A Methodology for Extracting Bone Marrow from Cadavers

by Christiana Fakhri, Lauren Rudie, Stephanie Baker, Meredith Mann, Sarah Bivens, Laura Spoonire, Nichole M. Ruble

Abstract: The current estimation of postmortem interval (PMI) relies on insect succession, state of decomposition, and the external microbiome. Therefore, PMI is influenced by external environmental conditions, such as climate and scavenger activity. However, the marrow cavity of skeletal elements is protected from the external environment and may be used to determine a more accurate estimate of PMI. As no published methodology exists for the extraction of marrow from human cadavers over extended periods, this study aimed to develop effective and consistent practices for marrow extraction. Three human cadavers were placed at the Southeast Texas Applied Forensic Science facility (STAFS) at Sam Houston State University. Prior to sampling, experimental protocol was established. However, changes were made to accommodate accessibility of the marrow cavity, weather conditions, insect activity, state of decomposition, and marrow content of the left and right femur, humerus, and pelvis. The resulting extraction procedure involved the use of medical-grade bone marrow biopsy tools and culture swabs in combination with a power drill to collect samples from each cadaver. Each sampling location was cleansed with alcohol wipes prior to extraction and sealed with glue after sample collection. Samples were collected over a six-month period, starting May of 2016. Bone marrow was present in the femur, pelvis, and humerus of each cadaver throughout and upon completion of the project.

Introduction

Estimation of postmortem interval, or time since death, is a valuable and practical tool used in field of forensic sciences. However, an accurate estimation of postmortem interval (PMI) is difficult to obtain, as the understanding of human decomposition is limited. In the past, researchers focused on insect succession, gross external changes, and the external microbiome to estimate PMI (Hyde, Haarmann, Lynne, Bucheli, & Petrosino, 2013). However, these methods are susceptible to external factors, such as temperature, scavenger activity, and moisture level (Megyesi, Nawrocki, & Haskell, 2005). Recently, use of the microbiome associated with human decomposition has shown to be a promising component of PMI estimation, as bacteria are present throughout all stages of decomposition and play a catalytic role in the process (Metcalf et al, 2013). Cataloging the succession of external bacteria is promising but complicated by the diversity of bacterial strains, number of species present, and the influence of environmental conditions (Vass, 2001).

Marrow cavities, however, are protected from the external environment. While soft tissues have variable decay rates and are only present for a few weeks or days, skeletal elements persist for weeks to months (Megyesi et al., 2005). The longevity of skeletal elements, coupled with the protective nature of the bone, should allow the bacterial succession of marrow to provide a more accurate estimation of PMI over longer periods (Schwarcz, Agur, & Jantz, 2010).

While various studies have been conducted to catalog external bacterial succession in animal carcasses, no known studies have examined human marrow containing bones for extended periods (Howard, Duos, & Watson-Horzelski, 2010). As such, a method for extracting bone marrow is needed to undertake a study of bacterial succession inside marrow cavities. Therefore, this study seeks to establish a protocol for the effective and continued extraction of marrow from the humerus, femur, and pelvis of human cadavers.

In a clinical setting, bone marrow biopsy samples are extracted from patients manually via biopsy needles (Voigt & Mosier, 2013). The sample is obtained from the patient's iliac crest of the pelvis, usually near the posterior superior iliac spine (Bain, 2001). The pelvis is preferred, as it is a relatively safe access site, and the area is easily penetrated using the biopsy needle (Bain, 2001). Once an incision has been made to expose the superficial surface of the ilium, the biopsy needle is inserted slowly towards the anterior superior iliac spine (Bain, 2001). The needle is turned clockwise and counterclockwise in order to dislodge the sample from the marrow matrix. Once an adequate sample has been obtained, roughly a 20 mm specimen, the needle is carefully removed from the patient and the insertion site is closed (Voigt & Mosier, 2013).

Accordingly, the clinical methodology for marrow extraction was adapted for experimental use on human cadavers. In this study, sampling locations include the humerus, femur, and pelvis as these bones not only contain marrow but also persist on crime scenes longer than most other elements (Galloway, 1996). The humerus is the forearm bone located in the upper limb, the femur is the thigh bone located in the lower limb skeleton, and the iliac crest is the most superficial portion of the ilium. The humerus and femur are classified as long bones and have high marrow concentrations in the metaphysis and epiphysis, or the ends of the bone (Clarke, 2008). The ilium is an irregular bone, with relatively high marrow concentrations throughout the bone. The dense, compact nature of bone stems from cortical bone, its outermost layer (U.S. National Library of Medicine, n.d.). Cortical bone is composed of many small sub-units called osteons, which give bone its tensile strength. Deep to cortical bone is trabecular bone, which has a spongy appearance and houses bone marrow (U.S. National Library of Medicine, n.d.).

Methods

On May 11, 2016, three specimens, one female and two males, were placed at the STAFS facility (Figure 1). Each specimen was placed in the supine position for continued access to the pelvis, humeri, and femora with minimal disturbance during early decomposition. Each specimen was protected by a caged enclosure to prevent scavenging.

Figure 1. (From left) Specimens 027 and 033; (In front) Specimen 109 during late decomposition.

Each sampling day, enclosures were removed so that the specimens could be accessed. Ideally, three to four personnel were available for sampling to keep two sets of clean hands (driller, record keeper/documenter, tool handoff) and two sets of dirty hands (samplers). Clean hands were used to drill, document, hand off sampling materials, and seal extraction sites. Dirty hands were used to collect bone marrow.

Before entering the enclosure and coming into physical contact with the cadavers, proper protective measures were taken to prevent exposure to biohazards. Personal protective equipment (PPE) such as non-porous PVC suits, long-cuffed gloves, face shields, surgical masks, face shields, and rubber boots were donned prior to entering the enclosures. The PPE was disposed of as biohazardous waste, and boots were cleaned and stored in a designated location at the facility for continued use.

On the day of placement, an I-shaped incision was first made on each specimen at the most proximal point of the humerus, pelvis, and femur in order to retract the soft tissues and provide controlled access to skeletal elements. Starting from the most proximal point of each bone and moving distally, a 3 to 4-inch incision was made using a scalpel. The incision was extended distally to provide additional sampling sites, thereby minimizing the amount of exposed bone at a given time.

Prior to extracting marrow, each sampling location on the bone was cleansed with an alcohol wipe, starting centrally at the point of entry and moving radially away from the extraction site. Medical grade T-Lok bone marrow biopsy needles

(Figure 2) were used to pierce the cortical bone of the pelvis, femur, and humerus on each specimen (Argon Medical Devices, n.d.). However, the biopsy needle was unable to pierce the long bones due to thickness of the cortical bone. Therefore, we used a power drill and carbide bit to drill a hole into the humeral and femoral marrow cavities. The T-Lok biopsy needle, alone, was still used to access the pelvis.

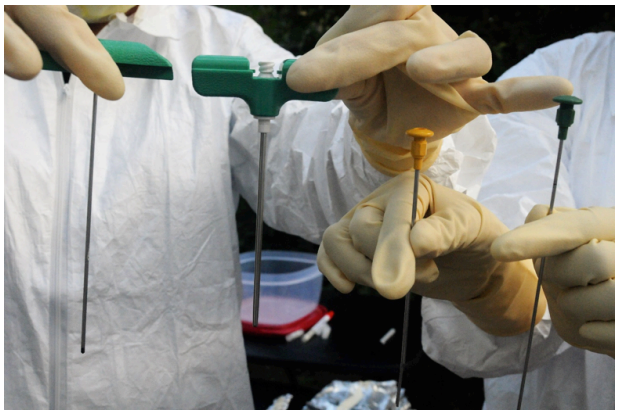

Figure 2. Medical grade T-Lok bone marrow biopsy kit.

After each use, the carbide bit was cleansed with 70% alcohol spray and an alcohol wipe. To access the pelvis, the T-Lok biopsy stylet and needle cannula, as a unit, were inserted into the pelvic crest. Sampling moved from the pelvic crest toward the medial border of the ilium to allow continued access to new extraction points throughout the duration of the study . Once inside the pelvis, the T-Lok unit was rotated counterclockwise and clockwise to separate the sample from the marrow matrix, allowing for sample isolation and extraction. The stylet was then removed by rotating the needle section 90° and pulling it straight up, leaving the needle cannula in the bone for access to the marrow cavity. At this point, the extraction cannula could then be used to extract bone marrow from each sampling location by inserting the extraction cannula into the bone cavity through the needle cannula and into pelvis (Figure 3a), or directly into the bone (Figure 3b) through the drilled hole for the long bones.

As decomposition progressed, collection of marrow with the extraction cannula became more difficult, particularly in the humerus and femur. To combat this, a sterile cotton swab (BBL CultureSwaEZ) was used to obtain a marrow sample if the extraction cannula could not collect any material. If the sample was collected with the T-Lok rather than the swab, material was transferred into a labeled cryotube by inserting the probe into the extraction cannula to

Figure 3a. Sampling on long bone with T-Lok.

Figure 3b. Sampling on long bone with EB Swab.

expel the marrow (Figure 4). If the swab was used to collect marrow, then it was placed back into its plastic transport container, labeled according to specimen identification number, sampling date, and sampling location.

Figure 4. Transfer of marrow sample into labeled cryotube.

After extracting the sample, the hole of entry was sealed before moving to the next bone (Figure 5a, Figure 5b). Initially, sampling locations were sealed with a combination of putty and Elmer's probond glue. Putty was used to fill any opening to the marrow cavity left by the extraction process, and glue was used to seal the putty and adhere it to the bone. Once sealed, a sampling location was not used again. However, insect activity and rain/moisture levels rendered the putty ineffective in sealing the extraction site. Therefore, the use of putty was discontinued, and glue alone was used throughout the remainder of the experiment. Complications only arose when the glue was applied during heavy and continuous rainfall, as it was unable to properly dry and set before being washed away. After sampling was completed, each specimen was checked to ensure that the extraction site was sealed. Any trash or biohazardous waste from the sampling process was cleaned, and the cages were placed back over each body.

Extraction tools were reused. Therefore, dirty tools (T-Lok kits, scalpels, and drill bits) were placed in a sealed container for transport back to the microbiology lab at Sam Houston State University (SHSU) for cleaning and autoclaving. The power drill was sprayed with ethanol and wiped down after use. Samples were placed in a separate container for

Figure 5a. Sealing of long bone.

Figure 5b. Sealing of pelvis.

transport. At SHSU, the samples were placed and stored in a -20°C freezer. The process for cleaning dirty tools was performed in four steps: manual washing, soaking in ethanol, repackaging, and

autoclaving. First, the tools were washed with water and soap. Care was taken during this step to remove dirt and tissue. A pipe cleaner was used to clean the cannulas. Next, the cleaned tools were placed in a tub of 70% ethanol. This tub was placed on a shaker set to 2-3 x 1000 rpm for at least 24 hours. Then, the tools were rinsed with Millipore water. The rinsed tools were packaged in biopsy kits using foil sheets, then labeled, dated, sealed, and initialed. A strip of autoclave tape was also used on each kit to ensure that the appropriate pressure and heat level was obtained during the autoclave process. Finally, the prepared kits were placed in a large metal tray and placed in the autoclave. The autoclave was set to Gravity 2 and allowed to cycle. Dirty transport containers were washed, sprayed with ethanol, and left to dry.

Discussion

Over a period of six months, the left and right femur, pelvis, and humerus of each cadaver was sampled per the described protocol. To varying degrees of success, different methods and procedures were utilized to obtain bone marrow and keep the marrow cavity protected. Initially, extraction protocol called for the use of medical grade biopsy needles to obtain marrow samples from the femur, pelvis, and humerus. However, the needles were unable to pierce through the cortical diaphysis, or shaft, of the long bones. To address this problem, a cordless drill was used to create the hole in the long bones. The drill was disinfected between each use. The integration of a power drill was an effective and easy amendment to the sampling protocol, ensuring access into the marrow cavity of long bones without complicating extraction procedure.

However, the most problematic issue was keeping the cavity protected after sampling. At first, putty and glue were used, in combination, to seal the extraction site. The putty was intended to act as a plug while the glue acted as a seal. However, the putty-glue combination proved ineffective under the stress of rainfall and insect activity. The material would either wash away or be chewed through by flies (maggots) and beetles, leaving the cavity exposed to the external environment. We suspect that the putty did not form a strong enough bond with the bone and that the surface of the bone was too soft for the glue to adhere to. Therefore, Elmer's Pro-bond glue was used to seal the holes without use of the putty. The glue, as the primary means of sealing extraction points, proved effective under most conditions. Scavengers were unable to eat through or displace it, and the glue dried quickly. Problems only occurred under extreme conditions of heavy rainfall, as the glue was unable to set before being washed away.

Relative to the methodology described are the four stages of decomposition: fresh, early, advanced, and skeletonization. Each stage is characterized by qualitative observations, such as appearance, and general characteristics of the remains. During the fresh stage, the body does not have any discoloration of the skin. In early decomposition, the skin's appearance becomes discolored, progressing from a pink/white appearance to a brown/black leathery appearance. The advanced decomposition stage is characterized by minimal bone exposure and mummification of the skin. The skeletonization stage includes extensive bone exposure with minimal tissue and body fluids remaining (Megyesi et al., 2005). Depending on environmental factors and bodily conditions upon death, the decomposition process can proceed at different rates and spend variable amounts of time in each stage. Specific to our specimens, boney elements became fragile and brittle at the onset of advanced decomposition stage, around the third month of sampling. When obtaining samples with the biopsy needles and drill, bones were prone to breaking. If breaks occurred, the pieces were glued back together to prevent any further exposure of the bone marrow cavity.

Despite complications, the established protocol for extraction and sampling of bone marrow in human cadavers proved effective and practical for extended use.

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