Micron 92 (2017) 32–38

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

A method for measuring the three-dimensional orientation of cortical canals with implications for comparative analysis of bone microstructure in vertebrates

Isaac Vorster Pratt, David Michael Lane Cooper*

University of Saskatchewan, Anatomy & Cell Biology, 2D01 Health Sciences, 107 Wiggins Rd., Saskatoon, SK, S7N 5E5, Canada

ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form 31 October 2016
Accepted 31 October 2016
Available online 2 November 2016

Keywords:
Micro-CT
Bone
Cortical porosity
Laminarity
Cortical canal orientation

ABSTRACT

The orientation of vascular canals in cortical bone can reveal information about the growth rate and loading environment of a bone. For example, in birds it has been proposed that a high proportion of circumferential canals (a laminar cortex) is related to fast growth or torsional loading related to active flight. In this paper we present a method to measure the three dimensional (3D) orientation of vascular canals. Image data are obtained from micro-CT and two angles are measured: phi, determining how longitudinal a canal is; and theta, determining whether a canal is radial or circumferential. This method can measure the orientation of each canal contained in the scanned images. Here we demonstrate the approach on two samples – a rat tibia and a hawk humerus. This method offers a direct (3D) method for quantifying features of canal orientation, such as the degree of laminarity, and can be applied easily and non-destructively to multiple species and bones. The growth and development of the cortical canal network and its impact on factors such as bone strength and bone quality remains relatively unexplored. Our method provides a new tool to examine the impact of the orientation of cortical bone canals on bone and explore the origins of cortical canals formed during modelling and remodeling. This method has applications in comparative bone biology, small animal models, and human bone studies.

1. Introduction

The importance of cortical porosity on bone strength has been increasingly studied over the last decade as three dimensional (3D) x-ray imaging techniques have become able to quantify porosity *in vivo* in animals and humans (Buie et al., 2007; Burghardt et al., 2010; Harrison and Cooper, 2015; Jorgenson et al., 2015; Kawalilak et al., 2016; Nishiyama et al., 2010; Pratt et al., 2015; Vilayphiou et al., 2016; Zebaze et al., 2013, 2010). One aspect of cortical porosity that remains relatively unexplored is the orientation of the cortical canals. The orientation of primary and secondary canals has been proposed to reflect mechanical loading of the bone in humans (Hert et al., 1994; Petrtyl et al., 1996) and animals (de Margerie, 2002; de Margerie and Rakotomanana, 2007; de Margerie et al., 2005; Marelli and Simons, 2014; Simons and O'Connor, 2012), or the growth rate of a bone (de Margerie et al., 2002, 2004; Lee and Simons, 2015). de Margerie (2002) proposed that a cortex containing predominantly circumferential canals in birds was a functional response to a torsional load from active flight. Primary canals are formed during the initial growth and development of the bone through modelling (Maggiano, 2012), while secondary canals are bored in cortical bone throughout life during remodeling (Frost, 1963). In most mammals and birds, the cortical bone is lamellar and contains many primary canals incorporated into the bone during development (Mitchell and van Heteren, 2015). It is predominantly these primary canals that we examine in this paper. In mature human tissue the predominant canal type is secondary. de Margerie (2002) measured the orientation of canals in histological slices by classifying canals into four categories: radial, longitudinal, oblique, or circumferential. A radial canal appears to extend from the centroid of the bone, like the spokes on a bicycle wheel. A longitudinal canal is aligned with the long axis of the bone. A circumferential canal is parallel to the contour of the diaphysis. An oblique canal is intermediate between a radial and a circumferential canal. They calculated a 'laminarity' index, the ratio of the area of the circumferential canals to the total area of all canals. Measuring the 3D orientation of cortical canals has

* Corresponding author.
E-mail addresses: isaac.pratt@usask.ca (I.V. Pratt), dml.cooper@usask.ca (D.M.L. Cooper).

http://dx.doi.org/10.1016/j.micron.2016.10.006

historically used histology, a primarily two dimensional method. Two dimensional methods are inherently weaker as a result of the measurement being taken only in one plane. The method used by de Margerie (2002) misses the fact that many canals are oblique (sometimes referred to as 'reticular'), and as a result overestimate the number of true radial, longitudinal, and circumferential canals. Attempts to create more accurate indices (De Boef and Larsson, 2007) remain limited by the histological method used, as the longitudinal angle can only be estimated from the cross-section, and not directly measured. This estimation is based on how circular the canal's cross-section appears in a histological section, with circular canal cross-sections interpreted to be longitudinal, and more oval canal cross-sections are interpreted to represent canals that are more transverse. Estimating measurements of the 3D orientation of a canal or osteon from a cross-sectional image can be misleading. Osteons tend to be more oval than circular in cross-section (Hennig et al., 2015), leading to difficulty in analysis. Canal shapes are also not always circular and therefore the orientation that is estimated may differ significantly from the actual orientation. Prior to the development of micro-computed tomography (micro-CT), the 3D methods available were difficult, tedious, and destructive (Dehoff, 1983; Odgaard et al., 1990). However, micro-CT offers easy access to the canal network, and computer technology allows automated methods which can measure many canals in short order. Micro-CT is an x-ray imaging method that uses successive images of a rotating object to reconstruct a 3D volume. Micro-CT typically relies on contrast created by differences in absorption of x-rays by different materials, although phase information is increasingly used (Pratt et al., 2015). Micro-CT was first used to study bone in late 1980s (Feldkamp et al., 1989), and has proliferated explosively since then due to the high quality of the data it produces. It is now used extensively to study both trabecular and cortical microstructure (Bouxsein et al., 2010; Cooper et al., 2003; Holdsworth and Thornton, 2002). Micro-CT allows for direct visualization of cortical porosity, and quantification of the canal network. The limitation of micro-CT is that commercial systems are often unable to differentiate primary and secondary canals. Dechow et al. (2008) used confocal microscopy and micro-CT to examine cortical structure, including some analysis of canal orientations. They imaged rectangular cut bone samples and compared the canal orientation with the axes of mechanical stiffness. They found that in the human femur average canal orientation tends to align with the axis of maximum stiffness in the plane of the cortical bone plate, suggesting a link between function and canal orientation as well. However, their work was limited to analysis of cut samples, and they did not identify the morphological axes of the bone. Previous work in Cooper's bone imaging group has measured the orientation of canals with respect to bone's long axis (Britz et al., 2012), showing the ability to differentiate between radial and longitudinal canals, and significant differences in orientation between normal and immobilized rats. The currently described method builds on that work, by adding the ability to measure orientation in the orthogonal plane, thus providing the full 3D orientations of each canal in the cortical network. Adding this angle allows differentiation not only between radial and longitudinal canals, but also between radial and circumferential canals. It also provides the ability to identify the different types of oblique canals. These 3D orientations allow for a more accurate calculation. This method provides the ability to investigate questions about how canal orientations are built-in during growth (primary), and how the remodeling process alters the (secondary) canal network in humans and in comparative animal studies. An improved understanding of the processes by which canals develop and how their direction is determined will add to our ability to interpret cortical microstructure and infer behaviour in both extant and extinct animals.

2. Methods

2.1. Micro-CT

We used two micro-CT systems for this study. The first is a SkyScan 1172 desktop microtomograph (Kontich, Belgium) with a <5μm x-ray source size and an 8.83 μm camera pixel size. The second is a Hamamatsu C9300-124 optical camera paired with a Hamamatsu A40 x-ray converter at the BioMedical Imaging and Therapy (BMIT) facility at the Canadian Light Source Synchrotron. This set-up provides a 4.3 μm pixel size, from a <5 μm source size. The BMIT synchrotron micro-CT set-up has an open gantry, and a field of view of 15 mm that is wider than that of the desktop system. Offset scanning methods can also be used to increase the field of view further to 30 mm. As a result, synchrotron micro-CT is ideal for imaging whole bones from larger animals/humans. The BMIT facility offers a number of different camera set-ups, with pixel sizes ranging from 0.9–100 μm. Any micro-CT system may be used with this method, although resolutions of < 10 μm are needed to accurately capture the morphology of the canal network (Cooper et al., 2007; Matsumoto et al., 2006). Frame averaging is typically used to improve the signal-noise ratio, and aluminum filtration of the beam and beam-hardening correction algorithms are used to minimize beam hardening. While the method is constructed with micro-CT in mind, other imaging methods that produce 3D data sets could potentially be used.

2.2. Reconstruction & skeletonization

We use NRecon 1.6.10.5, a commercial reconstruction software package (Bruker SkyScan, Kontich, Belgium) to reconstruct micro-CT data for analysis. The reconstructed micro-CT slices are then imported into Amira 6.0 (FEI Company, USA), where the canals are segmented and skeletonized. The canals are segmented using a global threshold to separate bone from soft tissue and air in the Amira Segmentation Editor. The value of the threshold was chosen to maximize the canal structure visible and minimize noise. The exact value of the threshold will vary depending on the imaging set-up used. They are then skeletonized using the Auto Skeleton module. The skeletonization process in Amira calculates a distance map of the segmented canals and then performs a thinning of the canals which preserves the topology of the canal network, including the length and orientation of the canals. The end result of the skeletonization process is a lineset which contains a set of co-ordinates for each canal. This lineset file is then simplified using a custom ImageJ (NIH; http://rsb.info.nih.gov/ij/) macro. This process identifies the branch points in the canal network and breaks each canal down into a series of shorter canal segments. Simplification of the canals into canal segments better captures the variability in orientation of the canals and provides a more accurate representation of the canal network. In particular, curved canals need to be broken up to capture the detail of their orientations. The length of the canal segments is a user choice, but it is recommended to keep the canal length at approximately 100–150 μm, the thickness of a typical histological ground section. Using shorter canal segments provides a more robust estimate of the orientation of curved canal sections, though with diminishing returns (Britz et al., 2012). This lineset will form the basis for the orientation measurements (see Fig. 1). Other architectural parameters such as canal length and canal connectivity density can be measured from the skeleton as well (Cooper et al., 2003). For a scan of a whole bone as in the examples provided here, the micro-CT slices are also used to create a lineset of co-ordinates of the centroid of the bone, producing a lineset with one centroid (x,y) co-ordinate for each integer z value. If the scan is only of a section of the bone, the centroid position can be reconstructed from the endosteal profile of the bone or defined