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The effects of freezing, boiling and degreasing on the microstructure of bone

S.L. Lander*, D. Brits, M. Hosie

School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg 2193, South Africa

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ABSTRACT

The histology of bone has been a useful tool in research. It is commonly used to estimate the age of an individual at death, to assess if the bone is of human or non-human origin and in trauma analysis. Factors that affect the histology of bone include age, sex, population affinity and burning to name but a few. Other factors expected to affect bone histology are freezing, boiling and degreasing but very little information is available for freezing and the effect thereof, and it is unknown if boiling and degreasing affects bone histology. The aim of this study was to assess the effects of freezing, freezing and boiling, and freezing, boiling and degreasing on the histological structure of compact bone. Five cadaver tibiae were frozen at −20 °C for 21 days followed by segments being boiled in water for three days and degreased in trichloroethylene at 82 °C for three days. Anterior midshaft sections were prepared as ground sections and for Scanning Electron Microscopy (SEM). Quantitatively, there were no significant differences between freezing, boiling and degreasing; however, qualitative differences were observed using SEM. After being frozen the bone displayed cracks and after boiling the bones displayed erosion pits on the surface. It is suggested that further research, using different durations and temperatures for boiling and freezing be undertaken on bone samples representing different ages and various skeletal elements.

* Corresponding author. Tel.: +27 724465881.
E-mail address: stacey.lander@vodamail.co.za (S.L. Lander).

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Introduction

The histological structure of compact bone consists of long cylindrical Haversian systems, also known as osteons, that have circumferentially arranged lamellae and a centrally placed Haversian canal which runs longitudinally through the compact bone (Kramer and Allan, 2005; Robling and Stout, 1999). The histomorphometry of bone has been used to estimate the age of an individual at death (Robling and Stout, 2000; Rösing et al., 2007; Singh and Gunberg, 1970), whether a bone fragment is human or non-human (Hillier and Bell, 2007), and to assess differences between the sexes and populations in bioarchaeology and in trauma analysis (Ubelaker, 1998).

Many factors affect the histological interpretations of bone such as the sectioning angle, specific bone examined and bone portion sampled, where Haversian systems, for example, can be described as oval or elliptic in shape if not sectioned perpendicularly. Additionally different skeletal elements are known to vary histologically with differences in Haversian system sizes observed between ribs, femora and fibulae (Evans and Bang, 1966; Pirock et al., 1966). Similarly, a single bone can exhibit random and systematic histological differences as it is seen in areas with and without muscle attachments, where an increased number of Haversian systems are present where muscles insert (Currey, 2003). Other factors include age (Curtis and Nawrocki, 2010; Hillier and Bell, 2007; Singh and Gunberg, 1970; Smith and Walker, 1964), sex (Currey, 2003; Hillier and Bell, 2007; Mulhern and Van Gerven, 1997), pathology (Schultz, 2001), diagenesis (Hollund et al., 2012; Jans et al., 2002) and burning (Cattaneo et al., 1999). For example, females have larger Haversian systems while males have more numerous Haversian systems (Hillier and Bell, 2007) and when assessing burnt bone it is found that shrinkage of the bone occurs at specific temperatures (Cattaneo et al., 1999).

The dry storage of skeletal collections found in some museums and universities is due to the cleaning and preparation of bone for analytical research in forensic science, zoology and anthropology, to name but a few disciplines (Simonsen et al., 2011). The preparation of bone requires specific procedures and particular techniques to ensure good quality dry bones that can provide researchers with a plethora of information. Frequently used methods to prepare dry bones include boiling, degreasing and bleaching which removes all the soft tissue and fat present. These techniques or combination of techniques might affect the appearance of bone as research has shown that dehydration that occurs between 100 °C and 600 °C, results in severe cracks and fractures (Shipman et al., 1984; Thompson, 2004). Moreover, little information on the effects of boiling and degreasing temperatures is available and as research on bone is done in institutions that employ these techniques, it is important to assess such effects, if any.

Small animal specimens such as cats and chickens are often frozen prior to dissection and undergo subsequent boiling and degreasing, instead of being embalmed. Similarly, cadavers and forensic remains are often frozen for short periods of time prior to analyses. Research on frozen bone has shown an area enlargement of cells and nuclei, osteocytes disappearing, collagen disorganization (Andrade et al., 2008) and cracking found around the centre of the Haversian canal due to liquid expansion (Tersigni, 2007). However, no information is available on the effects of freezing in conjunction with boiling and degreasing on bone histomorphometry. It is thus important that all these preparation techniques (freezing, boiling, degreasing) are assessed consecutively to establish if bone that had been frozen, boiled and degreased shows any microscopic alterations that could potentially affect the conclusions drawn from the bone microstructure. Therefore, the aim of this study was to assess the effects of freezing, freezing and boiling, and freezing, boiling and degreasing on the histological structure of compact bone.

Materials and methods

Five left tibiae were dissected from randomly selected cadavers housed at the School of Anatomical Sciences, University of the Witwatersrand at the onset of this research. The demographic information of these individuals i.e., age, sex and ancestry, is known and is documented in Table 1. The ages of the selected cadavers ranged between 66 and 92 years with an average of 82.2 and SD = 10.5 years. These ages are in line with the normal acquisition ages seen for white individuals at the University of the Witwatersrand, which are most often represented by elderly individuals (Dayal et al., 2009). After the