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Inter-trabecular angle: A parameter of trabecular bone architecture in the human proximal femur that reveals underlying topological motifs

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ABSTRACT

Trabecular bone is an intricate 3D network of struts and plates. Although the structure-function relations in trabecular bone have been studied since the time of Julius Wolff, controversy still exists regarding the architectural parameters responsible for its stability and resilience. We present a parameter that measures the angle between two connected trabeculae – the Inter-Trabecular Angle (ITA). We studied the ITA values derived from μCT scans of different regions of the proximal femora of 5 individuals of different age and sex. We show that the ITA angle distribution of nodes with 3 connecting trabeculae has a mean close to 120°, nodes with 4 connecting trabeculae has a mean close to 109° and nodes of higher connectivity have mean ITA values around 100°. This tendency to spread the ITAs around geometrically symmetrical motifs is highly conserved. The implication is that the ITAs are optimized such that the smallest amount of material spans the maximal 3D volume, and possibly by so doing trabecular bone might be better adapted to multidirectional loading. We also draw a parallel between trabecular bone and tensegrity structures – where lightweight, resilient and stable tetrahedron-based shapes contribute to strain redistribution amongst all the elements and to collective impact dampening.

Statement of Significance

The Inter-Trabecular Angle (ITA) is a new topological parameter of trabecular bone. The ITA characterizes the way trabeculae connect with each other at nodes, regardless of their thickness and shape. The mean ITA value of nodes with 3 trabeculae is close to 120°, of nodes with 4 trabeculae is just below 109°, and the mean ITA of nodes with 5 and more trabeculae is around 100°. Thus the connections of trabeculae trend towards adopting symmetrical shapes. This implies that trabeculae can maximally span 3D space using the minimal amount of material. We draw a parallel between this motif and the concept of tensegrity – an engineering premise to which many living creatures conform at multiple levels of organization.

1. Introduction

Trabecular bone, also known as spongy or cancellous bone, is a porous, reticulate osseous tissue. The ends of long bones consist almost entirely of trabecular bone, enclosed in a thin shell of compact bone [1,2]. The fine trabecular structure reduces the weight of the skeleton [3–6], but trabecular bone is nevertheless capable of bearing significant mechanical loads [1,7,8]. Many studies have aimed at understanding the structure-function relations in trabecular bone that can account for its mechanical properties. Perhaps the best known are the studies by Julius Wolff, who suggested that individual trabeculae of the proximal human femur line up and strengthen the structure along the principal tension and compressional stress lines (trajectories), thus revealing the pattern of loading of the femur [2]. This hypothesis implies that bone structure adapts in response to the stresses exerted on it. Wolff's law has

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since stimulated many structure-function studies of trabecular bone, which either confirm the causality between the function and the form, for example [9,10] or question that connection [11,12]. For an insightful discussion, see Hammer [13].

The preferred orientation of the thickest trabeculae is sometimes macroscopically visible on sections, and especially when 2D projections are examined. However, the interconnecting struts and plates in trabecular bone form a 3D fabric (i.e. a meshwork), and the analysis of a 3D structure in 2D projections may yield incomplete information about its architecture. The 3D studies of trabecular bone architecture based on volumetric imaging methods focus on such averaged parameters as trabecular volume, number, thickness, spacing, morphology and connectivity [14–20]. The anisotropy of trabecular bone is often calculated as the fabric tensor, which is a quantitative characteristic of the trabecular structure as a whole and is related to the global axes of the specimen [7,21–23].

At the micrometer scale, trabecular bone material is composed of overlapping lamellar packets. The 3D structure of trabecular bone lamellae is similar to the 3D structure of lamellae in compact bone [24]: 2–3 μm-thick layers of ordered collagen of varying orientations are separated by thinner layers of disordered collagen [25,26]. In a study of 3D collagen organization in lamellar packets of individual trabeculae of human trabecular bone we found that alternating layers of ordered collagen fibrils are aligned either with the long axis of the trabecula or are aligned obliquely to its long axis [24]. The frequently observed angle between differently aligned layers of collagen fibrils is around 70°. We hypothesized that this angular offset between the alternating layers of ordered collagen might be similar to the angular offset between contiguous trabeculae (connected struts) at a much higher hierarchical level of organization [24]. This led us to measure the so-called "inter-trabecular angle" (ITA) in trabecular bone of the human proximal femur, and indeed it was found to center around 110°. Since 110° and 70° are complementary (summing to 180°), an intriguing structural correlation was found between these two different hierarchical levels – the micrometer level and the millimeter level. This observation was the inspiration for the current study.

Here we use micro-CT scans to examine one aspect of the 3D architecture of trabecular bone, namely the values of the inter-trabecular angles (ITAs) as a topological descriptor of trabecular bone architecture. Topology is the manner in which the constituents of the whole are related, unaffected by size and shape of individual parts. Hence, the ITA represents trabecular bone architecture at the "blueprint" level, without considering the thickness and shape of individual trabeculae and without linking their orientation to the anatomical (global) axes of a sample. Here we report the results of a study involving a cohort of five individuals of different age and sex.

2. Materials and methods

We analyzed proximal femora from five adult humans (Table 1). The study was performed on cadaver tissue obtained from an accredited non-transplant tissue bank, and the biological profile (i.e., age and sex) of the donors was known. Four donors did not have any skeletal pathology. One donor (F54) was diagnosed with osteoporosis and had a fracture of the femoral neck followed by surgical reposition and placement of orthopaedic screws. We avoided using trabecular bone from the close proximity of the screws (ca. 4.5 mm in all directions). The femoral heads of only two specimens were available. For the analyses, we selected functionally and anatomically distinct areas in each reconstructed CT-volume: the metaphysis (available in 4 specimens) the neck (available in 2 specimens) and the head of the femur (available in 2 specimens) (Table 1). The bones were scanned using a micro-CT (Micro XCT-400, Zeiss X-ray microscopy, California USA) at 40 kV and 200 mA with the pixel size between 43 and 47 μm, depending on the size of the whole sample and the required acquisition time. Note that the 10% variation of the pixel size did not affect the results because the average thickness of a trabecula includes at least 4 pixels within its smallest dimension and therefore can be effectively resolved in an image despite the 10% variation in pixel size.

Table 1
Summary of the 8 analyzed parts from 5 different human proximal femora plus 1 presented in Supplemental materials.

Sex	Age	Specimen name	Scanned parts
Male	63	F63	Femoral head; metaphysis; neck
Male	20	M20	Femoral head
Female	20	F20	Metaphysis; neck
Female	79	F79	Metaphysis
Female	54	F54	Metaphysis; neck in Supplemental materials

In each reconstructed 16 bit trabecular bone 3D image the cortical shell was digitally removed around the trabecular interior by manual labeling of the trabecular bone, excluding compact bone, in every 20th slice, followed by "interpolate selection" and "smooth all slices" functions of the graphic editor (Avizo, FEI, Oregon, USA). Each 3D image was thresholded at grey levels in the range of 28,000–32,000 depending on the global and local brightness levels of the image, so that trabeculae would not be disrupted (as may occur at a too high threshold) and mottled false-positive noise would not be introduced between trabeculae (too low a threshold) (Avizo, FEI, Oregon, USA). Note that the threshold level could not be exactly the same for different samples, as the selection of the threshold grey value relied on the individual expertise of an operator and was iterated until an agreement between at least two operators was achieved. Then the "Magic Wand" (Avizo, FEI, Oregon, USA) thresholding tool was applied to the whole tomogram ("all slices" checked) to create a selection. The thresholded and selected trabecular interior was saved as a binary 3D image in Fiji/ImageJ [27]. The binary volumes were additionally despeckled in Fiji/ImageJ (a median filter that effectively deletes single pixels that are not connected to other pixels of the same value) to minimize the noise when needed. The sample was then skeletonized (Fiji, Skeletonize3D plugin [28], http://fiji.sc/Skeletonize3D), which means applying a thinning algorithm to the trabeculae and representing each trabecula as an edge with exactly one voxel thickness, roughly corresponding to the longitudinal axis of each trabecula (Fig. 1A). Since all the struts and plates are symmetrically reduced to lines, the skeletonization protocol is sufficiently insensitive to subtle variations in binarization threshold [29]: a slightly thicker (lower threshold) or thinner (higher threshold) trabecula is converted into the same topological element – a line. The axes of the trabeculae are not absolutely straight, and for the ITA calculations their simplified linear (1D) analogue, Euclidean distance, was used. The ratio between the "real" trabecular axis (edge length) and the Euclidean distance was roughly 12:11. Depending on the size of the femur specimen, each volume contained between 30,000 and 400,000 trabeculae, i.e., struts limited in length by their branching points. The resulting "skeleton" – a continuous network of branches (or edges) – was interrogated using the AnalyzeSkeleton plugin, Fiji [30],http://fiji.sc/AnalyzeSkeleton. The output of AnalyzeSkeleton is a list of vectors (we refer to them as 'edges') represented by (x,y,z) coordinates of start points and end points. Each such edge is topologically equivalent to a trabecular strut in the original 3D image. Therefore, the 3D matrix of edges is topologically equivalent to the 3D fabric (meshwork) of the trabecular bone sample analyzed. The edge matrix was loaded into