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Short communication

A novel specimen-specific methodology to optimise the alignment of long bones for experimental testing

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A R T I C L E I N F O

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A B S T R A C T

The choice of coordinate system and alignment of bone will affect the quantification of mechanical properties obtained during in-vitro biomechanical testing. Where these are used in predictive models, such as finite element analysis, the fidelic description of these properties is paramount. Currently in bending and torsional tests, bones are aligned on a pre-defined fixed span based on the reference system marked out. However, large inter-specimen differences have been reported. This suggests a need for the development of a specimen-specific alignment system for use in experimental work. Eleven ovine tibiae were used in this study and three-dimensional surface meshes were constructed from micro-Computed Tomography scan images. A novel, semi-automated algorithm was developed and applied to the surface meshes to align the whole bone based on its calculated principal directions. Thereafter, the code isolates the optimised location and length of each bone for experimental testing. This resulted in a lowering of the second moment of area about the chosen bending axis in the central region. More importantly, the optimisation method decreases the irregularity of the shape of the cross-sectional slices as the unbiased estimate of the population coefficient of variation of the second moment of area decreased from a range of (0.210–0.435) to (0.145–0.317) in the longitudinal direction, indicating a minimisation of the product moment, which causes eccentric loading. Thus, this methodology serves as an important pre-step to align the bone for mechanical tests or simulation work, is optimised for each specimen, ensures repeatability, and is general enough to be applied to any long bone.

1. Introduction

When characterising material properties of bone at the tissue level, samples are machined into regular specimens to enable the application of Euler–Bernoulli equations to study the effects of applying loads, producing shear stress in torsion and direct stress in bending. Bending and torsion tests are used to characterise the mechanical behaviour of whole bones. In bending, a load is applied laterally while the bone is supported between two external rollers. In torsion, the epiphyses are mounted to enable a torque to be applied along the diaphysis. To study the mechanical behaviour of bone at the structural level, its complex geometry needs to be taken into account.

Currently, anatomical reference frames are used to mount bones for experimental testing in four-point bending and torsion (Cristofolini et al., 1996; Cristofolini, 1997; Cristofoli et al., 2000). These experiments have large standard deviations in the results (Cristofolini and Viceconti, 2000). The Ruff and Hayes (1983) method is the most repeatable method known to assign a standard coordinate system for in-vitro testing (Conti et al., 2008) and has been used in structural testing of bones (Finlay et al., 1995; Ebacher et al., 2007; Varghese et al., 2011). The ability of this system to align bones to optimally resist bending and torsion has never been compared to analytical results calculated from solid mechanics. The aims of this study are to:

- develop a method that calculates the principal directions of bone using imaging data,
- compare the second moment of area and its coefficient of variation across the cross-section using the anatomical reference frame and the new method in the principal and anatomical directions, and
- obtain landmarks to define bone alignment for in-vitro testing in four-point bending and torsion.

2. Materials and methods

11 Ovine tibiae from 5 months old British Texel lambs weighing approximately 20 kg were harvested immediately after slaughter. The bones were cleaned of soft tissues, wrapped in cloth soaked in 1% Phosphate Buffer solution and frozen at −20 °C in sealed bags. Bones were thawed and scanned at a resolution of 115 μm in a Metris X-Tek 225 CT System (Nikon Metrology, Tring, UK). Manual segmentation was

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conducted in Mimics 15.0 (Materialse NV, Leuven, Belgium), to create cortical and endocortical masks. Geomagic Studio 12.0 (3D Systems, North Carolina, USA) was used for repair and smoothing to produce approximately 10,000 triangles for export in STL format.

Euler–Bernoulli objects have a set of principal moments (I_1, I_2 and I_3, in descending order), and their corresponding principal axes, where they would experience maximum and minimum stresses in the absence of eccentric loading (Ruff and Hayes, 1983):

$$I = \begin{bmatrix} I_1 & 0 & 0 \\ 0 & I_2 & 0 \\ 0 & 0 & I_3 \end{bmatrix} \tag{1}$$

The volume second moment, I, is a measure of the distribution of the material points with respect to the axis. When defined about cartesian axes, they are:

$$I_{xx} = \int_A y^2 + z^2 dV$$
$$I_{yy} = \int_A x^2 + z^2 dV$$
$$I_{zz} = \int_A x^2 + y^2 dV \tag{2}$$

When the load to an object is not applied about the principal axis, or if the object is asymmetrical, a product moment of area is experienced:

$$I_{xy} = \int_A xy\, dV$$
$$I_{yz} = \int_A yz\, dV$$
$$I_{zx} = \int_A zx\, dV \tag{3}$$

The product and area moments can be assembled to solve for the principal moments I:

$$\begin{vmatrix} I_{xx} - I & -I_{xy} & -I_{xz} \\ -I_{yx} & I_{yy} - I & -I_{yz} \\ -I_{zx} & -I_{zy} & I_{zz} - I \end{vmatrix} = 0 \tag{4}$$

An area moment is maximised when its product moment is minimised. As bone geometry is irregular, the object will be optimised to experience *near* pure shear in torsion, and *near* pure direct stress in bending. Here the principal directions will be referred to as D_1, D_2 and D_3, representing the initial coordinate system used in aligning the bone, and corresponding to I_1, I_2 and I_3.

The algorithm (Fig. 1) to align a bone along its principal axes was developed in RhinoScript and carried out in Rhinoceros (Robert McNeel & Associates, Seattle, USA). Firstly, the two masks were converted to a single solid body, using standard CAD procedures. Then, the volume centroid of the body was calculated to act as the origin for the principal axes. The principal moments and their corresponding axes were computed and sorted in descending order. The bones were then rotated so that their first principal directions coincide with the global axes, using the dot and cross product. This was repeated until the error between the current and target axis equals zero (Fig. 1, loop A) and repeated for the second principal axis D_2 (Fig. 1, loop B).

In bending, only the span of the bone between the external two rollers and in torsional loading only the free region that is not embedded contribute to the strength of the bone in testing (Ebacher et al., 2007). The location of this free region (and span) for experimental testing was found by minimising the variation in the second moment of area along the segment. Firstly, the first cut, which defines the proximal diaphysis, is allowed to be located between 20% and 50% of the bone length. The width at this cross-section was used to locate the distal diaphysis at five times the distance of the initial width. (Fig. 2A). The widest width throughout the produced segment is then calculated to ensure that the segment fulfils the minimum span-to-width ratio of 4:1, shown by Hardy and Pipelzadeh (1991) to be the minimum required to minimise shear. The segment is then passed through the algorithm again for alignment (Fig. 1).

In both the second and third sections of the algorithm, cutting planes are used to section the bones and bone segments at 5% interval along the long axis (Fig. 2B). The intersection between neutral axis and each slice is calculated (black dots). These are used as the reference points for the calculation of the second moment of area.

Code was written to locate the mesh vertices that were furthest away in the first principal axes, to yield the location of six reference landmarks for aligning the bone during experimental testing, with three on each end of the bone (Fig. 3). Physically, the landmarks are located with the aid of an optical tracker, to avoid misalignment.

Data were not normally distributed and so an unbiased estimate of the population coefficient of variation (γ_v) of the second moment of area for each alignment

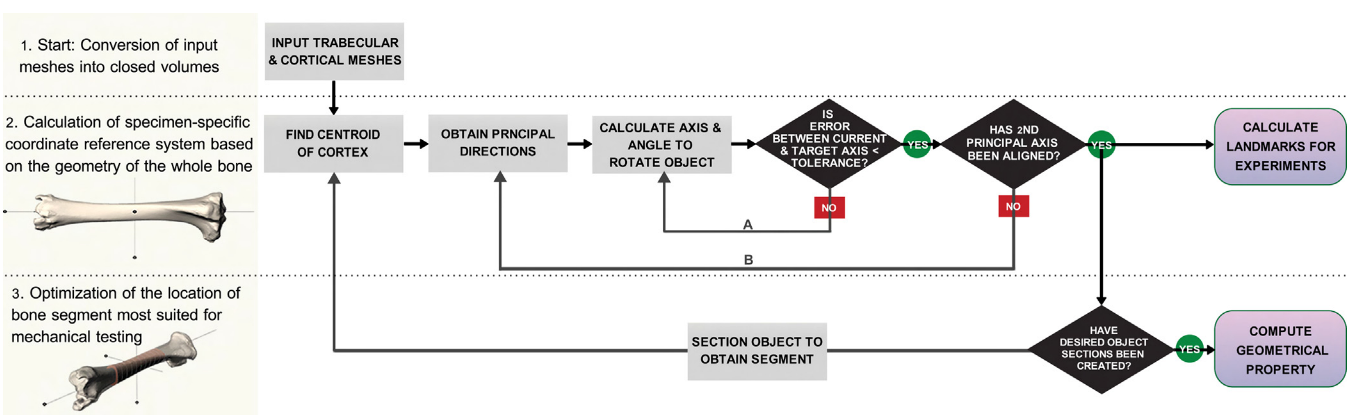

Fig. 1. Optimisation of bone segment best suited for bending and torsional tests can be obtained by running this algorithm. The code first processes input STL meshes so that they are suitable for later calculations, before optimising the alignment based on the whole bone and bone segment.

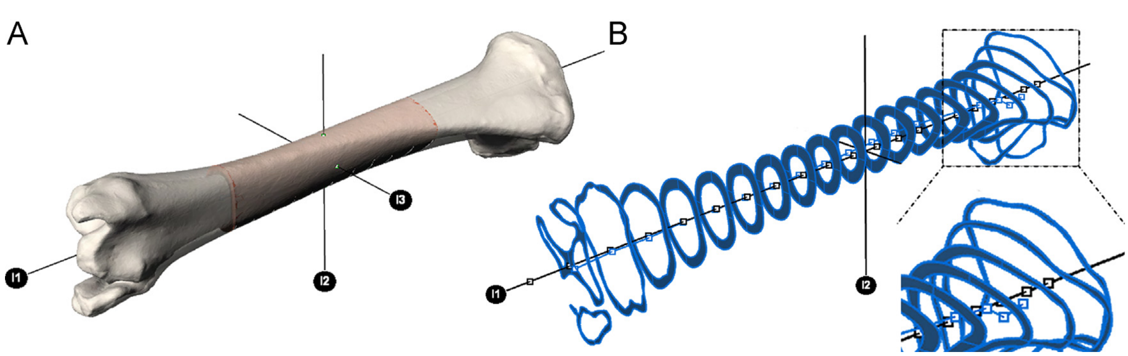

Fig. 2. (A) A segment of a bone calculated based on the minimum length-to-width ratio and aligned to its principal axes, as shown in pink. (B) Slices of bone sectioned at 5% interval along the long axis, after the bone (or bone segment) has been aligned to its principal directions. The blue (or light grey) dots show the position of the centroid of each slice while the black dots show the intersection between the first principal axis and each slice (termed neutral axis point). The difference between the two lines is seen most clearly in the proximal region. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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