A41 Fluorescence in Heat-Altered Bone Under Ultraviolet Light

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After attending this presentation, attendees will be knowledgeable about the use of fluorescence under ultraviolet light in the examination of burned and heat-altered remains. Attendees will also learn what fluorescence can reveal about the bone properties and the parameters of the heat event.

This presentation will impact the forensic scientific community by exploring the use of fluorescence under ultraviolet light as a method for analysis of burned and cremated remains. The description of these types of cases has been largely qualitative; this research attempts to provide a low-cost, easily interpreted method of analysis of this type of anthropological casework.

Alternative light sources and fluorescence are methods that have a long tradition within the forensic sciences. Few studies have addressed fluorescence in burned and cremated human remains. Warren et al. mention it as part of the examination of commercially cremated remains and Harbeck et al. examine how fluorescence changes with differing temperatures of the heat event.[1,2] In addition to understanding fluorescence changes with heat exposure, it is important to know what is underlying the fluorescence patterns to ensure reliability and applicability of the technique. Fluorescence results from an interaction of the excitation of light and the material under examination. The specific combination of excitation wavelength and fluorescence emission peak is indicative of the material under examination. Thus, fluorescence in burned bone can not only reflect the temperature of heat exposure, it also indicates what changes are taking place to bone properties.

The diaphysis of five pig femora were cleaned and segmented, with each exposed to a differing time and temperature combination. One segment was held at ambient temperature for a control; five segments were each subjected to temperatures between 100°C and 900°C in 200° increments for 60 minutes. To examine the impact of duration, a segment was heated at 500°C or 900°C for one of the following durations: 15, 30, or 45 minutes. Fluorescence data was collected using a spectrofluorimeter, as well as bone chemistry and composition analyses, to document changes to the skeletal tissue. Ultraviolet light between 300nm and 395nm at 5nm increments was used for excitation, with emission collected across the visible spectrum (400nm and 700nm).

Under excitation light similar to most Ultraviolet (UV) light sources (365nm), fluorescence changed based on temperature of exposure but not duration. The control samples and those heated to 100°C showed a bright blue fluorescence (~450nm peak). No fluorescence was seen in samples heated to 300°C and 500°C, with red fluorescence (>640nm) noted in samples heated to temperatures of 700°C and 900°C.

To investigate what excitation and emission combination resulted in fluorescence of the samples, an Emission Excitation Matrix (EEM) was collected for each bone segment. Parallel factor analysis of the EEMs for samples at all temperatures indicated two components best describe the dataset. The first component of the model (excitation=330nm, emission peak=696nm) is a red fluorescence. More research is needed to determine the underlying mechanism for this fluorescence. The second component (excitation=365nm, emission peak=458nm) characterizes blue fluorescence corresponding to the collagen and organic components in samples of low temperature exposure.[3,4]

This research indicates that fluorescence under ultraviolet light can be used to assess not only the temperature of the heat event, but also indicate the bone properties. In order for fluorescence to be a reliable method, it is necessary to determine what changes in bone properties underlie the fluorescence after heat exposure. Based on this research, it is expected that bone exposed to low temperatures would exhibit blue fluorescence linked to collagen content; with complete or near-complete loss of fluorescence in the middle range temperatures, and a red fluorescence is expected at the higher temperatures.

References:

1. Warren MW, Falsetti A, Hamilton WF, Levine LJ. Evidence of arteriosclerosis in cremated remains. *Am J For Med Path*. 1999;20(3):277-280.
2. Harbeck M, Schleuder R, Schnieder J, Wiechman I, Schmahl WW, Grupe G. Research potential and limitations of trace analyses of cremated remains. *For Sci Inter*. 2011;204(1-3):191-200.
3. Bachmann L, Zezell DM, Ribeiro AC, Gomes L, Ito AS. Fluorescence spectroscopy of biological tissues—A review. *Appl Spectrosc Rev* 2006;41(6):575-590.
4. Bachman CH, Ellis EH. Fluorescence of bone. *Nature* 1965;206(4991):1328-1331.

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