous and dental tissue from non-bone materials of similar chemical compositions. P Am Acad Forensic Sci 2013;19:453.

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