THE APPLICATION OF CONSOLIDATION MATERIALS TO BURNED BONE: A COMPARATIVE APPROACH

by

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ABSTRACT

Fire-altered bone presents a unique challenge to anthropologists and bioarchaeologists due to its characteristically increased friability. A potential solution to this problem is the standardized use of consolidant materials on fragile osteological material, including burned remains. Although anthropologists and odontologists have employed a variety of consolidant materials in the past, there is no consensus regarding which material is most appropriate.

Acryloid™ B-72, Acrysol™ WS-24, Rhoplex™ B-60A, and Butvar® B-98, four easily obtained and commonly used consolidants, were compared to assess their ease of material application and their ability to keep friable, burned bone intact through mechanical testing.

Based on both the qualitative and quantitative data collected, Acryloid™ B-72 is the most suitable consolidant for use on burned bone during in situ recovery. If the dry time for Rhoplex™ B-60A could be significantly reduced through the use of a different solvent, such as acetone, Rhoplex™ B-60A would also be appropriate. However, before using consolidants on friable bone, any additional analyses that may be performed including DNA analysis, stable isotope analysis, and radiocarbon dating need to be considered, and a subset of samples set aside which do not receive consolidant treatment.

I. **INTRODUCTION**

Taphonomic agents and postdepositional activities can significantly influence bone's ability to withstand the stress of scene removal and analysis. In the field of paleontology and paleoanthropology, low permineralization and incomplete fossilization can result in skeletal specimens fragmenting under the increased stress of their own incompletely or partially fossilized weight once removed from the surrounding matrix (Biscula et al. 2009; López-Polín et al. 2008). Archaeologically, bone may exhibit varying degrees of degradation and skeletal decomposition due to soil composition, including moisture content and pH, which can contribute to significant loss of bone's organic component (Stone et al. 1990). Furthermore, fire-altered bone presents a unique analytical challenge to forensic anthropologists and bioarchaeologists, owing to increased friability and fragmentation (Mincer et al. 1990; Shipman 1984), since specimen completeness enhances the ability to generate a biological profile, which includes data on ancestral affiliations, personal identification, and reconstruction of peri- and postmortem events.

This necessity is apparent in a medicolegal setting, where the comparison of antemortem and postmortem records can contribute to a relatively quick method of positive identification, when the integrity of the structures under examination is maintained. Hill et al. (2011) discuss two case studies where individuals were exposed to extremely high temperatures for a prolonged period of time during a brushfire and then recovered and handled in different manners. The first case was recovered with no special precautions to prevent fragmentation during scene removal and transportation to the lab.

This ultimately resulted in complete destruction of the dental structures to be examined, requiring a DNA match to positively identify the individual and release them from the death investigator's office. Although a positive identification was ultimately achieved, DNA analysis can contribute to increased investigative costs and a delay in positive identification and repatriation. In the second case presented, the head and neck of the recovered individual were wrapped in a protective plastic covering. While this method also resulted in the destruction of dental structures, all elements were contained within the wrapping and could be reconstructed, enabling radiographic analysis.

Furthermore, two primary factors that complicate the recovery of human remains from a forensic setting include the general complexity of a fatal fire-scene (i.e. monochromatic coloration of thermally altered materials, structural collapse resulting in compressed strata, further scene modification due to fire suppression efforts) and the propensity for increased fragmentation of burned bone during recovery efforts (Dirkmaat et al. 2012; Ubelaker 2009). The four phases of the Bridgeville Fatal Fire Recovery Protocols (Dirkmaat et al. 2012) were developed to maximize recovery and mitigate the fragmentation of human remains at fire scenes, under a US National Institute of Justice Grant (Symes et al. 2012). Two primary factors that complicate the recovery of human remains from a forensic setting include the general complexity of a fatal fire-scene (i.e. monochromatic coloration of thermally altered materials, structural collapse resulting in compressed strata, further scene modification due to fire suppression efforts) and the propensity for increased fragmentation of burned bone during recovery efforts (Dirkmaat et al. 2012; Ubelaker 2009). Phase 1 consists of an initial large-scale search of the area to determine the general location of any remains. This typically will include a transect or

line search. Phase 2 begins once evidence of human remains is encountered and focuses search efforts on the area adjacent to and surrounding the remains. This area should be searched using a modified "cake-cutting" excavation method to locate the boundary of the crime scene. Excavated matrix should be sorted quickly by hand, with a detailed, more traditional "top-down" excavation technique commencing when the first piece of evidence is encountered. This progression marks the start of Phase 3, the purpose of which is to fully expose the remains and any associated evidence. All matrix excavated moving forward is to be collected and carefully screened off site in an effort to recover the remains completely. Upon completion of the excavation, Phase 4 commences and is concentrated on the preparation for and actual removal of all forensically significant fire-altered biological remains from the scene. Furthermore, Dirkmaat and colleagues (2012) offer several recommendations to prevent further fragmentation of remains during transport including the use of a broad rigid insert (e.g. plywood) at the base of body bags and wrapping fragile areas, including the head, with a layer of clean fabric and plastic wrap or aluminum foil to provide added structural support. Although wrapping areas of intense thermal alteration may reduce the degree of fragmentation during transport, the removal of these coverings prior to analysis, as well as any repeated handling, may result in fragmentation in the lab. However, the use of aluminum foil as a covering disrupts the standard operating procedures for some Medical Examiner's Offices, as cases receive full body radiograph prior to autopsy or examination, thus making the removal of foil dressings upon arrival a necessity prior to resuming normal intake procedures (Dana Austin, personal communication 2015).

A potential solution to the problem of fragmentation during recovery and transport is the standardized use of consolidant materials on fragile osteological material, including burned remains. Bohnert et al. (1998) document the chronology of calcination for various body parts during a cremation event. After 40 minutes of exposure to fire (670-810°C), the facial bones fragmented, ribs were calcined, and the lower arms were almost completely destroyed. Despite the fact that the temperature of a house fire would fluctuate while the cremation event it is held constant, the authors conclude that the general timeline observed is relevant within a forensic setting. Therefore, prolonged exposure to high temperatures could thermally alter human remains to the point where the application of consolidant materials at the scene prior to recovery may be necessary. Although anthropologists and odontologists have employed a variety of consolidant materials to avoid the accidental destruction of friable archaeological and forensic remains during excavation and transport (Bisulca et al. 2009; Johnson 1994; Kres and Lovell 1995; Mincer et al. 1990; Rossi et al. 2004; Stone et al. 1990), there is little to no consensus or research available regarding the most appropriate consolidant material to use when burned bone is encountered.

The research objectives of this project are to quantitatively and qualitatively compare the ability of four commercially available conservation-grade consolidants to increase the ultimate strength and toughness of thermally-altered bone. Quantitative assessment of total dry time per specimen and ultimate strength was conducted using a drop weight test and forced vibration test. Qualitative variables related to consolidant application such as ease of solution preparation, dry time, solution storage needs in the field, ease of application, and any changes to the bone's appearance were examined.

Additionally, mode of deformation and mechanical response under loading was qualitatively assessed through nanoindentation.

This research is being conducted to identify and recommend the regular use of a consolidant material when encountering friable burned bone in the field, where stabilization would be required to keep elements intact upon removal and handling. The four consolidant materials chosen for comparison in this research include Acryloid™ B-72, Acrysol™ WS-24, Rhoplex™ B-60A, and Butvar® B-98, all of which are commonly used, easily obtained, and relatively inexpensive (Kres and Lovell 1995; Mincer et al. 1990; Rossi et al. 2004).

BACKGROUND

Previous research concerning burned bone has focused on the replication of burning and cremation events (Bennett 1999; Bohnert et al. 1998; Schultz et al. 2008; Thurman and Willmore 1980; Wells 1960), chromatic changes (Devlin and Herrmann 2008; Shipman et al. 1984), changes to the microstructure and macromorphology (Bradtmiller and Buikstra 1984; Gonçalves et al. 2011; Herrmann and Bennett 1999; Shipman et al. 1984; Stiner et al. 1995), and modifications to bone biomechanical properties (Kalsbeek and Richter 2006; Nelson 1992; Thompson 2005). The following literature review will elaborate on research concerning the mechanical properties of bone, physical changes to bone after thermal alteration, and a review of consolidant materials and their use on fragile bony material in variable contexts.

Bone Biology and Mechanical Properties

Bone is both a viscoelastic (exhibiting viscous and elastic characteristics under deformation) and anisotropic (having different mechanical values when measured in different directions or along different planes) composite material made up of water and an organic and inorganic component of collagen and the mineral hydroxyapatite, respectively (Seeman 2008; Viguet-Carrin 2006). Mineral content contributes to bone's stiffness, or rigidity, and is brittle, while collagen contributes to bone's toughness, or ability to absorb energy and resist fracture, and is therefore more ductile (Turner and Burr 1993; Viguet-Carrin 2006). However, because bone is anisotropic, the way in which bone behaves to a force depends on the direction of load application (Bankoff 2012, Martin et al. 1998), and because it is viscoelastic, its mechanical response to a loading event is both speed and time dependent (Bankoff 2012; Martin et al. 1998).

Additionally, biomechanical research has indicated that the Young's modulus of elasticity, a measure of intrinsic material stiffness, is strongly correlated with the mineral content of bone, and that the loss of bone strength may be due to collagen degradation and an increase in porosity (Currey 1988, Currey 1990, Currey 1999; Turner-Walker and Parry 1994) associated with various taphonomic agents.

Heat-induced alteration to bone

Thompson and Chudek (2007) note a two-tier approach when addressing heat-induced changes to bone. The primary level involves the underlying microscopic changes to bone, including mineral content and the organic to inorganic components ratio. The

secondary-level concerns macroscopic changes such as color and fracturing. A review of research summarizing both levels follows.

Primary level: Microscopic fire related changes to bone

Since fire-altered bone presents a unique set of challenges to investigators due to its extreme propensity to fragment, understanding the potential causes for decreased strength in burned bone is necessary before research into a method for mitigating friability can commence.

Stiner et al. (1995) compared microstructural changes to burned bone in both modern and archaeological settings. They set out to answer a series of research questions, including how burning events change bone strength, durability, and preservation. A modern sample of fresh goat and cow bones, along with an archaeological sample from the Paleolithic strata of Hayonim Cave in Israel, were examined. The authors found that crystal size and organization of hydroxyapatite in bone increases significantly during fire related diagenesis. These findings are consistent with Shipman (1984). This process of recrystallization reduces the bones' ability to resist pressure, resulting in decreased overall strength.

Additionally, Viguet-Carrin et al. (2006) analyzed the extent to which bone collagen is involved in overall bone strength. Type I collagen is arranged in fibrils, which are stabilized by inter/intrafibillar crosslinks and the presence of hydroxyapatite crystals on, within, and between collagen fibers in bone. The authors conclude that this complex relationship between collagen and the mineral components of bone undoubtedly influence mechanical properties, including overall strength.

Kalsbeek and Richter (2006) investigated the impact that bone composition has on hardness and how that is affected by increasing temperature. Their sample population consisted of 3-4 centimeter (cm) fragments from thirty-five deer metapodials, which were then subdivided and heated in a furnace at incremental temperatures between 100°-1000° Celsius (C) for three hours. X-ray diffraction spectra were recorded to assess relative inorganic composition. The authors concluded that while bone deterioration and preservation involves several parameters, hardness is primarily based on burning temperature. Low hardness measurements occur particularly after collagen denatures, around 155°C, then hardness sharply increases for samples heated above 900°C. However, increased hardness does not necessarily translate to an increase in ultimate strength. Hardness denotes a material's resistance to force exerted on the surface, while ultimate strength is an intrinsic measure of the maximum amount of stress a bone can withstand during plastic deformation (Turner and Burr 1993). Although Kalsbeek and Richter (2006) reported an increase in hardness above 900°C, the calcined samples remained brittle and susceptible to mechanical degradation.

The research discussed above provides background information concerning the reaction of collagen and hydroxyapatite to increased temperatures. Conclusions indicate that the denaturation of collagen from exposure to high heat inherently decreases the ultimate strength of carbonized and calcined osseous material. Furthermore, the increase in crystal size and reorganization of the lattice structure of bone's inorganic component due to burning significantly affects the material's ability to withstand stress. Understanding the heat-induced changes in bone microstructure provides an understanding of the mechanisms contributing to burned bone's friable nature and

increased fragmentation, which then stimulates ongoing research in methods for stabilization and consolidation. In addition to microstructural changes, significant macroscopic changes to bone are observed after prolonged exposure to heat.

Secondary level: Macroscopic fire related changes to bone

Thermally-altered bone undergoes a series of chromatic changes, and will eventually crack, fracture, warp, and shrink after exposure to heat. Shipman et al. (1984) aimed to provide a framework to reconstruct a burning event based on these changes. Microscopic attributes, including osteon size and crystal structure, were assessed; however, since results are in agreement with previously discussed research, this summary will focus on the macroscopic changes assessed by Shipman and colleagues. The study population consisted of six animal samples composed of bone and dentition, exposed to different maximum temperatures in a furnace for four hours. Color was assessed using a Munsell Soil Color Chart (Munsell 2009), and was divided into five stages, exhibiting increased color diversification, roughly based on increasing temperature. Shrinkage was calculated as a percentage change in overall dimension based on maximum lengths and circumference of the element measured. Degree of shrinkage was determined to be a function of maximum temperature reached during the firing event.

In addition to color and size changes burned bone will warp and fracture, significantly altering element morphology. Thurman and Willmore (1981) performed a replicative cremation to investigate differences in the fracture patterns of "green" defleshed bone from fleshed bone. Their samples were placed at regular intervals on an elongated oak fire and allowed to burn until partially or fully calcined. Serrated,

transverse fractures, and diagonal cracking alongside pronounced warping, as well as parallel fractures along "checking" lines and slight warping were observed depending on the pre-cremation treatment of the bone.

Gonçalves et al. (2011) also analyzed heat-induced fracture patterns from dry bone cremations, contradicting Thurman and Willmore's (1981) findings. Gonçalves et al. (2011) observed similar warping and thumbnail fractures on dry bone cremations that Thurman and Willmore (1981) reported for fresh, fleshed bone, indicating that their presence in a forensic or archaeological setting does not necessarily indicate the state of the remains prior to prolonged heat exposure. Even though these results seem contradictory, warping and fracturing of whole bone occurs under both conditions, thus highlighting the fragmentary and friable nature of burned bone.

Fracture patterns in burned remains are further complicated when attempting to reconstruct perimortem events. Understanding and interpreting perimortem trauma and taphonomic fractures is crucial to the analysis of osteological remains, and differentiating between the causes of various morphological fracture patterns is imperative (Herrmann and Bennett 1999). Although the differentiation and analysis of fracture types is possible after exposure to fire, the importance of a thorough and exhaustive method for recovery of burned remains, particularly in a forensic context where fires can be purposefully set to disguise or obliterate perimortem trauma, cannot be overstated (Dirkmaat et al. 2012; Herrmann and Bennett 1999; Symes et al. 2015). A possible solution to maximize recovery at a fire scene is the regular use of consolidation materials in the field in an attempt to prevent further fragmentation during recovery, transport, lab analysis, and handling.

Consolidation materials

As previously stated, anthropologists and odontologists have employed a variety of consolidant materials to friable bone in numerous contexts; however, there is little to no consensus or research available regarding the most appropriate consolidant for burned bone. Johnson (1994) presents detailed information about the chemical properties of several popularly used consolidation materials, and she evaluates some of the problems encountered when dealing with these materials in a field or lab setting. Specifically, she focuses on materials that have been published as being applied to bone found in the archaeological record including: natural resins, cellulose nitrate resins, poly(vinyl) acetyl resin, poly(vinyl) butyral resin, poly(vinyl) acetate resins, poly(vinyl) acetate emulsions, acrylic emulsions, acrylic colloidal dispersions, and acrylic resins.

Johnson (1994) brings up several characteristics that should be considered when choosing a consolidation material, including reversibility, long-term archival quality, and the ability for specific analytical techniques to be performed. In addition to these considerations, she suggests inexpensive, readily available materials and specific solution concentrations for stabilization in various environmental contexts. Johnson's assertions are corroborated in other conservation manuals, which also include recommended solution concentrations for consolidants being used as adhesives (Sease 1994). Although Johnson (1994) only discusses the use of consolidants in unburned archaeological material, stabilization was required due to the poor preservation of the recovered artifacts and their likelihood of fracturing after excavation. Therefore, it is expected that the effect of consolidants on friable burned bone should mirror the effects reported for archaeological materials.

Consolidant application to bone

Mincer et al. (1990) try to determine which materials, commonly used by forensic odontologists and forensic anthropologists, are the most efficient in preserving incinerated teeth. To determine which materials to test, a questionnaire was mailed to 81 forensic odontologists and 73 forensic anthropologists, from which they received 97 replies. Common materials cited by survey participants include cyanoacrylate (e.g., superglue), polyvinyl acetate, acrylic spray, hair spray, clear fingernail polish, epoxy cement, and orthodontic acrylic resin. All of these materials were tested on sets of three teeth, which were incinerated for 20 min at 427°C. A control group remained untreated, while the remaining ashed teeth were treated with one of the various stabilizing substances. Three coats of the material were applied by brushing, spraying, or dropping in the case of cyanoacrylate cement, and were then tested for fragility by agitation.

Mincer et al. (1990) concluded that all of the materials tested satisfactorily increased physical stabilities; however, they proposed that acrylic spray was the best option, but they did not discuss why the other materials were dismissed. Rather than examining each individual material tested, they simply stated that some showed disadvantages including a long drying time, difficult application, and a remaining sheen or patina after impregnation with each stabilizing agent.

Kres and Lovell (1995) conducted similar qualitative research concerning the application of consolidation materials to archaeological bone. Using Acryloid™ B-72 dissolved in toluene, Acrysol™ WS-24 diluted in distilled water, Butvar® B-98 dissolved in ethanol, and Rhoplex™ AC-33 in a solution of isopropanol and acetone at a 20%

concentration, they applied these materials to tibial and vertebral fragments from an adult female excavated at Tell er-Ruba in the Eastern Egyptian delta.

Kres and Lovell (1995) applied the consolidant solutions to the bone fragments either by brushing or full immersion using metal trays. Although Acryloid™ B-72 seemed to strengthen the fragments; the glossy finish left behind was undesirable. Acrysol™ WS-24 and Rhoplex™ AC-33 had extremely long drying times, sometimes up to three hours for a single layer of application. They concluded that taking drying time, finish appearance (i.e., glossy and matte), and non-toxic and non-flammable properties into account, Butvar B-98 was the most suitable consolidant they tested for stabilizing fragmentary archaeological remains.

Additionally, Rossi et al. (2004) conducted a comparative study of two consolidation solutions to investigate their ability to decrease friability and increase the strength of burned osseous remains. Acryloid™ B-72 and Butvar® B-98 were tested on fifteen fresh bovine femora. Femora were placed in one of three groups and burned in an electric furnace at different degree ranges (346°C-357°C, 610°C-755°C, 684°C-838°C), roughly corresponding to firing stages 3-5 in Shipman et al. (1984), for 2.5, 1.0, and 1.5 hours respectively.

Consolidant solutions were then applied to the burned remains either by brushing the solution onto the bone fragments or through immersion. A control group was maintained where no consolidant was applied to the remains; however, all other fragments tested had either two or three coats of material. Strength of consolidation was measured based on the ability to prepare histomorphology slides. Rossi et al. (2004) concluded that Acryloid™ B-72 provided better results for decreasing the friability of the

cremated remains, allowing for cross-sectioning and grinding for slide preparation. Based on these findings I expect Acryloid™ B-72 to be the most appropriate consolidant material for stabilizing the bone and tooth samples included in the present research by increasing hardness and toughness.

PILOT STUDY

Prior to data collection for the present research, a pilot study was conducted during the summer of 2015 to finalize the burning process as well as determine the best method for material application. An open fire cell made from wooden pallets, gypsum drywall, and fiberglass insulation (Figure 1) was used for two separate burning events.

Figure 1. Plan view of open fire cell

During these events I determined that bone specimens should be adequately separated on the floor of the fire cell to minimize commingling of each bone during recovery.

Furthermore, the concentration of consolidant materials was increased from 4% to 10% because qualitative assessment in the field indicated 4% concentration was not sufficient in stabilizing the burned specimens as they continued to fragment during the recovery process. Finally, the brush and immersion application techniques outlined previously (Kres and Lovell 1995; Rossi et al. 2004) were shown to be impractical in the field. Immersion involves complete submersion of a specimen in a tray of solution and is too difficult for *in situ* application. On the other hand, the brush method of application proved to be extremely time consuming. After testing various mechanisms for a spray application, a squeeze application using plastic bottles was decided upon because it allowed for the easiest application of material with minimal overspray.

Although previous research qualitatively assessed several consolidants' efficacy in reducing fragmentation of friable archaeological and heat-altered bone, the research presented here quantitatively elaborates on Kres and Lovell (1995) and Rossi et al's. (2004) results by testing four consolidant materials on calcined bone in an attempt to identify the most suitable material for use during field recovery. Variables investigated in this research include ease of solution preparation and application, dry time, any semi-permanent or permanent changes to the appearance of the bone, as well as the consolidant's ability to keep burned bone intact after recovery through mechanical testing of ultimate strength and plasticity.

The following chapters will include an outline of my materials and methods, a summary of my findings, a discussion of the implications of the results, including research limitation and future directions, and a brief conclusion reiterating the significance of this research.

II. **MATERIALS AND METHODS**

The study sample population consisted of domestic pig (*Sus scrofa domesticus*) femora (n = 58) and skulls (n = 5). Because I used pig remains designated for human consumption and obtained from a local butcher, I did not require IACUC approval to conduct my research. The sample was divided into five subgroups (Table 1), including a control group of burned bone with no consolidant added, to comparatively test four different consolidant materials in terms of their ability to increase the strength and toughness of the burned bone to which they were applied (Turner and Burr 1993).

Table 1. Sample Group Composition

Subgroup	Treatment	Consolidant Added	Elements included
1	Burned	None	1 cranium with mandible 10 femora
2	Burned	Acryloid™ B-72	1 cranium with mandible 12 femora
3	Burned	Acrysol™ WS-24	1 cranium with mandible 12 femora
4	Burned	Rhoplex™ B-60A	1 cranium with mandible 12 femora
5	Burned	Butvar® B-98	1 cranium with mandible 12 femora

THERMAL ALTERATION TO SAMPLE

In order to simulate a structure fire, an open fire cell was constructed using wood pallets, gypsum drywall, and fiberglass insulation (see Figure 1); however, because fire propagation is not being investigated with the research, materials typically found in a structure fire (i.e., furniture and home goods) that would contribute to flashover were not included in the fire cell. Due to the potential wildfire hazard the pallet structure was

placed in a drained, concrete lined retention tank located at the Forensic Anthropology Research Facility at Texas State University (Figure 2). Bone samples were weighed and placed on the floor of the structure (Figure 3), which was subsequently lit on fire and allowed to extinguish naturally.

Figure 2. Drained concrete pit used for fire events

Paraffin wax coated bundles of straw (brand name: Tumbleweeds) and lumber by-product (brand name: Lightning Nuggets) were used as a fire starters and placed at several points along the exterior pallet walls. Fire temperature was recorded in real time using a hi-temp flexible ceramic fiber-insulated type-K thermocouple probe placed in a corner of the structure and attached to a data collection device with data points being collected every second. Both the sensor and the cable for the probe are rated from -58-1200°C (-50-2200°F). Three separate fire events were conducted to thermally alter the entire study population, and duration of the fire was recorded as the change in time from

when fire starters were lit and the last time smoke was observed emanating from the remnants of the structure.

Figure 3. Plan view of structure used in Burn Event 1

CONSOLIDANT MATERIALS AND SOLUTION PREPARATION

Consolidant materials applied during this research include Acryloid™ B-72 (Paraloid™ B-72, a methyl methacrylate/ethyl acrylate copolymer, Acrysol™ WS-24, an acrylic colloidal dispersion solution, Rhoplex™ B-60A, an acrylic emulsion, and Butvar® B-98, a poly(vinyl) butyral resin(Acrysol™ Technical Data Sheet 2007; Butvar® B-98 Manufacturers Guide 2013; Paraloid™ Technical Data Sheet 2007; Rhoplex™ Technical Data Sheet 2012). These materials were chosen because

anthropologists have utilized them in the past, and they are relatively inexpensive (Johnson 1994; Kres and Lovell 1995; Mincer et al. 1990; Rossi et al. 2004).

Acryloid™ B-72, also known as Paraloid™ B-72, is bought in pellet form and must be dissolved in acetone before application (Johnson 1994). Although Johnson (1994) suggests Rhoplex™ AC-33 as a suitable acrylic emulsion consolidant, and it has been applied to archeological bone in the past (Kres and Lovell 1995; Stone et al. 1990), the manufacturers Rohm and Haas recently discontinued the product and suggest Rhoplex™ B-60A as a replacement. Both Rhoplex™ B-60A and Acrysol™ WS-24 are purchased as premade aqueous solutions that can then be diluted in distilled water to obtain desired concentration (Acrysol™ Technical Data Sheet 2007; Conservation Resources International 2014; Rhoplex™ Technical Data Sheet 2012). Butvar B®-98 is bought in powder form and is soluble in various alcohols including isopropyl alcohol (Butvar Manufacturers Guide 2013). All four materials were prepared at a 10% concentration, within the range suggested by Johnson (1994) (Table 2). Acrysol™ WS-24 and Rhoplex™ B-60A were prepared using the dilution equation below, where V_2 = the volume of diluted solution being made; C_2 = the desired concentration of the solution; C_1 = current concentration of solution; V_1 = volume of solution to be added to solvent.

$$V_1 = \frac{V_2 * C_2}{C_1} \quad \text{(Eq 1)}$$

The volume of the solvent to be mixed with the volume of the solution meant for dilution could then be calculated as:

$$\text{Volume of solvent} = V_2 - V_1 \quad \text{(Eq 2)}$$

Table 2. Preparation of 100 mL of consolidant (10% C)

Consolidant	Solvent Type	Amount of Solute	Amount of Solvent
Acryloid™ B-72	acetone	10 g	100 mL
Acrysol™ WS-24	distilled water	28 mL	72 mL
Rhoplex™ B-60A	distilled water	22 mL	78 mL
Butvar® B-98	isopropyl alcohol	10 g	100 mL

Material Application

Each polymer was applied to a subgroup of the study population *in situ* (Table 1). Prior to consolidant application, debris from the fire event had to be removed to expose the thermally altered bone samples on the floor of the fire cell. Larger pieces, such as sheetrock, charred wood, and nails, were removed manually, while small debris was gently brushed or blown away from bony material using soft taklon bristled makeup brushes (brand name: EcoTools) and a one directional negative pressure silicon bulb meant to clean camera lenses (brand name: Giottos Rocket Air Blaster). Consolidant material was then applied to all visible surfaces of the bone using a graduated polyethylene squeeze wash bottle with a straw-like spout to minimize the amount of overspray, and then it was allowed to dry. Dryness was defined for this research as a lack of moisture and tackiness on the external bone surface due to the difficulty in assessing internal dryness. After the second layer of consolidant dried, the bone was turned over in order to apply material to previously inaccessible surfaces for two additional layers. Although Kres and Lovell (1995) applied two layers of consolidant to their sample in the lab, four total layers of consolidant were applied to each thermally treated bony element in this research to ensure complete coverage when applied *in situ*. Dry time for each layer was then summed together to obtain a total dry time per specimen.

MECHANICAL TESTING AND SAMPLING STRATEGY

Although the specific anatomical location of sampling has been shown to exhibit variation in mechanical properties (Cioffi et al. 2007; Cuy et al. 2002; Turner and Burr 1993), the fragmentary nature of the samples made controlling for this variable difficult. Rather, general anatomical regions, specifically the anterior diaphysis when it could accurately be assessed, and bone type (i.e., trabecular bone from the epiphyses, cortical bone from the diaphyses, and enamel) were controlled for during nanoindentation and drop weight impact testing. A stratified random sample of relatively smooth and flat fragments was selected for these tests to ensure accuracy by keeping the sample perpendicular to the applied force. The remaining intact proximal portion of the femur and the condylar epiphysis of burned specimens were then selected for forced vibration testing. These mechanical test were chosen over traditional tensile testing, which requires sample preparation into a dumbbell shape (Currey 1990). Samples in Stages IV and V, defined in Shipman et al. (1984) according to colors present (i.e., white, light gray, light blue gray, and gray as the predominant color; Munsell 2009), were preferentially chosen for mechanical testing as a means of controlling for the degree of calcination.

Nanoindentation

To qualitatively test how each of these materials affects the mechanical response of the friable bone fragments, load-displacement curves were analyzed from indents produced by the Hysitron Ubi 1 Nanoindenter® in conjunction with Triboscan™ software (Figure 4). Nanoindenters operate by pressing a small probe into the surface of a

sample and measuring probe displacement versus applied force in order to understand the mechanical properties of the material tested (Hysitron 2016). Due to monetary and time constraints, only one sample from each of the five groups investigated were indented for three different mineralized tissue types (i.e., enamel, cortical bone, and flat bone from the frontal). In addition to the five sample groups, unburned control samples were tested to

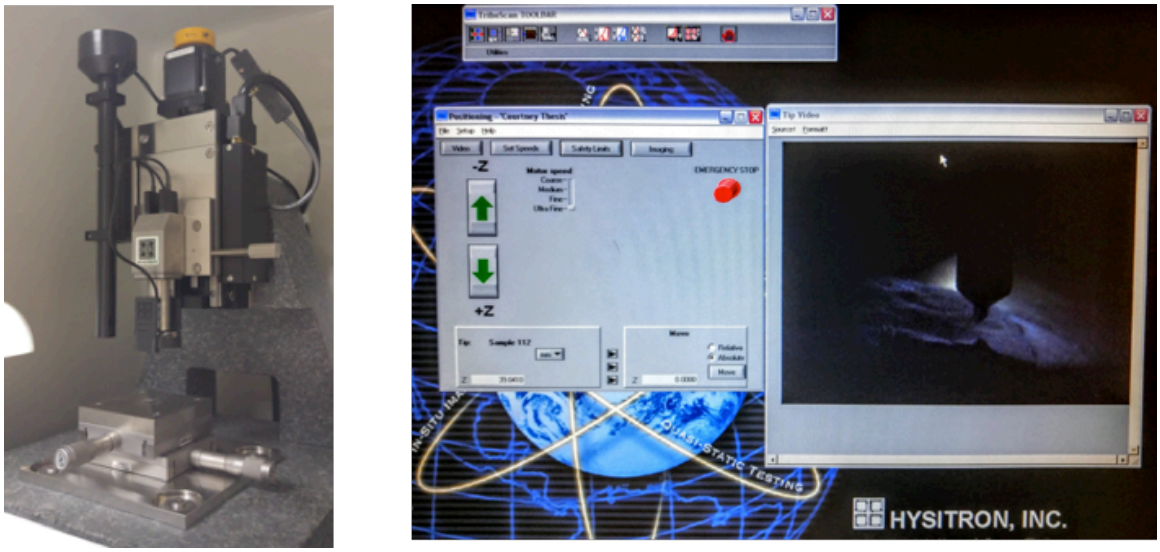

Figure 4. Ubi 1 Hysitron Nanoindenter (left) and Triboscan™ Software (right)

establish baseline load-displacement data. Prior to indentation, samples were mounted on magnetic wafers to prevent the sample from moving during the indentation process. Because most samples were curved, sandpaper was used to obtain a flat, smooth surface to adhere to the magnet. Since there is not a standard method for nanoindentation of bone, a modification of the method outlined in Paietta et al. (2011) using a 50μm 90° conical tip was applied. A trapezoidal ramp-and-hold curve, with a controlled load and unload rate of 30 μm/sec to a peak force of 700μm, and held for 120 seconds (sec) prior to unloading, was used. Each sample was indented between 3 and 12 times in a linear

array to ensure that consistent, generalized load-displacement curves were obtained for qualitative analysis of the mode of deformation occurring in each tested sample.

Mode of deformation was determined using the decision tree presented in Oyen and Cook (2009), which classifies the shape of a load-displacement curve as purely elastic deformation, brittle fracture deformation, elastic-plastic deformation, visco-elastic deformation, and visco-elastic-plastic deformation (Figure 5). An elastic response does

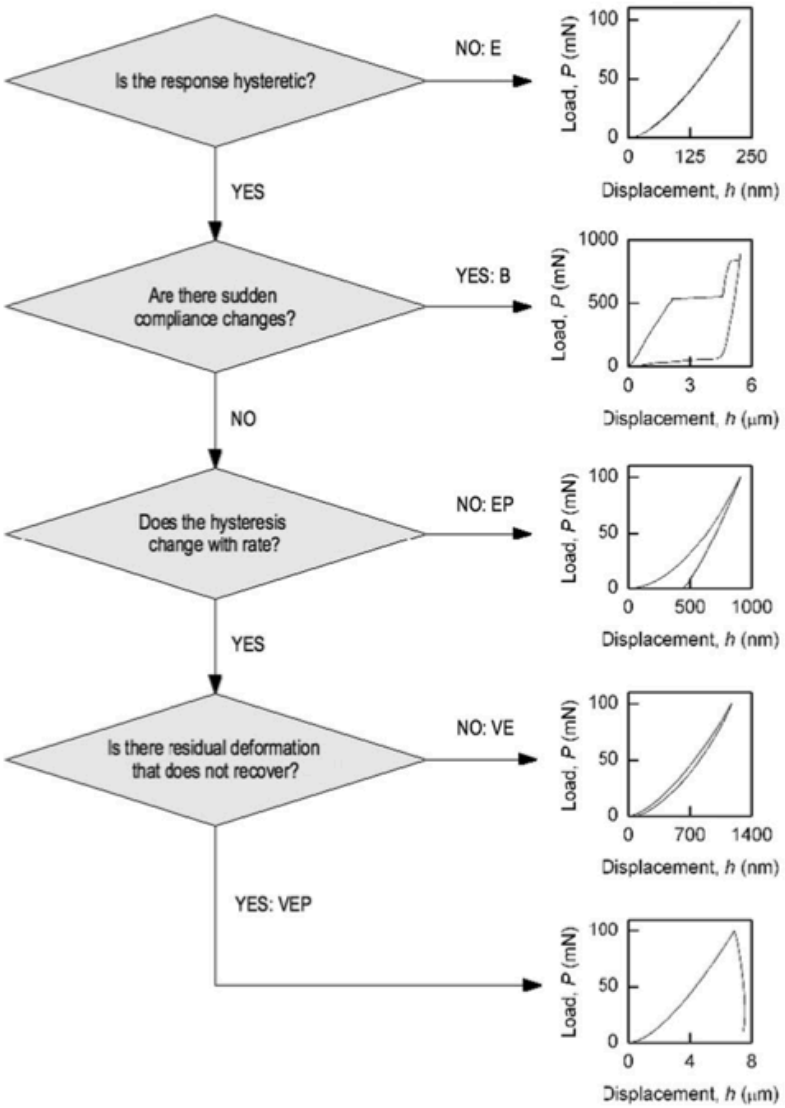

Figure 5. Oyen and Cook (2009) decision tree to assess the mode of deformation in a load-displacement curve where: E, elastic response; B, brittle response; P, plastic response; V, viscous response

not exhibit hysteresis, a loop between input and output, and will produce a load-displacement curve where the loading and unloading segments are indistinguishable because displacement instantly reverses at the same rate as the force is unloaded. A brittle response will exhibit immediate displacement due to a fracture event producing a curve with a stair-stepped appearance. An elastic and plastic response produces a hysteresis that is consistent regardless of loading rate resulting in permanent deformation. A viscous and elastic response does not cause permanent displacement, but will change depending on the rate at which the load force is applied. Finally, a visco-elastic and plastic response is rate dependent and results in permanent displacement. Although the general shape of an elastic-plastic load-displacement curve does not differ from a visco-elastic and plastic response, previous research has shown that bone deformation is rate dependent (Martin et al. 1998); therefore, a hysteresis with no fracture events exhibiting permanent displacement will be considered as a visco-elastic and plastic mode of deformation.

Furthermore, although Young's modulus and hardness can be calculated using load-displacement data and indenter contact area (Oliver and Pharr 1992), research suggests the anisotropic quality of bone contributes to vastly different values depending on the sample orientation (Swadener et al. 2001). Although samples were indented on the external bone surface, the extreme warping that occurred during thermal alteration make correcting for anisotropy, according to the rule of mixtures suggested in Swadener et al (2001), extremely problematic. Therefore, load-displacement data from nanoindentation were only used to investigate deformation response. Each indent was placed 1 millimeter (mm) apart, unless an uneven surface was encountered and then avoided prior to indent in

order to obtain more accurate displacement data. Prior to testing bone samples, the nanoindenter was calibrated using fused silica and copper.

Drop Weight Impact Test

Drop weight impact testing was conducted on 176 cortical and 43 trabecular samples (Table 3) to assess differences in sample toughness by evaluating the impact

Table 3. Summary of samples used in drop weight impact test

Treatment	Sample Location	Total Samples Tested
Burned (no consolidant)	31 epiphyses 8 diaphyses	39
Acryloid™ B-72	36 epiphyses 10 diaphyses	46
Acrysol™ WS-24	38 epiphyses 8 diaphyses	46
Rhoplex™ B-60A	37 epiphyses 9 diaphyses	46
Butvar® B-98	34 epiphyses 8 diaphyses	42

strength, or impact energy required to cause fracture (Ku et al 2005). A ball bearing of .010 gram (g) mass was dropped through a guiding tube, set perpendicular to the sample stage, beginning at 0.10 meter (m) away from the sample, by increments of 0.01 m to a maximum height of 0.85 m height until the sample fractured (Figure 6). Drop energy was then calculated by:

$$E = mgh \quad \text{(Eq 3)}$$

where E is energy in Joules (J), m is the mass of the ball bearing in kilograms (kg), g is gravitational pull (9.81 m/s^2), and h is the drop height of the ball bearing (m). Although some energy is lost due friction as the ball bearing travels through the guide tube prior to sample impact, the effect is considered negligible (Ku et al. 2005). Impact energy was

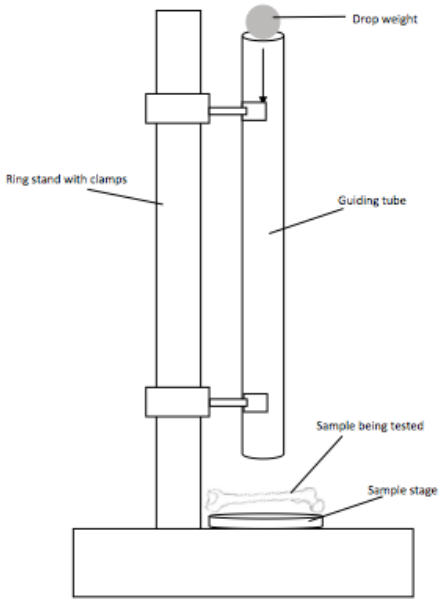

Figure 6. Schematic of mechanism used for drop weight impact testing

adjusted for cortical samples by adapting the equation from Lee et al. (2011) in an attempt to control for varying sample dimensions (i.e. length, width, thickness) by:

$$\mathbf{E_{adj}} = \frac{\mathbf{E}}{\mathbf{d_s * t}} \quad \text{(Eq 4)}$$

where d_s is the minimum dimension of the sample (mm), either length or width, and t is the thickness of the sample (mm). Furthermore, because the samples had varying shapes, a typical minimum dimension for each sample was used to normalize the impact energy. Because the femoral heads used to test trabecular bone were very homogenous in diameter, height, and hemi-spherical shape, impact energy was calculated using Eq. 3 and not adjusted.

Vibration testing

To further assess the consolidant's ability to keep friable burned samples intact during transport and repeated handling in a laboratory setting, samples were exposed to forced vibration using a Jintai R&D dental lab vibrator (ISO9001:2000) meant to agitate air bubbles from plaster while making dental casts. Samples tested (Table 4) were placed in 4mil polyethylene bags and placed on the stage of the lab vibrator, after it was turned to a maximum vibration frequency of 60 Hz +/-10%, for a 30sec exposure time. Although

Table 4. Summary of samples used in vibration test

Treatment	Sample Location	Total Samples Tested
Burned (no consolidant)	10 proximal diaphyses 10 condylar epiphyses	20
Acryloid™ B-72	12 proximal diaphyses 12 condylar epiphyses	20
Acrysol™ WS-24	12 proximal diaphyses 12 condylar epiphyses	24
Rhoplex™ B-60A	12 proximal diaphyses 12 condylar epiphyses	22
Butvar® B-98	12 proximal diaphyses 12 condylar epiphyses	24

Young's modulus and bending or torsional stiffness can be calculated using Beam Theory (Landro and Lorenzi 2009; Muller et al. 2008), dimensions necessary for calculations (i.e., length, thickness, and density) were not recorded due to the irregular shape of the samples. Instead a percentage of specimen weight loss due to fracturing as a result of the applied force was calculated and interpreted as a measure of strength.

STATISTICAL ANALYSIS

Descriptive statistics including measures of central tendency and dispersion were calculated for all sample variables tested. The Shapiro-Wilkes test for normality and a Levene's test for homogeneity of variance was used to determine whether an analysis of variance or non-parametric equivalent (i.e., Kruskal-Wallis) was more appropriate for the dataset being investigated (Rogerson 2015). Post hoc tests were performed when significant differences were found, and effect size was calculated to interpret the impact of the variable being analyzed. Effect sizes of 0.5 for high impact, 0.2-0.3 for moderate impact, and 0.1 for low impact were used for interpretation of the statistical significance of each test (Fritz et al. 2012).

III. **RESULTS**

STAGED FIRE EVENTS

As previously stated, three fire events were conducted to burn all bone samples. A summary of the temperature data is presented in Table 5 and Figure 7.

Table 5. Summary of temperature data

Fire Event	Duration (min)	Peak Temperature (°C)	Duration ≥200°C (min)	Duration ≥400°C (min)	Duration ≥600°C (min)	Duration ≥800°C (min)	Duration ≥900°C (min)
1	225	998	123.75	83.67	32.20	20.75	5.70
2	360	897	45.97	25.43	20.68	5.42	0.00
3	310	648	91.27	50.57	0.23	0	0

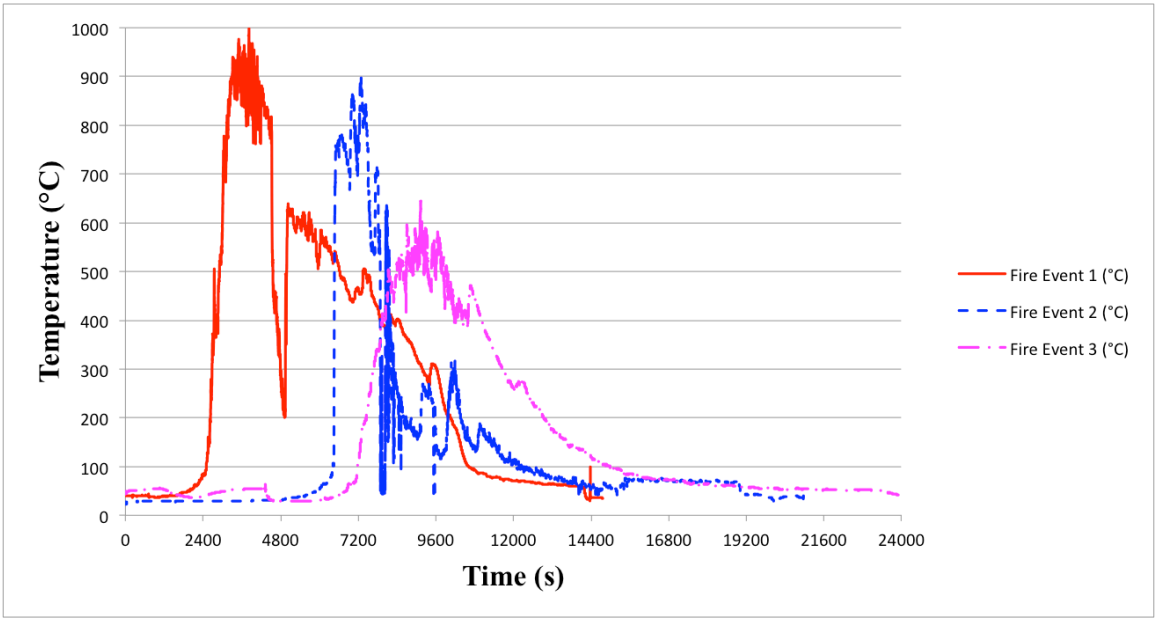

Figure 7. Smooth-line scatterplot of temperature data (x-axis intervals convert to 40 min)

Based on the data collected, fire event 1 reached a higher peak temperature than events 2 and 3; however, the range of colors present on the samples from each fire indicates a comparable range of temperature and exposure achieved among fire events (Figure 8). Furthermore, the second burn conducted initially did not thermally alter the sample adequately, and two pallets were added to the structure in a "teepee" formation to reignite and sustain the fire to obtain a friable, calcined sample (Figure 9 and 10).

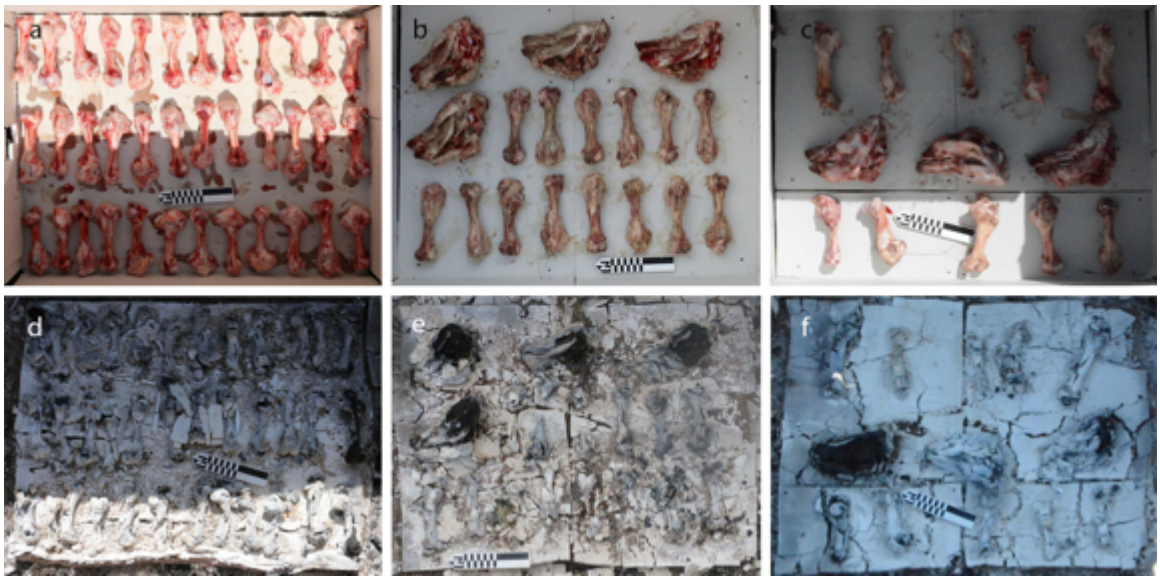

Figure 8. Bone color (a-c)before and (d-f) after Fire Event 1-3 (left to right)


Figure 9. Insufficient burning of specimens during Fire Event 2

Figure 10. “Teepee” structure viewed (a)anteriorly and (b)laterally to sustain Fire Event 2

CONSOLIDANT MATERIAL AND APPLICATION

Several characteristics were qualitatively considered for each material being tested including: ease of solution preparation, viscosity and ease of application, storage of material in a field setting, absorption by bone samples, and any alteration to the samples appearance (Table 6).

Table 6. Summary of consolidant qualitative data

	Acryloid™ B-72	Acrysol™ WS-24	Rhoplex™ B-60A	Butvar® B-98
Solution Preparation	Minimal	Minimal	Minimal	Laborious
Viscosity	Low	Low	Low	High
Easily Applied	Yes	Yes	Yes	No
Storage Needs	Shade	None	None	Shade
Degree of Absorption	Complete	Moderate	Moderate	Minimal
Permanent Color Change to specimen	None	None	None	Darkens specimen
Surface Finish	Matte	Semi-Glossy	Semi-Glossy	Glossy; bubbly

Acryloid™ B-72

Acryloid™ B-72 was prepared by dissolving copolymer pellets in pure acetone. Although preparation time was not recorded, the material was dissolved completely by the time I arrived at the site of recovery indicating a relatively quick mixing time (e.g., 10-20 minutes) once the solute and solvent have been measured out. Once dissolved the solution has a low viscosity, similar to that of water, making application using the squirting method easy. However, because acetone is highly volatile and I experienced extremely high ambient temperatures during the *in situ* application and recovery period of data collection, the Acryloid™ B-72 solution had to be wrapped in cloth and kept out of direct sunlight to minimize evaporation and reduce the probability of a potential change

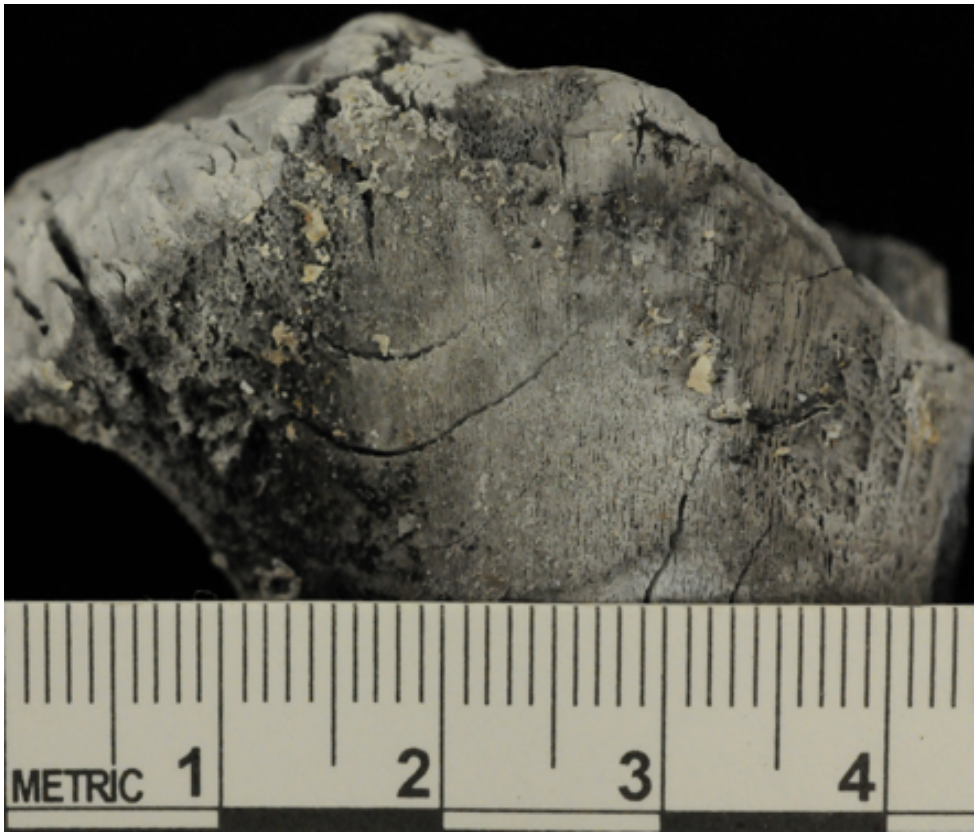

Figure 11. Matte bone surface after Acryloid™ B-72 application

in solution concentration during recovery. The consolidant appeared to completely absorb into the bone specimens once applied, leaving a matte finish after all layers had completely dried. Furthermore, no permanent change to bone color was observed (Figure 11).

Acrysol™ WS-24 and Rhoplex™ B-60A

Acrysol™ WS-24 and Rhoplex™ B-60A are qualitatively very similar to each other, as well as to Acryloid™ B-72. Both materials were prepared by diluting a premade solution (particle weight 36% and 46% respectively) in distilled water, and a uniform mixture was obtained immediately after minimal agitation. The solutions also have a low, watery viscosity and were easily applied to all bone surfaces. Furthermore, special

Figure 12. Semi-glossy bone surface (arrows) after Acrysol™ WS-24 application

consideration for storage during recovery is not required since distilled water is not as volatile as acetone, making any change in solution concentration improbable. Both consolidants appeared to completely absorb when initially applied; however, subsequent layers tended to maintain surface tension of the solution causing most of it to bead and roll off the bone surface. Finally, a semi-glossy finish and no permanent change to bone color were observed for both materials (Figure 12 and 13).

Figure 13. Semi-glossy bone surface (arrows) after Rhoplex™ B-60A application

Butvar® B-98

Unlike the previous three materials, Butvar® B-98 involves a lengthy and complex solution preparation. When preparing Butvar® B-98 solution, the solvent should

be placed in a container prior to adding the solute. When prepared by adding the solvent to the solute, the Butvar material did not completely dissolve and instead coagulated into a gelatinous mass that was unusable. Furthermore, Butvar® B-98 should be added to the solvent slowly to allow particles to completely dissolve before adding more material, drastically increasing the time required for solution preparation. Once prepared into a homogeneous mixture, Butvar® B-98 is highly viscous, making application to uneven or curved surfaces extremely difficult. Because isopropyl alcohol has volatility more similar to acetone, and will evaporate quickly, Butvar® B-98 solutions were also kept out of direct sunlight during the recovery process. Furthermore, Butvar® B-98 did not readily absorb into the bone samples during material application. Rather, the mixture appeared to run down the bone surface, becoming an outer coating or skin once dry. Finally, Butvar®

Figure 14. Glossy bone surface (arrows) with bubbles and bone darkening (circle) after Butvar® B-98 application

B-98 caused a permanent change in bone color, making the samples darker, while leaving a very glossy finish and hardened bubbles obscuring the bone surface (Figure 14).

Quantitative Assessment

Dry time for each layer applied in the field was recorded, and later combined to obtain an aggregate dry time for each specimen recovered in the field. Measures of central tendency and dispersion for each consolidant sample are summarized in Table 7.

Table 7. Descriptive statistics of total dry time (minutes) per specimen

	Acryloid™ B-72	Acrysol™ WS-24	Rhoplex™ B-60A	Butvar® B-98
Minimum	27.50	63.25	200.00	42.25
Maximum	62.25	368.00	347.00	81.00
Mean	40.769	260.365	312.077	66.25
Median	35.750	313.00	319.00	67.00
Variance	163.286	11205.423	1364.410	114.521
Std. Deviation	12.778	105.856	36.938	10.701
Median Absolute Deviation (MAD)	5.25	42.00	11.00	7.00

A Shapiro-Wilks test of normality was applied to each distribution, and results show that Acryloid™ B-72, Acrysol™ WS-24, and Rhoplex™ B-60A are not normally distributed; therefore, the median is a more accurate statistic for central tendency. Acryloid™ B-72 has the fastest total dry time per specimen, followed by Butvar® B-98, then Acrysol™ WS-24, and finally Rhoplex™ B-60A. Furthermore, the median absolute deviation (MAD) rather than a standard deviation based on means, gives a more accurate measure of dispersion for each distribution. Acryloid™ B-72 has the lowest MAD, followed by Butvar® B-98, then Rhoplex™ B-60A, and finally Acrysol™ WS-24. Although Acrysol™ WS-24 tends to dry slightly faster than Rhoplex™ B-60A, the

sample exhibits a wider degree of dispersion around the median, indicating that total dry time for Acrysol™ WS-24 is more variable than Rhoplex™ B-60A (Figure 15).

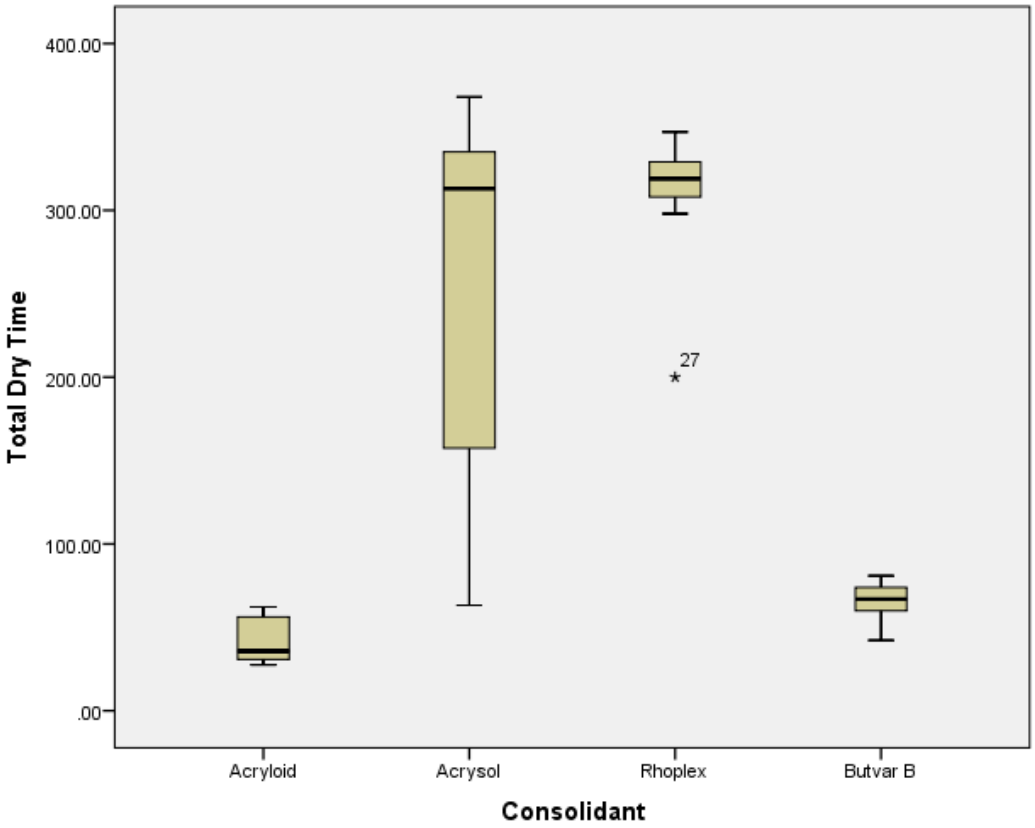

Figure 15. Box plot of total dry time (min) including outliers (*)

MECHANICAL TESTING OF SAMPLES

Nanoindentation

Load-displacement curves produced using the Hysitron Ubi 1 Nanoindenter® and Triboscan™ software were analyzed to qualitatively assess the tested samples mechanical response to deformation. For all three bone types tested the unburned control sample

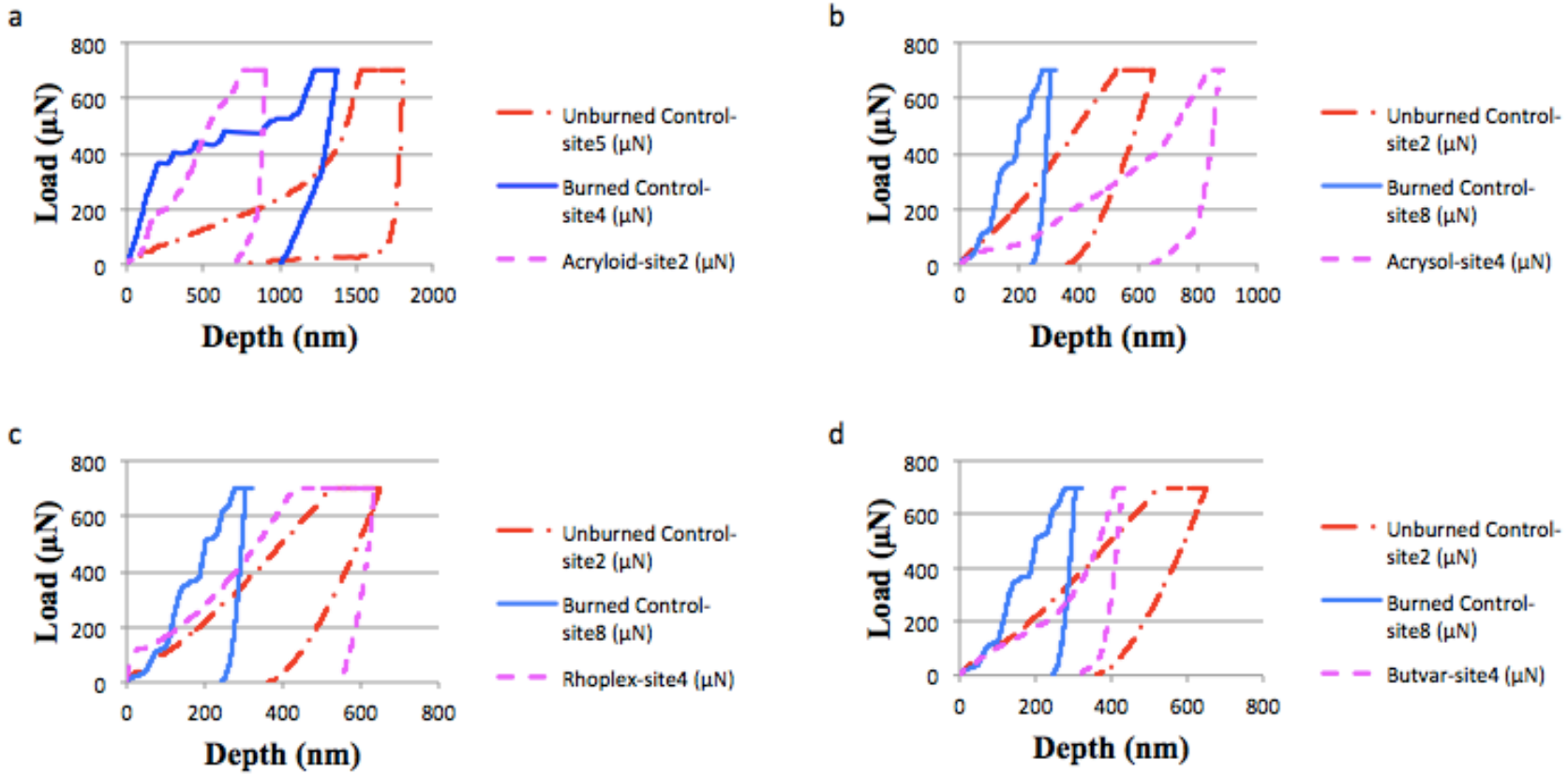

Figure 16. Representative Load-Displacement curves for cortical samples treated with Acryloid™ B-72 (a), Acrysol™ WS-24 (b), Rhoplex™ B-60A (c), Butvar® B-98 (d)

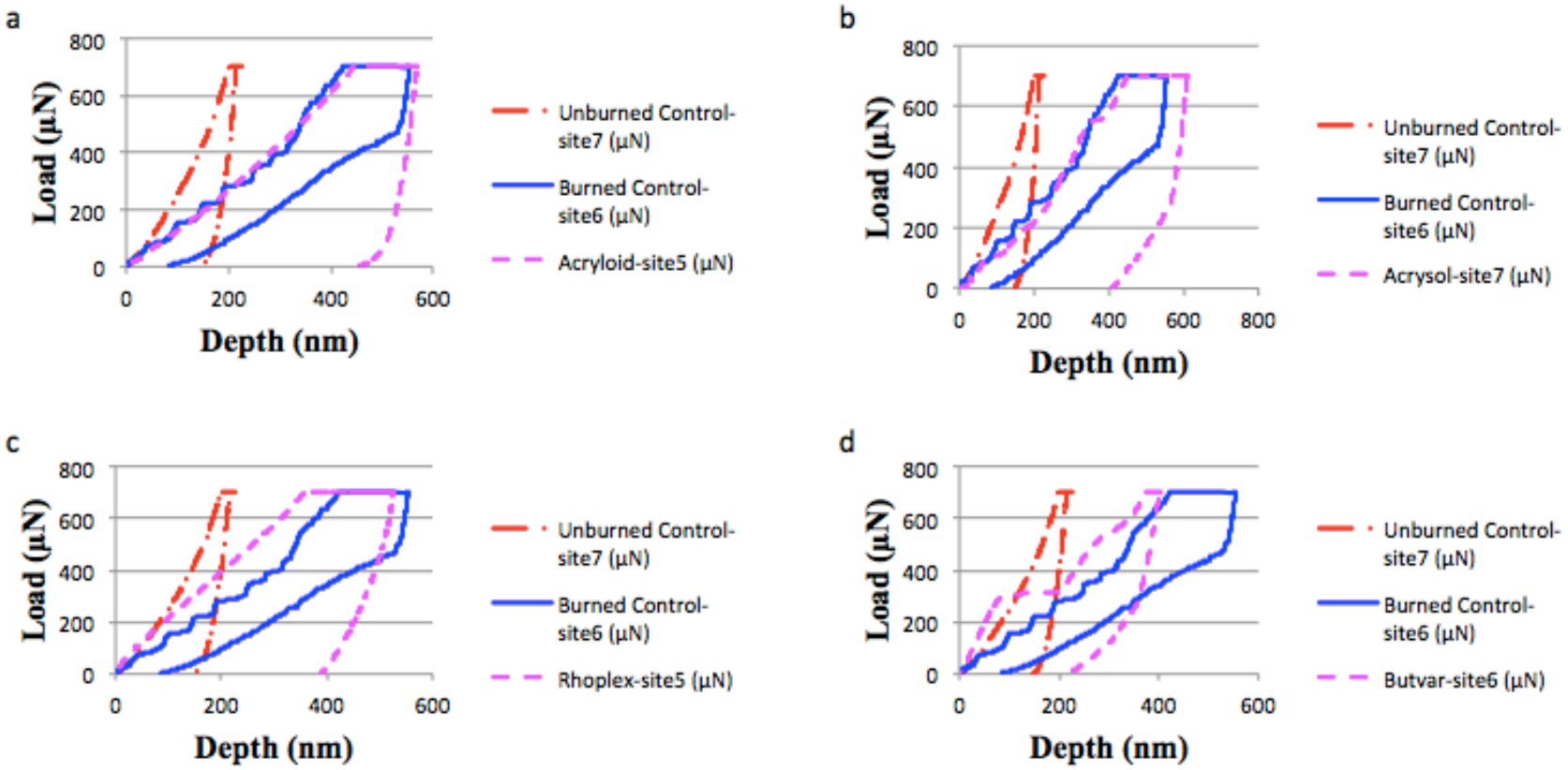

Figure 17. Representative Load-Displacement curves for enamel samples treated with Acryloid™ B-72 (a), Acrysol™ WS-24 (b), Rhoplex™ B-60A (c), Butvar® B-98 (d)

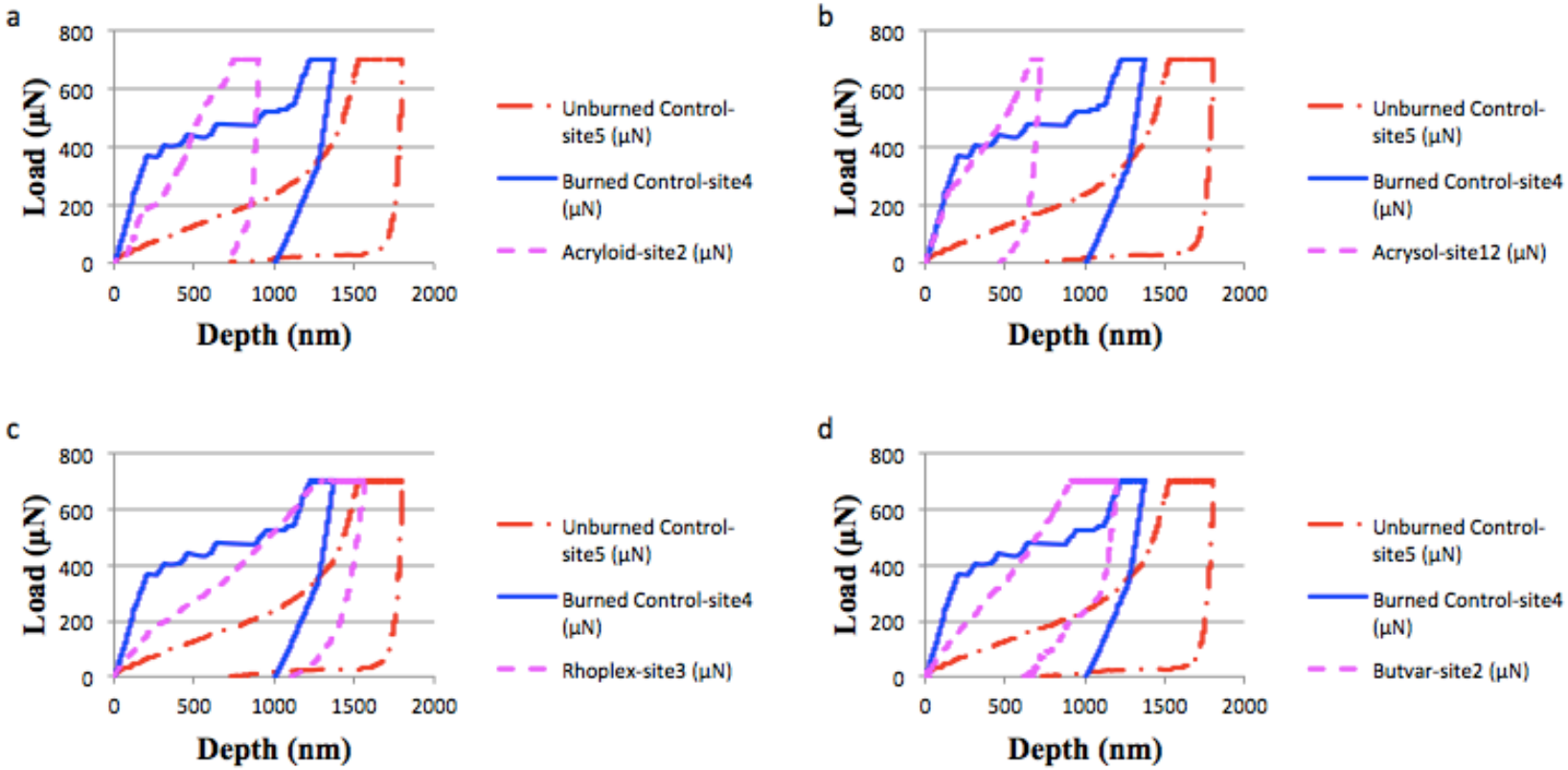

Figure 18. Representative Load-Displacement curves for cranial samples treated with Acryloid™ B-72 (a), Acrysol™ WS-24 (b), Rhoplex™ B-60A (c), Butvar® B-98 (d)

exhibited a visco-elastic and plastic mode of deformation, while the burned control had a brittle response (Figures 16-18). This brittleness is evident as fracture events during the loading and unloading curves, making the hysteresis have a stair-step appearance. Although the dental samples treated with Acrysol WS-24 and Butvar B-98 had a brittle mechanical response to deformation, the number of fracture events during loading is less than that of the burned control. Generally, the samples treated with consolidant material behaved in a visco-elastic and plastic manner, more similar to the unburned control sample than the burned control.

Drop Weight Impact Test

Although both cortical and trabecular samples were assessed using drop weight impact testing, they were analyzed separately because of the difference in bone type. Descriptive statistics for cortical sample distributions are summarized in Table 8.

Table 8. Descriptive statistics of adjusted impact force (J) for cortical samples

	Burned Control	Acryloid B-72	Acrysol WS-24	Rhoplex B-60-A	Butvar B-98
Minimum	0.218	0.218	0.491	0.654	0.327
Maximum	1.472	2.943	2.453	4.905	5.886
Mean	0.727	0.992	1.117	1.735	2.226
Median	0.654	0.981	0.981	1.635	1.962
Variance	127.772	326.357	225.919	664.849	1697.927
Std. Deviation	0.357	0.571	0.475	0.815	1.303
MAD	0.218	0.327	0.327	0.332	0.652

A Shapiro-Wilkes test of normality and a Levene's test for homogeneity of variance ($F(4,171)=12.159$; $\alpha = 0.05$; $p=1.01\text{x}10^{-8}$) indicate that all distributions do not meet the assumption of normality, and they do not have equal variance. Since the

ANOVA assumptions of normality and homogeneous variances are violated, a Kruskal-Wallis omnibus test was conducted to test whether the median differs across consolidant groups. Results indicate there is a statistically significant difference across samples ($H=63.654$; $\alpha = 0.05$; $p=4.94x10^{-13}$). Post hoc Mann-Whitney U-tests were performed for multiple pairwise comparisons (Table 9). To reduce the probability of making a Type-1 error and falsely rejecting the null hypothesis, the Dunn-Šidák correction was used to interpret the resulting p-values by adjusting the α-confidence level ($\alpha_{SID} = 0.005116$; Šidák 1967). Effect size (η^2) was also calculated to investigate the strength of the pairwise comparison, and can be interpreted similar to a correlation coefficient (Table 10). Thus a larger effect size indicates a higher portion of the observed difference is due to the variable being tested (i.e, consolidant treatment; Fritz et al. 2012).

Table 9. Summary of p-values from post hoc Mann-Whitney U-Test of adjusted impact energy for cortical bone

	Burned Control	Acryloid™ B-72	Acrysol™ WS-24	Rhoplex™ B-60A	Butvar® B-98
Burned Control	———				
Acryloid™ B-72	0.073	———			
Acrysol™ WS-24	0.915	0.087	———		
Rhoplex™ B-60A	0.001	0.037	0.002	———	
Butvar® B-98	0.002	0.117	0.003	0.398	———

Table 10. Summary of η^2 from post hoc Mann-Whitney U-Test of adjusted impact energy for cortical bone

	Burned Control	Acryloid™ B-72	Acrysol™ WS-24	Rhoplex™ B-60A	Butvar® B-98
Burned Control	———				
Acryloid™ B-72	0.238	———			
Acrysol™ WS-24	0.213	0.000	———		
Rhoplex™ B-60A	0.500	0.234	0.329	———	
Butvar® B-98	0.525	0.137	0.201	0.066	———

Although the Kruskall-Wallis omnibus test indicates there is a statistical difference in adjusted impact strength across cortical samples, post hoc Mann-Whitney U-test of pairwise comparisons using a Dunn-Šidák alpha level correction show that the Rhoplex™ B-60A and Butvar® B-98 are the only samples that statistically differ from the burned control. Although Acryloid™ B-72 and Acrysol™ WS-24 are statistically similar to the burned control, the η^2 for both comparisons suggest this statistic should be interpreted cautiously as there is only a moderate correlation in the variable assessed. Furthermore, a box plot of the distributions shows that although there may not be a statistical difference, all four consolidant materials increased the adjusted impact energy that the cortical samples could withstand prior to catastrophic fracture (Figure 19).

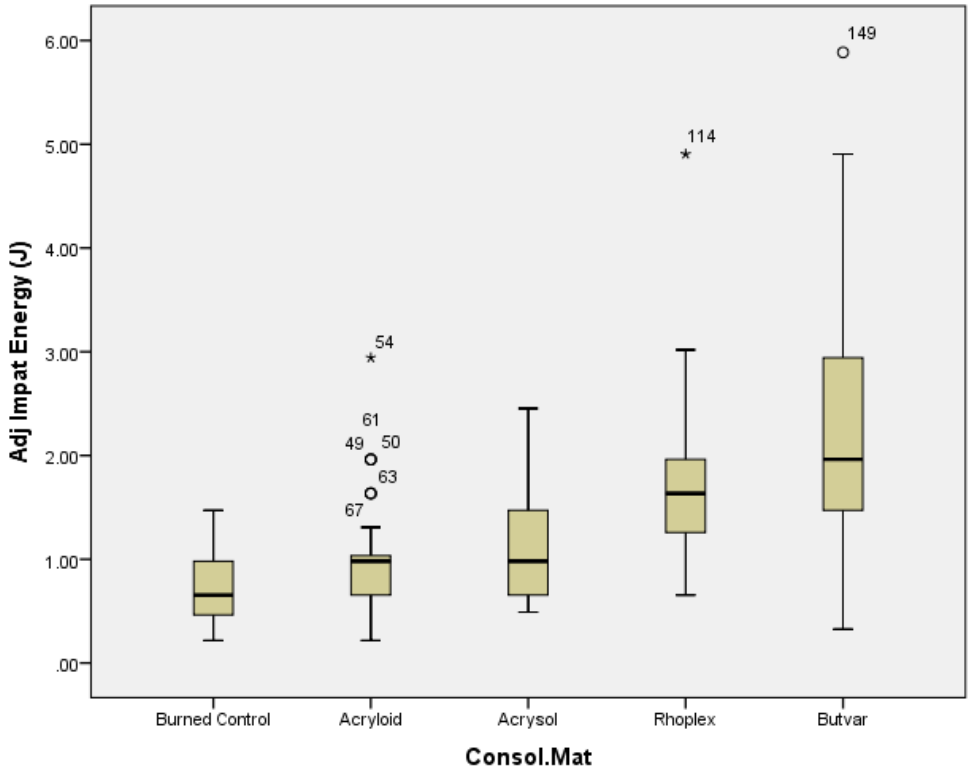

Figure 19. Box-plot of adjusted impact energy(J) for cortical bone including outliers (*°)

Although trabecular bone initially deformed due to compression, forming a dimple under the impact site, testing continued at increasing heights until catastrophic fracture occurred, breaking the sample into multiple fragments. Descriptive statistics for trabecular bone are summarized in Table 11. It should be noted that although impact

Table 11. Descriptive statistics of impact force (J) for trabecular samples

	Burned Control	Acryloid™ B-72	Acrysol™ WS-24	Rhoplex™ B-60A	Butvar® B-98
Minimum	0.028	0.018	0.019	0.048	0.048
Maximum	0.057	0.082	0.067	0.082	0.082
Mean	0.041	0.059	0.043	0.075	0.074
Median	0.038	0.067	0.043	0.082	0.082
Variance	0.000	0.001	0.000	0.000	0.000
Std. Deviation	0.012	0.023	0.018	0.013	0.013
MAD	0.010	0.015	0.015	0.000	0.000

energy from a height of 0.85 m is the maximum value recorded, several samples required multiple drops from this height to fracture; including one with Acryloid™ B-72, seven with Rhoplex™ B-60A, and five with Butvar® B-98 applied. The Kruskal-Wallis omnibus test was performed for trabecular samples as well since all distributions did not satisfy the assumption of normality required for ANOVA through the Shapiro-Wilks test. Results indicate there is a statistical difference across consolidants for trabecular bone ($H=20.82$, $df=4$, $p=3.44\text{x}10^{-4}$). Furthermore, similar results to the cortical samples were obtained from post hoc Mann-Whitney pairwise comparisons and calculated effects size ($\eta 2$) for the impact energy required to cause catastrophic failure to the trabecular samples (Table 12 and 13, Figure 20).

Table 12. Summary of p-values from post hoc Mann-Whitney U-Test of impact energy for trabecular bone

	Burned Control	Acryloid™ B-72	Acrysol™ WS-24	Rhoplex™ B-60A	Butvar® B-98
Burned Control	———				
Acryloid™ B-72	0.073	———			
Acrysol™ WS-24	0.915	0.087	———		
Rhoplex™ B-60A	0.001	0.037	0.002	———	
Butvar® B-98	0.002	0.117	0.003	0.398	———

Table 13. Summary of η^2 from post hoc Mann-Whitney U-Test of impact energy for trabecular bone

	Burned Control	Acryloid™ B-72	Acrysol™ WS-24	Rhoplex™ B-60A	Butvar® B-98
Burned Control	———				
Acryloid™ B-72	0.179	———			
Acrysol™ WS-24	0.001	0.163	———		
Rhoplex™ B-60A	0.624	0.228	0.566	———	
Butvar® B-98	0.613	0.137	0.566	0.042	———

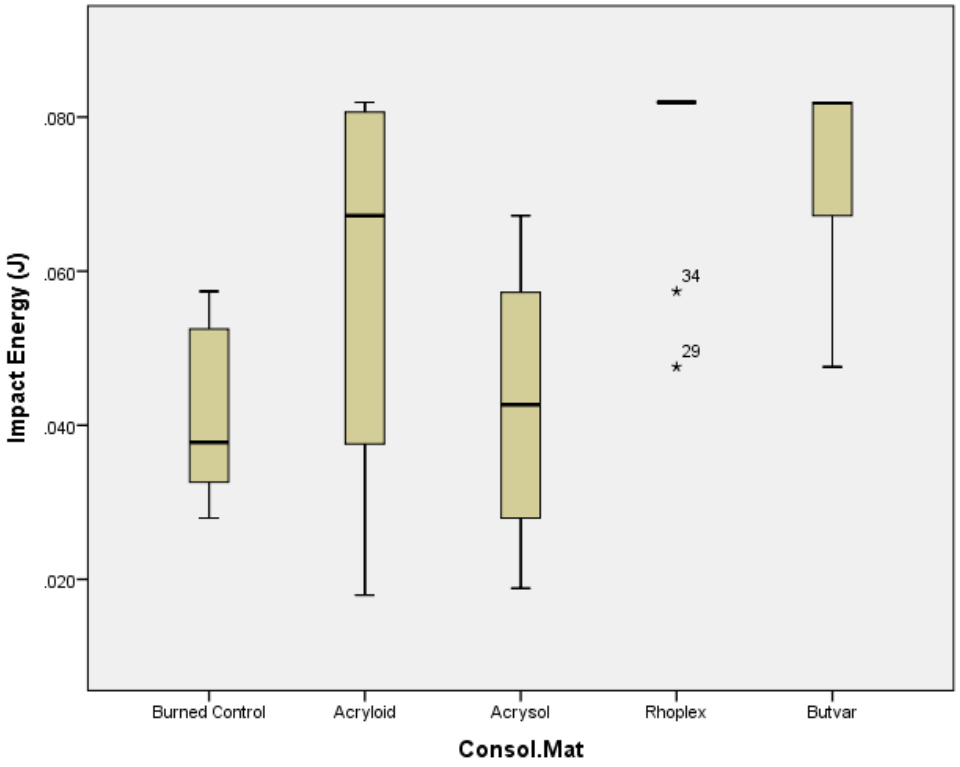

Figure 20. Box-plot of impact energy(J) for trabecular bone including outliers (*)

Forced Vibration Strength Test

Measures of central tendency and dispersion were calculated for each consolidant and are presented in Table 14. A Shapiro-Wilks test of normality indicates the data are not normally distributed, therefore the median and the MAD give a better indication of central tendency and spread for each distribution. All four samples with consolidant material added have a lower median percent of weight change after the forced vibration test; however, Acrysol™ WS-24 exhibits more dispersion about the median than Acryloid™ B-72, Rhoplex™ B-60-A, Butvar® B-98, or the burned control sample (Figure 21).

Table 14. Descriptive statistics of change in weight after forced vibration test

	Burned Control	Acryloid™ B-72	Acrysol™ WS-24	Rhoplex™ B-60A	Butvar® B-98
Minimum	1.148	0.037	0.053	0.008	0.000
Maximum	63.726	39.258	61.320	40.058	39.586
Mean	23.304	8.419	10.658	4.027	3.751
Median	19.148	3.830	1.606	0.812	0.348
Variance	340.896	146.549	285.026	115.287	110.013
Std. Deviation	18.463	12.106	16.882	10.737	10.489
MAD	10.369	2.744	48.683	0.069	0.193

Prior to investigating any differences between sample groups, a Levene's test for homogeneity of variance was executed ($F(4,105) = 4.386$, $p = 0.003$). Since the ANOVA assumptions of normality and homogeneous variances are violated with this dataset, a Kruskal-Wallis omnibus test was conducted, indicating there is a statistically significant difference across samples ($H = 41.801$; $\alpha = 0.05$; $p<0.000$). Post hoc Mann-Whitney U-tests were performed for multiple pairwise comparisons (Table 15), and interpreted using the Dunn- Šidák correction to adjust the α-confidence level ($\alpha_{SID}= 0.005116$; Šidák 1967)

as well as effect size (η^2) to investigate the strength of the pairwise comparison (Table 16).

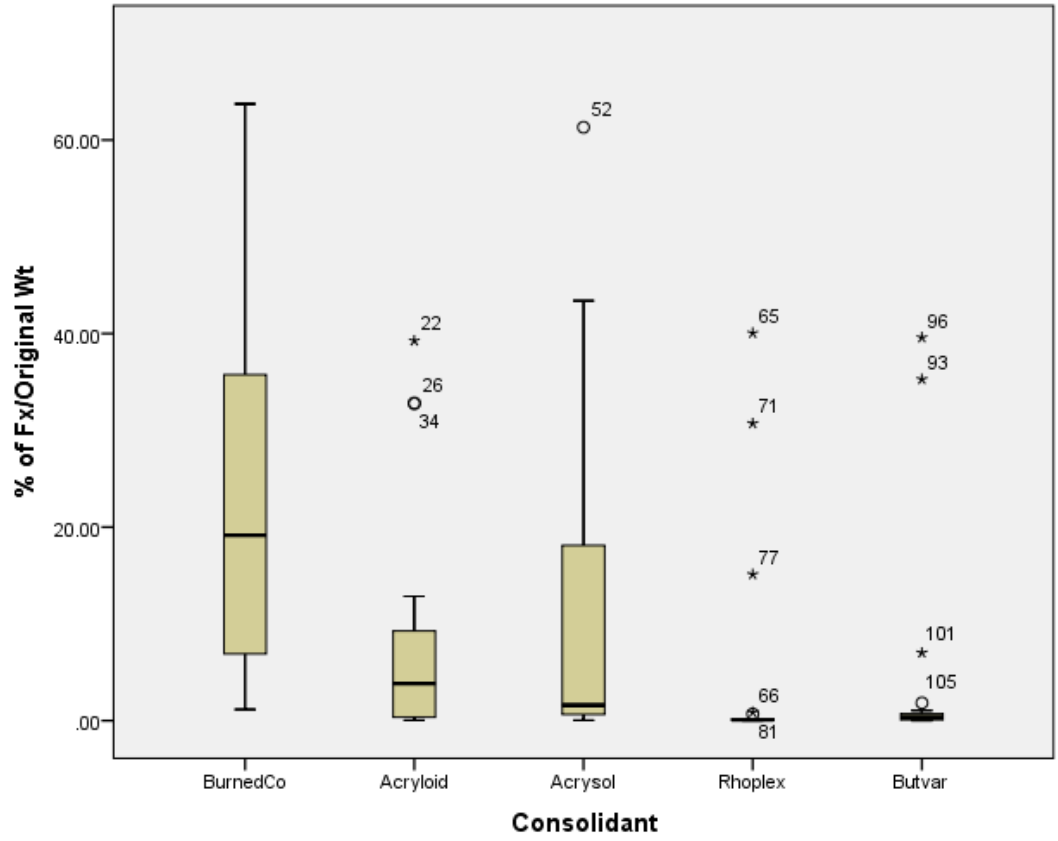

Figure 21. Box-plot of change in weight after vibration test including outliers (*°)

Table 15. Summary of p-values from post hoc Mann-Whitney U-Test after forced vibration testing

	Burned Control	Acryloid™ B-72	Acrysol™ WS-24	Rhoplex™ B-60A	Butvar® B-98
Burned Control	———				
Acryloid™ B-72	0.002	———			
Acrysol™ WS-24	0.002	0.953	———		
Rhoplex™ B-60A	0.000	0.002	0.000	———	
Butvar® B-98	0.000	0.014	0.002	0.080	———

Table 16. Summary of η^2 from post hoc Mann-Whitney U-Test after forced vibration testing

	Burned Control	Acryloid™ B-72	Acrysol™ WS-24	Rhoplex™ B-60A	Butvar® B-98
Burned Control	———				
Acryloid™ B-72	0.238	———			
Acrysol™ WS-24	0.213	0.000	———		
Rhoplex™ B-60A	0.500	0.234	0.329	———	
Butvar® B-98	0.525	0.137	0.201	0.066	———

The relative change in weight of the sample after exposure to a forced vibration, a proxy used to assess the sample's ultimate strength, statistically differs from the burned control for all consolidant materials tested indicating an increase in strength for samples treated with consolidant material over the burned control. Acryloid™ B-72 statistically differs from Rhoplex™ B-60A exhibiting a lower ultimate strength; however, there is no statistically significant difference between Acryloid™ B-72 and Acrysol™ WS-24. Although the Dunn- Šidák corrected p-value for a comparison of Acryloid™ B-72 and Butvar® B-98 indicate no difference in ultimate strength, the effect size is relatively low and can be interpreted cautiously. Similar interpretation should be used when considering the lack of statistical difference between Rhoplex™ B-60A and Butvar® B-98. Furthermore, the statistical difference observed for Rhoplex™ B-60A and Butvar® B-98 from the burned control samples exhibits a high effect size, indicating that the consolidant material has a strong impact on the statistical difference. Although all four consolidant materials keep burned bone more intact than when no materials were added, Butvar® B-98 and Rhoplex™ B-60A faired better than Acryloid™ B-72 and Acrysol™ WS-24 when samples were exposed to forced vibration.

IV. **DISCUSSION**

CONSOLIDANT RECOMMENDATIONS

Treatment with the materials tested here kept burned bone more intact than when no consolidation is attempted; however, each material has advantages and disadvantages. Acryloid™ B-72 exhibited a quick total dry time per specimen (median = 35.75 min) and leaves no visible change to the bone after application. Acrysol™ WS-24 and Rhoplex® B-60A leave a somewhat glossy finish after material application; however, their extremely long dry time (median > 5 hours) makes their use in the field completely unreasonable. Butvar™ B-98 has a fast total dry time similar to Acryloid™ B-72, but the change to the bone's appearance is particularly undesirable, specifically the permanent darkening to the bone samples since color can inform on the conditions of thermal alteration.

Furthermore, the samples' mechanical response to loading indicates that all four materials increase the ultimate strength of burned bone. Rhoplex™ B-60A and Butvar® B-98 performed better than Acryloid™ B-72 and Acrysol™ WS-24 during drop weight impact and forced vibration testing. This outcome is significant since these types of mechanical loading are likely to be encountered during recovery efforts from transportation vibration and potential mishandling of remains resulting in further postmortem damage. Additionally, Acryloid™ B-72 and Rhoplex™ B-60A consistently exhibited visco-elastic and plastic deformation similar to the unburned control across all bone types tested using nanoindentation, while Acrysol™ WS-24 and Butvar® B-98 variably demonstrated a somewhat brittle and a visco-elastic and plastic response to loading.

Based on the total dry time per specimen, the ease of application, the finish left on the bone, and the results of mechanical testing, Acryloid™ B-72 is the most suitable consolidant tested for use on burned bone (Table 17). This recommendation takes into consideration both the qualitative and quantitative results of this research. Although Acryloid™ B-72 was not ultimately as strong as Rhoplex™ B-60A or Butvar® B-98 during mechanical testing, the quicker dry time and the lack of alteration to the bone's appearance after application make Acryloid™ B-72 a more suitable choice. However, because Rhoplex™ B-60A kept specimens more intact during mechanical testing, if the dry time could be reduced using a more volatile solvent it would be recommended for use on burned bone.

Table 17. Summary of Results

	Acryloid™ B-72	Acrysol™ WS-24	Rhoplex™ B-60A	Butvar® B-98
Required Solution Preparation	Minimal	Minimal	Minimal	Laborious
Viscosity	Low	Low	Low	High
Easily Applied	Yes	Yes	Yes	No
Storage Needs	Shade	None	None	Shade
Degree of Absorption	Complete	Moderate	Moderate	Minimal
Color Change with Application	None	None	None	Darkens Specimen
Surface Finish	Matte	Semi-Glossy	Semi-Glossy	Glossy; bubbly
Quick Dry Time	Yes	No	No	Yes
Mechanical Response upon Loading	Viscous-elastic-plastic	Viscous-elastic-plastic	Viscous-elastic plastic	Brittle
Median Impact Strength (J) from Drop Weight test	0.981 (cortical) 0.038 (trabecular)	0.981 (cortical) 0.067 (trabecular)	1.635 (cortical) 0.043 (trabecular)	1.962 (cortical) 0.082 (trabecular)
Median %Weight Loss from Forced Vibration test	3.830	1.606	0.812	0.348

The findings from this research contradict those from Kres and Lovell (1995), and are more similar to those reported in Rossi and collegues (2004). Although Kres and Lovell (1995) utilized the same consolidants at a 20% concentration, they report easy solution application of Butvar® B-98 with a matte finish to the bone, while Acryloid™ B-72 was difficult to apply and left a glossy finish on cortical bone samples. Furthermore, they report a much shorter dry time per layer for Rhoplex™ AC-33 and more difficult application due in part to it's increased viscosity; however, the differences in our results may be due to a slightly different chemical formula for Rhoplex™ B-60A, the suggested replacement for the discontinued Rhoplex™ AC-33. Additionally, according to Kres and Lovell (1995) Acrysol™ WS-24 dried much quicker than I experienced during data collection.

Rossi et al. (2004) on the other hand present results that are more similar to those reported here. The authors also recommend the use of Acryloid™ B-72 to stabilize burned bone over Butvar® B-98 because it produced harder and stronger bone samples, even though a glossy finish was observed after both material's application. Each consolidant's ability to maintain bone integrity was based on the degree of fracturing and splintering within the sample during histological slide preparation. Rossi et al. (2004) further state that Butvar® B-98 did not penetrate test specimens and instead formed a hard outer layer, likely contributing to the observed fracturing. Although not confirmed through microscopy, this characteristic may explain the variable mode of deformation assessed using nanoindentation.

CYANOACRYLATE AS A CONSOLIDANT

Although a cyanoacrylate was not specifically tested here, in an effort to mirror the research conducted by Kres and Lovell (1995) and Rossi et al. (2004), a discussion of its properties is necessary as forensic odontologists and anthropologists have routinely employed cyanoacrylate, known commercially as Super Glue, as a stabilization agent when thermally altered remains are encountered (Mincer et al. 1990; James Fancher, DDS personal communication, 2015). Cyanoacrylate offers a quick dry time and strong bond when administered correctly. However, Acryloid™ B-72 offers several benefits over the use of cyanoacrylate. Specifically, Acryloid™ B-72, a methylmethacrylate/ ethylacrylate resin, is archival grade, stable over-time, and highly recommended for use on bone (Johnson 1994), whereas the long-term effects of cyanoacrylate on bone, including color change such as yellowing or cloudiness, shrinkage, cracking, flaking, warping, and continued effectiveness as a bonding agent are not known.

While these long-term effects may not immediately be of concern in most forensic settings, where remains are returned to family members after identification and prepared for cremation or burial, they should still be considered when selecting a material for consolidation. For instance, long-term effects of cyanoacrylate on bone could become apparent when a forensic case cannot be identified at the time of investigation, making examination potentially years later necessary. Furthermore, burned bone and cremations are regularly encountered in archaeological settings (Irish et al. 2015; Minozzi 2015; Schmidt et al. 2015), and the known archival properties of Acryloid™ B-72 would be necessary when deciding on an appropriate consolidant material.

Additionally, Acryloid™ B-72 can be prepared to the users preferred concentration depending on the usage of the material. For instance, Acryloid™ B-72 is routinely used as a surface barrier to artifacts during labeling and curation, and it can also be used as an adhesive at a higher concentration (50% C; Sease 1994). Finally, because an Acryloid™ B-72 solution is prepared in-house prior to application, greater volumes of material can be mixed and applied to a larger area of calcined bone than cyanoacrylate, in a relatively short amount of time.

RESEARCH LIMITATIONS

Several limitations should be considered when interpreting the results of this research. The temperature reported is based on a single thermocouple placed in one corner of the structure used to simulate a house fire. Although temperature was not used in correlation with resulting bone color, the maximum temperatures recorded may not be representative. This is based on the fact that bones classified in lower stages according to definitions in Shipman et al. (1984) were located on the perimeter of the structure, as was the data collector. Future research on this topic would benefit from more thermocouples and data collectors in use to get a more representative temperature reading for the entire structure during the fire.

Furthermore, although four layers of material, totaling roughly 100 mL, were applied to each specimen, it is likely that there are areas with significantly more consolidant material due to variation in application that could not be accounted for. This may contribute to the wide dispersion in values for measured mechanical properties. Varying degree of mineralization within and between bone specimens likely also contributes to this variation, as an increase in mineral content is correlated with an

increase in ultimate strength (Currey 1990). Additionally, mechanical properties of bone have also been shown to vary by specific anatomical location (Cuy 2002), and although control for general location of indentation (e.g., anterior shaft) was attempted at all times, the fragmentary nature of specimens, the constraints required for sample preparation, and the requirement of color matching made this difficult. For this reason, and inability to control for the anisotophic nature of bone, no absolute values for mechanical properties could be reported, since direction could not be completely controlled for (Swadener et al. 2001). This issue was exacerbated when attempting to test samples using nanoindentation. Because there are no standard methods for testing bone in this manner, sample preparation was minimal. Other studies including nanoindentation analysis of bone prepare samples by embedding them in poly(methylmethacrylate) (Paietta et al. 2011). However, this process was avoided since it could potentially negatively impact the results by interacting with the consolidant being tested. Finally, the lack of sample preparation increased the likelihood of the nanoindenter producing false load-displacement curves because of uneven sample surfaces. This problem was aggravated due to the porosity of the samples resulting in indenting into voids.

CONSIDERATIONS WHEN USING CONSOLIDATION MATERIALS

Before using these materials ubiquitously to prevent further damage and mitigate fragmentation of burned bone, several considerations must be made concerning the analyses that may be employed. Eklund and Thomas (2010) tested the amount of breakage and degradation to DNA after treatment with common conservation solvents and materials. Although acetone, a solvent used in the present research, preserved DNA

fairly well (97.5%±8.4), the consolidant materials in this study were not tested for effects on DNA degradation directly. Furthermore, the use of consolidants has been shown to impact stable isotope analysis when they are incompletely removed prior to testing (López-Polín 2012). While genetic and isotopic analysis should still be considered when considering the usage of consolidation materials, Harbeck et al. (2011) have indicated that DNA testing from material heated above 600°C is not recommended due to difficulties associated with amplification due to collagen degradation and weight loss. Furthermore, Harbeck et al. (2011) state that isotopic analysis should only proceed with samples that exhibit no evidence of thermal alterations since their results were only reliable when samples were exposed to temperatures below 200°C. The use of consolidants may also negatively affect dates obtained through radiocarbon dating methods, because the polymer adds carbon to the sample (Johnson 1994; López-Polín 2012). Other research has shown that bones treated with consolidation materials do not exhibit an altered chemical composition through various microscopy spectroscopy analyses (Chadefaux et al. 2008). Therefore, until further research is conducted on consolidant's effects on these types of analyses, it is recommended that a subset of bone intended for these analyses be removed, labeled, and preserved appropriately prior to *in situ* consolidant application.

Additional consideration for when burned bone consolidation should be performed is required. For instance, in a forensic setting, calcined bone that could contribute to identification through non-destructive analysis (i.e. dentition, frontal sinus, biological indicators of sex, age, and ancestry) should preferentially be consolidated.

FUTURE DIRECTIONS

Future applications for this research include testing these materials on a wider number and degree of burned bone specimens and unburned bone exhibiting poor preservation. Preliminary results on the use of Acryloid™ B-72 on friable human remains recovered from a late archaic archaeological site (41VV166) in the Lower-Pecos region of West Texas indicate consolidant usage is appropriate to prevent continued fragmentation. In this case, the remains were excavated, with some elements containing their surrounding matrix, wrapped in foil, and packed in containers filled with toilet paper and paper towels for transport to the lab for cleaning and analysis. While removing sediment from the endocranial space, the facial skeleton separated from the neurocranium. Since the facial skeleton was in better condition, Acryloid™ B-72, prepared at a 10% C, was only applied to the fracture edges. The sediment still present and the paper material filling the space between bone and the plastic container maintained the structure of the neurocranium. Sediment was then removed incrementally, and consolidant applied to exposed bone surfaces, resulting in an intact neurocranium that could then be photographed and analyzed (Figure 22).

Investigation of variations to solution preparation, such as exploring the effects of differing consolidant concentrations, as well as testing of different solvents to decrease the required dry time for Acrysol™ WS-34 and Rhoplex™ B-60A should also be studied. Sease (1994) indicates that both Acrysol™ WS-24 and Rhoplex™ B-60A are readily soluble in acetone, the use of which could significantly decrease the dry time experienced during this research. However, Sease (1994) also indicates that these solutions may

become insoluble over time, thus restricting the potential duration for which a prepared solution can be used.

Figure 22. Left lateral (a) and posterior view (b) of individual from 41VV166

Lastly, scanning electron microscopy could be used to evaluate the degree to which consolidant material was able to permeate the sample. This may explain the brittle response found by nanoindentation of the Butvar® B-98 samples. If the material did not sufficiently absorb into the bone, instead forming a thin skin-like layer across the sample, it is probable that the load rate of 30μm/sec to a peak load force of 700μN caused displacement of the sample beyond consolidant permeation, resulting in a mechanical response more similar to the burned control.

V. **CONCLUSION**

Bone degradation can be caused by several taphonomic agents and can significantly reduce its ability to withstand stress once removed from its original context. However, fire-altered bone presents a unique challenge to anthropologists and bioarchaeologists due to its characteristically increased friability when completeness is necessary for analyses contributing to ancestral affiliations, personal identification, and a reconstruction of perimortem events.

The Bridgeville Fatal Fire Recovery Protocols (Dirkmaat et al. 2012), were developed to maximize recovery and mitigate the fragmentation of human remains at fire scenes. However, they do not address methods that could reduce the potential of fragmentation with repeated handling. Furthermore, the suggested use of aluminum foil as a protective covering may disrupt the standard operating procedures for some Medical Examiner's Offices, making intake procedures more complex. A potential solution to this problem is the standardized use of consolidant materials on fragile osteological material, including burned remains. Although anthropologists and odontologists have employed a variety of consolidant materials in the past, there is no consensus regarding which material is most appropriate.

My research sought to qualitatively assess variables associated with material application, including ease of solution preparation, dry time, solution storage needs in the field, ease of application, as well as any changes to the bone's appearance, and quantitatively compare four conservation-grade consolidant's ability to keep friable, burned bone intact by assessing ultimate strength and toughness through mechanical

testing. The materials chosen for comparison include Acryloid™ B-72, Acrysol™ WS-24 Rhoplex™ B-60A, and Butvar® B-98, all of which have commonly been used, are easily obtained, and are relatively inexpensive.

Based on both the qualitative and quantitative data collected, Acryloid™ B-72 is the most suitable consolidant for use on burned bone during in situ recovery. Should the dry time for Rhoplex™ B-60A be significantly reduced through the use of a different solvent, such as acetone, Rhoplex™ B-60A would also be appropriate. However, before the ubiquitous use of consolidant on friable bone, any additional analyses that could be affected need to be considered, and samples taken and labeled appropriately prior to removal from depositional context.

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