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A quantitative histological comparison of ground human bone preparation techniques

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ABSTRACT

To assess bone histology, ground bone sections can be prepared mechanically (automated technique) or manually by grinding the bone by hand (manual method). Recently the manual grinding method proposed by Maat et al. (2001) has received increased interest compared to other grinding techniques commonly used to investigate histochemical staining to diagnose pathological changes and age-at-death. Although automated techniques are thought to be qualitatively equivalent to Maat et al.'s (2001) method, a quantitative comparison has not been done. The aim therefore was to quantitatively compare Maat et al.'s (2001) manual method to an automated grinding technique by measuring the maximum and minimum diameters, and calculating the area, of Haversian systems and Haversian canals from the anterior midshaft of five cadaveric tibiae. Statistical tests were used to assess the differences between the variables. Quantitatively there was no significant difference between the two techniques, illustrating that the quality of the sections produced by the manual method was equally suitable for qualitative and quantitative examination. Future researchers interested in doing quantitative research on ground sections are therefore not limited by a lack of access to specialized automated equipment because manual ground sections are sufficient for histological assessment.

1. Introduction

Assessing the histology or microstructure of bone is a widely used technique in biological anthropology, especially in bioarchaeology, forensic anthropology and palaeopathology. It has been used in species determination (Harsanyi, 1993; Hillier and Bell, 2007), the assessment of diagenetic alteration (Turner-Walker and Jans, 2008; Hollund et al., 2012), the estimation of age-at-death (Robling and Stout, 2008; Keough et al., 2009), diagnosing disease (Schultz, 2001; Ortner, 2003) and assessing post-traumatic time intervals (de Boer et al., 2012a).

When preparing histological bone sections, thin sections are acquired by slicing the bone using a microtome or grinding it to the desired thickness. Depending on the purpose of the study, fresh or cadaver bone intended for microtome use is either decalcified (softening of hard tissue) and embedded in wax or resin, or left intact (not decalcified) and subsequently embedded for cutting purposes (de Boer et al., 2013). Considering that archaeological bone is generally more poorly preserved compared to fresh or cadaver bone, an automated grinding technique is preferred by bioarchaeologists as the "decalcification and microtome cutting of archaeological bone is considered obsolete" (de Boer et al., 2013: 83). The automated grinding technique involves the embedding of intact bone in resin, followed by grinding on silicon carbide paper until the desired section thickness is reached. Although this is a common grinding technique used by researchers using various modifications (Schultz, 2001), it is often described as time consuming and expensive (Maat et al., 2001). With this in mind, Maat et al. (2001) revised the 'rapid manual method' initially introduced by Frost (1958) and recommended it as an alternative grinding technique to the automated grinding of bone as it was inexpensive and very little time was needed to complete a section.

The rapid manual method described by Maat et al. (2001) involves manually preparing thin undecalcified archaeological bone sections by grinding the bone by hand using very basic materials including a hacksaw, silicon carbide paper, kitchen detergent and water. Additional instructions for fragile bone are also given using cyanoacrylate glue ("Super Glue") as an embedding medium. Recently many researchers have compared Maat et al.'s (2001) manual grinding method to other grinding techniques (Beauchesne and Saunders, 2006; Martiniakova et al., 2006; Haas and Stora, 2014) and utilised the technique to investigate histochemical staining to diagnose pathological changes (de Boer et al., 2012b, 2013) and age-at-death (Maat et al., 2006; Kim et al., 2007; Keough et al., 2009) with reasonable success. Beauchesne and Saunders (2006) tested Maat et al.'s (2001) method by producing high quality bone sections quickly and affordably from archaeological bones of good preservation for histological research purposes.

Qualitatively (histomorphoscopy) and quantitatively (histomorphometry) comparing the manual grinding method to an automated grinding technique is important in determining the

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quality of the bone section produced. This is done by assessing the bone's morphology and describing it, and measuring the histological structures of the bone to quantify it. For example, determining whether the cement line of the Haversian system is clearly visible, and whether this clarity is suitable for measurements to be taken and accurately repeated. In addition to this, Frost (1958) noted that the heat generated from sawing and grinding the bone affects the bone's microstructure which can be quantitatively assessed. Qualitative comparisons between an automated and Maat et al.'s (2001) manual grinding technique were investigated by Beauchesne and Saunders (2006) where each technique was found directly comparable to one another using both modern bone and archaeological bone of good preservation. For very fragile bone samples, the automated technique produced higher quality sections compared to the manual grinding technique (Beauchesne and Saunders, 2006; Haas and Stora, 2014). Although modern bone and well preserved archaeological bone were found qualitatively equivalent for the two techniques, a quantitative analysis comparing an automated grinding technique to Maat et al.'s (2001) manual grinding technique has not been attempted. In this study, due to the increase in popularity of Maat et al.'s (2001) method for research purposes, a quantitative comparison between it and an automated grinding technique was carried out.

2. Materials and methods

Five left tibiae were removed from dissection cadavers housed at the School of Anatomical Sciences, University of the Witwatersrand. These remains were donated to the school for research purposes and fall under the South African Human Tissues Act. Cadavers were used because Beauchesne and Saunders (2006) and Haas and Stora (2014) had very little success using Maat et al.'s (2001) manual grinding method to prepare very fragile bone samples. Beauchesne and Saunders (2006) also indicated that modern bone and archaeological bone of good preservation would be best suited for the manual method and defined good preservation as “bone that had not been greatly affected by taphonomic changes, thereby maintaining much of its original mineral and organic composition and thus its stiffness and strength” (Beauchesne and Saunders, 2006: 84). For the purpose of this study, cadaver bone was therefore well suited. The demographics of the individuals used were one black male, three white males and one white female of ages 92, 85, 66, 78 and 90 years respectively.

The tibiae were cleaned using water, a scrubbing sponge and dissecting instruments. Two adjacent sections of 1–2 mm were removed from the anterior midshaft of each tibia using a small handsaw. The anterior midshaft was selected as this region is often used in the estimation of age-at-death which utilises histomorphometry (Maat et al., 2006; Keough et al., 2009). One section from each tibia was prepared according to descriptions by Maat et al. (2001) and the other section was prepared using an automated grinding technique (Donath, 1995).

2.1. Manual ground sections

Using a sheet of P_{220} waterproof abrasive paper attached to a glass slide with Vaseline, one section from each tibia was manually ground by hand using a circular motion in distilled water until the sections became reasonably thin. ‘Frost's gripping device’ described by Maat et al. (2001), which involves folding a small piece of waterproof abrasive paper over a slide and gripping the two ends using your thumb and index finger, was used with distilled water to finally grind the section down until it became opaque. The sections were washed twice in distilled water to remove the dirt particles from the sections using a paint brush and allowed to dry at room temperature before being mounted onto slides and coverslipped using Entellan.

2.2. Automated ground sections

The automated technique selected for this study was the standard grinding technique used by the Bone Research Laboratory, situated at the University of the Witwatersrand. This grinding procedure was a good representative of most automated techniques and is known as the EXAKT cutting/grinding technique (Donath, 1995). The additional sections removed from the anterior midshafts of the tibiae were embedded in special embedding moulds using Technovit 7200 VLC resin (©EXAKT) and polymerised overnight in a water-cooled EXAKT Light-Polymerisation Unit. The polymerised blocks were removed from the embedding moulds and the non-tissue side of the blocks was mounted onto plexiglass slides using a mixture of Technovit 3040 powder and Technovit liquid resin in the ratio 1:0.5. A vacuum-adhesive apparatus was used for 20 min to complete the mounting procedure.

The tissue blocks were preliminarily ground using the EXAKT AW110 Micro-Wet Grinding System in order to level the block and bring the area of interest to the surface. The grinding table was initially covered with P_{1200} grit abrasive paper and finally with P_{4000} grit polishing paper to create a smooth surface. Once the grinding process was completed, the tissue blocks were dried in an EXAKT Block-Drying Unit at 40 °C under vacuum for 30 min.

Before the attachment of a second slide to the tissue surface of the blocks, the block surfaces and slides were cleaned with benzene to remove any grease. The attachment of the second slide was accomplished by using Technovit 7210 VLC adhesive and an EXAKT-Precision-Adhesive Press for 10 min for the adhesive layer to harden with a constant thickness. The EXAKT 310CP Precision-Parallel-Control-Sawing Unit was used to separate the two slides by measuring and sawing a distance of 300 μm from the surface of the second slide. This resulted in one slide having a 300 μm section and another with the excess tissue and resin. The 300 μm section remaining on the second slide was ground and polished on the EXAKT AW110 Micro-Wet Grinding System until opaque. The sections were allowed to dry at room temperature before being coverslipped with Technovit 7200 VLC and polymerised in the EXAKT-Precision-Adhesive Press for 15 min.

2.3. Microscopic analysis

A transect line was drawn down the middle of the ground sections from the most anterior aspect of the section to the most posterior aspect (Fig. 1). Using a Zeiss Axioskop 2 Plus at 100× magnification, three consecutive images were taken from the anterior margin of the section closest to the transect line on sides A and B; because one of the individuals had very little cortical bone present, only two consecutive images for A and B were taken. Using Adobe Photoshop Cs2 version 9 each image was overlaid with a sampling grid with squares containing two sets of numbers from 0 to 9 (Lander et al., 2014).

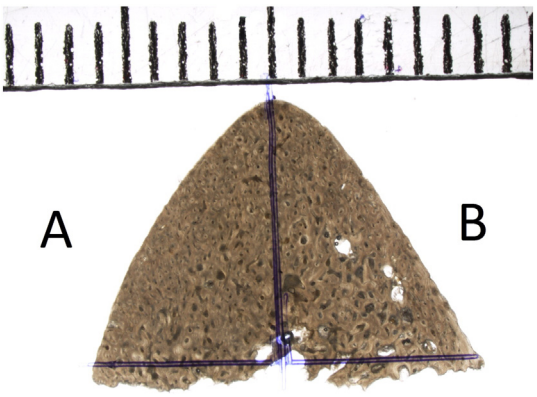

Fig. 1. Section illustrating transecting lines with millimetre ruler (5× magnification).