1

INJURY BIOMECHANICS RESEARCH
Proceedings of the Thirty-First International Human Subjects Workshop

Microstructural Analysis of Osteon Orientation in Human Rib Cortical Bone

J. M. Cormier, J. D. Stitzel, S. M. Duma, and F. Matsuoka

ABSTRACT

The orientation of osteons in cortical bone has been shown to correlate with the loading condition. The purpose of this study is to determine the orientation of osteons in the rib cage to better understand the behavior of the rib cage under mechanical load. A total of 37 rib specimens were removed from 21 rib locations from a male and female cadaver. Eight histological slides were created from each 5 mm rib section. A custom image analysis algorithm was created in Matlab to track the center of each osteon through the rib section. Results show variations in the osteon direction between samples taken from the anterior, lateral and posterior surfaces of the rib cage. The average offset angle between the osteon direction and the rib axis was determined to be 4.1, 3.2 and 1.9 degrees for the anterior, lateral and posterior surfaces respectively. The average offset angle for the anterior specimens was significantly higher than that of the posterior rib specimens (P=0.01). The lateral specimens also showed a significantly higher average offset angle than the posterior rib specimens (P=0.01). The results indicate a trend in osteon offset angle between the three locations studied. The offset angle is highest in the anterior region, then decreases in the lateral and is lowest in the posterior regions of the rib cage.

INTRODUCTION

Bone microstructure orientation plays an important role in determining the response of the bone as a whole. Bone microstructure has been shown to adapt to the mechanical forces applied. The orientation of osteons may also give insight into the anisotropic behavior of bone.

The majority of previous work has focused on the microstructure of the long bones in human and animal models. Early literature disagrees on whether osteons have a preferred direction along the long axis of the bone (Hert et al., 1994). Work showing an oblique osteon orientation began with Cohen and Harris (1958). An analysis of the canine femur showed a helical direction of the osteons about the bone circumference. This finding was reinforced later by Martin and Burr (1989). The oblique orientation of the osteons with respect to the long axis was also supported by Black *et al.* (1980) using human tibial specimens. The average angle of inclination with respect to the long axis of the bone was found to be 5.45 degrees. Work by Tappen (1977) did not show that osteons assumed an oblique pattern with respect to the bone axis. The results of Tappen (1977) also deviate from the accepted belief that bone adapts to forces it is exposed to *in vivo* (Cowin, 1986; Hayes et al., 1978; Wolff, 1892). The adaptation of cortical bone has been shown to take place through the growth of secondary osteons in cortical bone (Burr, 1993; Carter, 1984; Martin, 1992; Rubin et al., 1988).

The influence of osteon direction on the mechanical properties of bone was shown by Petrt?l (1988), Lanyon and Bourn (1979) as well as Hert *et al.* (1994). Petrt?l found the elastic modulus of the human femur to be highest in the helical directions described by previous researchers as the direction of osteons in the femur. More recently, the orientation of osteons in the humerus, radius, ulna, tibia, and femur were examined by Hert *et al.* (1994). The authors observed a 5 to 15 degree offset of the osteons from the long axis of the bone. The cortical bone exhibited sharp boundaries between osteons of different orientation. Hert also showed that the offset angle of the osteons matched the direction of the principal strains in the bone under a combination of bending and torsional loading. Lanyon and Bourn (1979), as a result of their study of sheep tibia found an oblique orientation of osteons that resemble those found later by Hert *et al* (1994). This orientation coincided with the direction of principal strains under the bending and torsional loading condition as well. The lack of knowledge of the complete stress state in the rib cage supports learning the orientation of osteons in the human rib cage.

The purpose of this study was to investigate the orientation of osteons in the human rib cage. It is hoped this will expand the current understanding of the behavior of the rib cage under mechanical load, and the adaptation provided by the osteons. The ribs represent a unique piece of the human skeleton, as they are continuously loaded during respiration. This loading and the articulation of the ribs with the sternum and vertebral bodies play an influential role in the microstructural adaptation of the ribs.

METHODOLOGY

Two cadavers were used for this study, a 71 year old male and a 61 year old female. Rib sections were removed from each of the two cadavers using an autopsy saw. A total of 19 and 18 sections were removed from ribs on the left side of the thorax for a female and male subject respectively (Figure 1). For the female, eight histological slides were created from each rib section removed from the thorax. For the male subject, nine histological slides were created from each of the 18 specimens. This resulted in a total of 152 histological slides for the female and 162 slides for the male subjects.

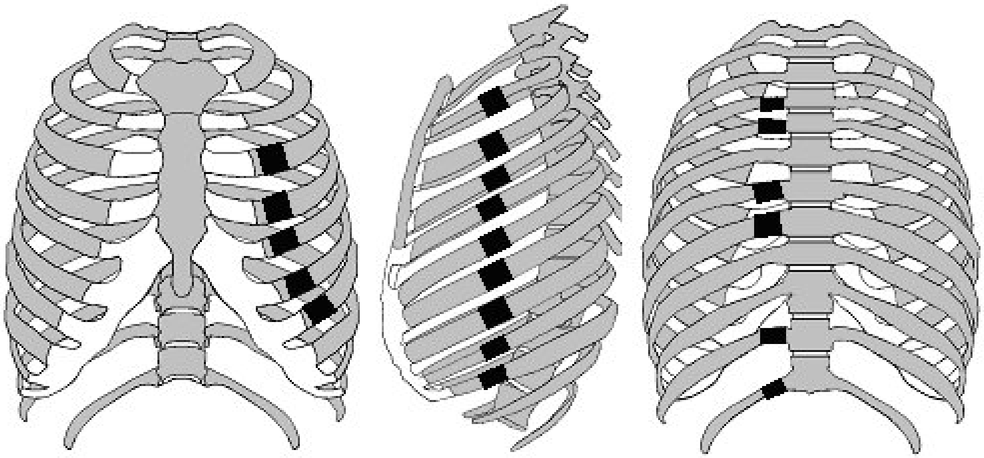

Figure 1: Locations from which sections were removed for histological analysis.

These sections were then cut again to reduce the specimens to a length of 3 mm. These secondary cuts were performed using a low speed diamond saw (Model 650, South Bay Technology, California, USA) equipped with a specially designed bone chuck and saline bath, producing a specimen with parallel edges while minimizing mechanical damage induced by the cutting process. Once the specimen was reduced to its final length, two cuts were made in the cortical bone of the specimen that served as reference markers for image analysis. These cuts were made in the interior and superior surface of the bone, using the low speed saw (Figure 2). Rib sections were then submerged in a 10% buffered Formalin solution to prevent decomposition of the bone until histological sectioning was performed.

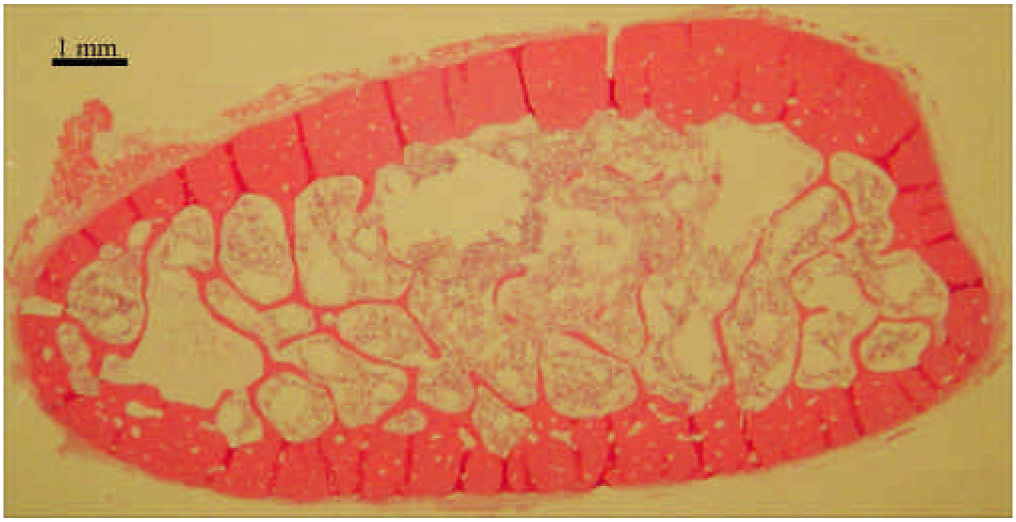

Figure 2: Histological slide of rib section with interior surface at the top and superior surface at the left.

Histological sectioning began by dividing the 3 mm rib specimens into 8 levels with a distance of 300 microns between each level for the female subject. The 3 mm specimens from the male subject were divided into 9 levels with the same 300 micron distance between each level. Two slices were taken at each of these levels at a slice thickness of 4 microns. Creating two slices at each level is not necessary, however, this increases the probability of having a useable histology slice at each level.

Slides were photographed for image processing using a bifocal microscope (Model LEG 05, Wild Heerbrugg, Switzerland) equipped with a digital camera (Model 3500, Nikon, Melville, NY). A magnification of 8-10X was used to ensure that Haversian canals in the cortical bone were visible. The camera was set to a resolution of 2048 by 1536 and the images were saved in Tagged Image File (.tif) format. Digitized images of an objective micrometer were used for scaling images.

Processing the images began by thresholding them to black and white images. This reduced the color depth of the images to 1 bit meaning that the images were basically an array of ones and zeros, one indicating white and zero indicating black. The center of each osteon in the cortical bone is marked by the Haversian canal (Hert et al., 1994). After creating histological slides of the rib cross section, these canals are visible as holes, or white areas in a black and white image. The trabeculae of the cancellous bone created a similar appearance, so to avoid erroneous results, the cancellous portion of each image was digitally removed. This created an image of a large white void in the center of the cortical shell that was then analyzed using computer algorithms.

Multiple algorithms were created in Matlab (Release 12, The Mathworks, Natick, MA) to load and process the black and white image of the histological sections. The first algorithm identified each Haversian canal and then determined its geometric center. This produced an array of x,y coordinates of the center position of the Haversian canals in the image. These coordinates were normalized using the reference cuts on the rib surface as datum markers for each section, Assuming the location of these cuts were consistent throughout the section. A homogenous transformation matrix was used to rotate and translate the coordinates of each Haversian canal based on the position of its corresponding datum points. Once complete, the Haversian coordinates were separated into two separate groups: those belonging to the interior surface and those belonging to the exterior surface.

To separate the canals into the two groups, black and white images of the center coordinates were created. The images generated were black with white dots indicating the center of each Haversian canal. The coordinates of each image were then rotated once more so that the interior and exterior surfaces of the rib were parallel to the horizontal, or x, axis. Thresholds for the distance along the y-axis were then established. Using the threshold specified for each rib section, the canals were divided into the interior and exterior surfaces based on their individual y-coordinate.

To determine which coordinates belonged to a single Haversian canal, an algorithm was used to compare the center coordinates from one image to those of the next, representing 300 microns of travel along the rib section. If the coordinates of one canal were within the established limits of a canal in the next image, these coordinates were paired together. The limits were set high enough so that the total travel of the canal between sections could be as high as 20 degrees. Based on previous research this upper limit would ensure that the algorithm would not incorrectly eliminate any canal coordinates. If more than one set of coordinates were paired together the algorithm chose the closest set of coordinates as the correct match.

Once paired coordinates were created from each section throughout the rib specimen, the Haversian canal angles were determined. Since the images had been rotated so that the interior and exterior surfaces were horizontal, changes in the x-coordinate of the Haversian canal directly indicated its motion with respect to the rib axis in the vertical plane. Canal translation to the left in the histological image indicated superior translation in the rib section. The angle of each canal between successive sections was determined by first dividing the displacement in the x-direction by the distance between histological sections (300μm), then taking the inverse tangent of this value (Figure 3).

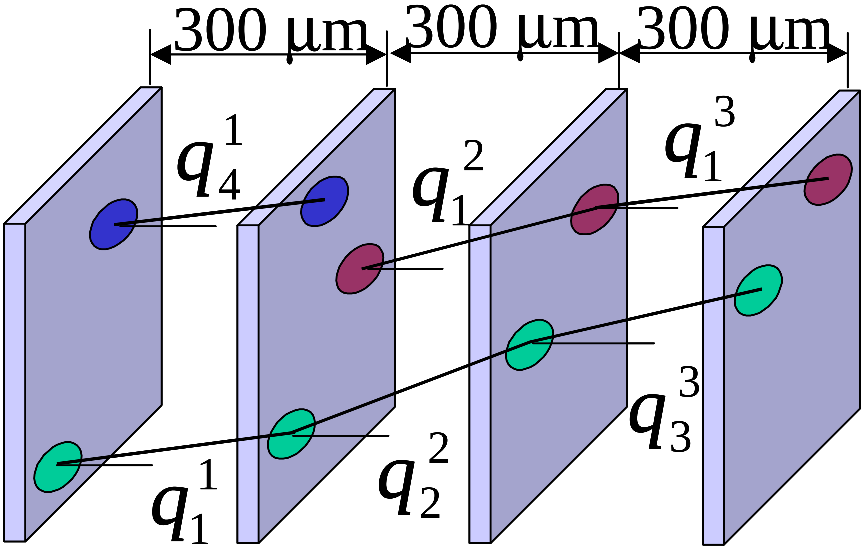

Figure 3: Diagram showing the translation of three separate Haversian canals through 4 histological sections and the determination of the average offset angle.

Equation (1)

$$q^{j}_{\sec tion} = \frac{\sum q_i^{j}}{n_i}$$

Equation (2)

$$q_{average} = \frac{\sum q^{j}_{\sec tion}}{n_j}$$

The Haversian canal offset angle ($\theta^j_{section}$) is calculated by taking the average offset angle for all canals between only two histological sections (Equation 1). The variable j represents the histological sections that are being considered for the calculation of $\theta^j_{section}$. The offset angle of each Haversian canal is represented by θ_ι and n_i is the total number of Haversian angles calculated. The average offset angle of all canals in the entire rib section ($\theta_{average}$) is then calculated (Equation 2). For this calculation, n_j represents the total number of histological sections created from the rib at the corresponding location.

RESULTS

The average offset angle of osteons in the human rib was determined for various locations from two cadaver subjects. The average absolute offset angle for both subjects at all locations was 3.1 degrees, with respect to the rib axis. The average absolute offset angle was 3.15 degrees for the female and 3.03 degrees for the male subjects. The highest offset angles were found in the anterior region of the thorax, with angles of -14.83 and 8.24 degrees for the exterior and interior surfaces respectively. The magnitude of the osteon offset angles in the anterior specimens was found to be significantly higher than those from the posterior locations ($p=0.02$). The magnitude of the osteon offset angles in the lateral specimens were also found to be significantly higher than those from the posterior locations ($p=0.01$).

DISCUSSION

The results from histological analysis of human rib specimens showed variability in osteon direction with respect to the rib axis, but overall trends were evident. This is similar to results obtained by Cohen *et al.* (1958) who also found a variation in osteon orientation, while still demonstrating an overall pattern of preferred direction. In this study, the short rib lengths analyzed creates difficulties in defining trends within each specimen, however, the average orientation within these specimens does reveal trends within the thorax as a whole.

The average offset angle in both surfaces was highest in the anterior region, then decreased in the lateral region and was lowest in the posterior region. This may reflect the anatomy of the sternal and vertebral attachments with the ends of the rib. The costal cartilage attachments at the sternal end of each rib creates a rigid joint. In contrast, the vertebral end of each rib is attached via ligaments, 7 for the second through tenth ribs and 3 ligaments for the remaining three ribs. These joints allow more movement of the rib to facilitate respiration and therefore may alter the loading conditions seen at the anterior and posterior portions of each rib. These different loading conditions may alter the way in which the bone adapts due to differences in the forces that the rib is exposed to at the vertebral and sternal joints. This may explain the statistically significant differences among the anterior, lateral and posterior osteon offset angles.

The quantification of osteon orientation in the human rib cage provided by the current study, contributes to the present understanding of rib anisotropic behavior and bone adaptation. This study represents a first step in determining the orientation of osteons in the rib cage and describes a methodology that can be applied to any histological analysis of bone. This knowledge can be used to expand current models used to predict thoracic trauma.

REFERENCES

BLACK, J., RICHARDSON, S. P., MATTSON, R. U., and POLLACK, S. R. (1980). Haversian Osteons: Longitudinal Variation of Internal Structure. J Biomed. Mat. Res., 14: 41-53.

CARTER, D.R. (1984). Mechanical Loading Histories and Cortical Bone Remodeling. *Calcif. Tissue Int.*, 36: S19-S24.

COHEN, J. and HARRIS, W. H. (1958). The Three-Dimensional Anatomy of the haversian System. J. Bone Joint Surg., 40A: 419-434.

COWIN, S. C. (1986). Wolff's Law of Trabecular Architecture at Remodeling Equilibrium. *J. Biomech. Eng.*, 108: 83-88.

HAYES, W. C., SWENSON, L. W., and SCHURMAN, D. J. (1978). Axisymmetric Finite Element Analysis of the Lateral Tibial Plateau. *J. Biomech.*, 11: 21-22.

HERT, J., FIALA, P., and PETRTYL, M. (1994). Osteon Orientation of the Diaphysis of the Long Bones in Man. Bone, 15(3): 269-277.

LANYON, L. E. and BOURN, S. (1979). The Influence of Mechanical Function on the Development and Remodeling of the Tibia. *J. Bone J. Surg.*, 61A: 263-272.

MARTIN, R. B. and BURR, D. B. (1989). Structure, Function and Adaptation of Compact Bone. New York: Raven Press.

MARTIN, R. B. (1992). A Theory of Fatigue Damage Accumulation and Repair in Cortical Bone. *J. Orthopaed. Res.*, 10: 818-825.

PETRT? L, M. (1993). Reactivities of the Compact Femoral Bone to the External Load. *Proceedings of the 24th Congress of Biomech.*, Paris: 1028-1029.

PETRT? L, M. (1988). Spiral Flow of Elastic Properties in the Compact Femoral Bone. *Proceedings of the 25th Congress of Eur. Soc. Artif. Organs*, Prague: 141-145.

RUBIN, C. T. and HOUSMAN, M. R. (1988). The Cellular Basis of Wolff's Law. Transduction of Physical Stimuli to Skeletal Adaptation. *Rheum. Dis. Clin. North. Am.*, 14: 502-517.

WOLFF, J. (1892). *Das Gesetz der Transformation der Knochen.* Berlin: Hirschwald.

BURR D.B. (1993). Remodeling and the Repair of Fatigue Damage. *Calcif. Tissue Int.*, 53: S75-S81.

TAPPEN N.C. (1977). Three-dimensional studies on resorption spaces and developing osteons, *Am J Anat.*, 149(3):301-17.

DISCUSSION

PAPER: **Microstructural Analysis of Osteon Orientation in Human Rib Cortical Bone**

PRESENTER: ***Joseph Cormier, Biodynamic Research Corporation for the Virginia Tech Wake Forest Center of Injury Biomechanics***

QUESTION: *Guy Nusholtz, DaimlerChrysler*

You're dealing with the microstructure of the rib and that would potentially have a relationship to the strength of the ribs and how it responds under load, which is a lot of what we're interested in, in terms of biomechanics. When we typically map from one size individual to another, we just assume--functionally we assume that it's a solid structure and we map differences in terms of the size and the material properties. But, you're dealing with a microstructure, and you're dealing with it somewhat at the cell level or at a much smaller or a miso level, and it may not map that way. Do you think this could have implications for the scaling rules that we use in terms of designing different dummies, of mapping from one size to another?

ANSWER: I don't think so and I don't think that we're dealing on the cellular level. We're dealing with more microscopic levels of the osteons. And, I don't think that would have an implication on when you're, when you're scaling because everybody's pretty much exposed to the same type of load conditions throughout their lives, unless you're really some kind of sports activity or something that's gonna change on a daily basis where your ribs are being exposed to--or where your bones are being exposed to. I think overall, the adaptation process that takes place in everybody is fairly consistent, or fairly consistent, and I don't think that would change or affect any type of scaling that you would apply.

Q: Okay. So you don't think that the way we do it now, which would be mapping any of that type of structure into the material properties will work fine.

A: I think so, yeah.

Q: Thank you.

A: Thank you.

QUESTION**:** *Barry Myers, Duke*

That was a very nice anatomical study. I'm interested: In '95, we took a look at the skull base and worried a lot about anisotropy. And at the end of the study, we realized that worrying about anisotropy was actually not all that important compared to things like inter-subject variation. Do you have a sense of how important anisotropy is in rib behavior, in terms of chest deflection or prediction of rib fracture?

A: As far as, you know, what we can tell by the orientation of the osteons, I don't think that the offset angles that we did find are high enough to give you a high level of anisotropy through the length of the rib. I don't--I don't see that being a factor in predicting rib fracture. I think it's fairly low, but I think if you want to develop a more accurate model, you may want to incorporate this. If you have the capability, you know, it would be nice to know the orientation of the microstructure to incorporate that in your model.

Q: Well, it would seem like it if it doesn't matter all that much, then why would I want to do all that detailed work?

A: Well, I just think to say that you do have a model that does incorporate the microstructure of the rib. And maybe if we do more, more analysis or maybe we do analysis taking into account the whole length of the rib, we may see some differences in between the regions that we looked at. Or, there may be something that has to do with the fact that how it changes--or, how the orientation changes along the length of the rib--that you might want to incorporate.

Q: Oh yeah. It's very worthwhile. But now that you have some data, you might do a sensitivity analysis to see--

A: Right.

Q: If you want to proceed with it.

A: Yeah, I agree.

Q: Thanks.

A: Thank you.

QUESTION**:** *Erik Takhounts, NHTSA*

My question may help you a little bit. I was wondering if you looked at the age of the cadavers that you studied and whether their orientation was changing with age? How age would affect the orientation of the osteons?

A: Well, I think up to a certain age--and some of the work that I've read, up to about 17, 18 years old, you know, the secondary osteons are done adapting the bone. And so, it's gonna be fairly--The orientation's gonna be fairly the same after, after these ages. One thing that is a problem with elderly people is that the diameter of the haversian canal gets smaller because you're depositing minerals inside of the haversian canal so maybe make it more difficult to find the center of the canal. But as far as the orientation changing throughout age: The effects of maybe osteoporosis and maybe the curvature of the spine can change the loads that the rib is exposed to throughout life and that may change that. But as far as overall, I think it's fairly same.

Q: Okay. Thank you.

A: Thank you.

QUESTION: *Frank Pintar, Medical College of Wisconsin*

Quick comment. We noticed in our cadaver tests that sometimes the, a healed fracture might influence the type of fracture on the outcome. So, maybe a suggestion is to look at those, if you can get cadavers that have healed fractures and see if those are appreciably different.

A: Yeah. That would be interesting because you're gonna get some kind of stress concentration in the area of the fracture which probably would change the loading that the rib is being exposed to in those areas. That's a good point. Thanks.