A24 The Use of High-Resolution Micro-Computed Tomography (micro-CT) for Quantifying Vascular Pore Networks Across Whole Cross Sections of Human Cortical Bone

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After attending this presentation, attendees will appreciate how regional patterns of cortical bone loss may be visualized and quantified in 3D space using high-resolution micro-CT.

This presentation will impact the forensic science community by demonstrating that high-resolution micro-CT facilitates extraction of vascular pore networks from within complete, thick cross sections of human bone tissue. Tracking how vascular pore structures accumulate and expand across the human lifespan has future forensic applications, including: (1) assessing whether porosity-related bone fragility could have played a role in an accidental or non-accidental fracture; and, (2) distinguishing fragments of bone types based on pore structure, which appears to be sensitive to age, strain mode, fragility, and human/non-human origin.

Bone remodeling takes place throughout life but declines with age. Osteoclast cells tunnel into old or damaged bone and osteoblast cells fill this tunnel with new bone, leaving a central pore to conduct a blood vessel. Osteoblasts' capacity for bone formation slows with age. They cannot keep pace in filling the resorbed spaces, which accumulate as large pores. Lowered mechanical strains trigger further resorption to expand and connect existing pores. Vascular pores concentrate stress, providing a target for microscopic damage to initiate and propagate into a spontaneous fracture.

Research into pore structure has been limited in either axial or lateral dimensions. Two-dimensional histological sections of bone can describe how pore cross sections, but not their three-dimensional structures, vary between regions of bone tissue. High-resolution three-dimensional imaging often limits the specimen dimensions laterally (e.g., synchrotron X-ray imaging) or axially (e.g., confocal laser scanning microscopy). This research implements three-dimensional vascular pore visualization of a whole cross section of a human rib with high-resolution micro-CT.

The sample chosen for this pilot study was a fresh cadaveric fourth right-side rib from a 72-year-old female. In order to fit within a single field of view in the scanner at an approximate resolution of 5µm, a midshaft segment of the rib was cut to 1cm and further quartered along its medial, lateral, superior, and inferior long axes. These rib segments were scanned in air using a SkyScan 1172-D High Resolution Desktop Micro-CT with source voltage of 59kV, camera pixel size of 8.87µm, and voxel size of 4.88µm. In Avizo Fire 8.1, the four rib segments were geometrically transformed to their original positions, extracted and united using the Segmentation Editor, then exported as an image sequence of cross sections. In ImageJ, the Slice Geometry plugin of BoneJ was used to annotate each cross section with the major axis, which divides the cutaneous (skin-side, low-strain) and pleural (lung-side, high-strain) regions of the rib.[1,2] Cross sections were imported back into Avizo Fire 8.1, where the major axis guided the creation of masks for these regions to extract them independently. Vascular pore structures were extracted from each region in the Segmentation Editor, then exported as cross sections.

The Analyze Particles tool in ImageJ was used to calculate pore number and area for each cross section, mirroring analysis of two-dimensional cross sections. The pleural region (high strain) had fewer (mean=52+/-5.4, range=31 to 71) but larger pores (mean=0.0074+/-0.0029mm^2, range=0.0030 to 0.020mm^2). The cutaneous region (low strain) had more numerous (mean=85+/-6.7, range=66 to 103) but smaller pores (mean=0.0046+/-0.0022mm^2, range=0.002 to 0.011mm^2). Both regions had similar total pore areas (pleural mean=0.387+/-0.161mm^2, range=0.159 to 1.036mm^2; cutaneous mean=0.392+/-0.196mm^2, range=0.171 to 0.915mm^2). The Analyze Particles plugin of BoneJ was used to extract pore volumes.[1] Again, the pleural region had fewer (2,923) but larger volume (mean=0.00247+/-0.0272mm^3, range=2.79x10^{-6} to 0.956mm^3) pores compared to the cutaneous region's more numerous (5,841) but smaller volume pores (mean=0.00125+/-0.0222mm^3, range=2.79x10^{-6} to 1.343mm^3).

High-resolution micro-CT is an accessible and effective technology for three-dimensional imaging of vascular pore structures across whole, thick cross sectional regions. These preliminary results suggest using this technology for further investigation into trade-offs between pore volume and number that are sensitive to strain mode during life.

Reference(s):

1. Doube M., Kłosowski M.M., Arganda-Carreras I., Cordeliéres F., Dougherty R.P., Jackson J., Schmid B., Hutchinson J.R., Shefelbine S.J. 2010. BoneJ: Free and extensible bone image analysis in ImageJ. *Bone.* 47:1076-9.
2. Agnew A.M. and Stout S.D. 2012. Brief Communication: Reevaluating osteoporosis in human ribs: The role of intracortical porosity. *Am. J. Phys. Anthropol.* 148: 462-466.

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