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Altered lacunar and vascular porosity in osteogenesis imperfecta mouse bone as revealed by synchrotron tomography contributes to bone fragility

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ABSTRACT

Osteogenesis imperfecta (brittle bone disease) is caused by mutations in the collagen genes and results in skeletal fragility. Changes in bone porosity at the tissue level indicate changes in bone metabolism and alter bone mechanical integrity. We investigated the cortical bone tissue porosity of a mouse model of the disease, *oim*, in comparison to a wild type (WT-C57BL/6), and examined the influence of canal architecture on bone mechanical performance.

High-resolution 3D representations of the posterior tibial and the lateral humeral mid-diaphysis of the bones were acquired for both mouse groups using synchrotron radiation-based computed tomography at a nominal resolution of 700 nm. Volumetric morphometric indices were determined for cortical bone, canal network and osteocyte lacunae. The influence of canal porosity architecture on bone mechanics was investigated using microarchitectural finite element (μFE) models of the cortical bone. Bright-field microscopy of stained sections was used to determine if canals were vascular.

Although total cortical porosity was comparable between *oim* and WT bone, *oim* bone had more numerous and more branched canals ($p < 0.001$), and more osteocyte lacunae per unit volume compared to WT ($p < 0.001$). Lacunae in *oim* were more spherical in shape compared to the ellipsoidal WT lacunae ($p < 0.001$). Histology revealed blood vessels in all WT and *oim* canals. μFE models of cortical bone revealed that small and branched canals, typical of *oim* bone, increase the risk of bone failure. These results portray a state of compromised bone quality in *oim* bone at the tissue level, which contributes to its deficient mechanical properties.

Introduction

Osteogenesis imperfecta (OI) or "brittle bone disease" is a genetic defect occurring in 1 in 20,000 human births [1,2]. Phenotypically, it is characterized by bone fragility and skeletal deformities due to mutations in collagen type I genes. The mutations cause collagen to fold improperly, disrupting fibril alignment and structure and providing an abnormal template for mineralization [3,4].

The *oim* mouse model (B6C3Fe a/a-Col1a2$^{oim/oim}$), a natural mutation in C57BL/6–C3HeB/Fe hybrid, mimics the biochemical and phenotypic features of the most debilitating non-lethal human forms of OI [5]. *Oim* mice have severe osteopenia early in life due to a spontaneous mutation in the α2 chain of collagen type I, which replaces the normal heterotrimer type I collagen α1(I)2α2(I)1 with the homotrimer type I collagen isotype α1(I)3. Homozygotes have impaired growth and skeletal fragility characterized by fractures, cortical thinning and bowing of the long bones, vertebral fractures and spine deformities, initiating in the fetus [5]. Studies have found that *oim* mice have high bone turnover, with a high resorptive activity [6] and lower bone formation rate [7], variable or heterogeneous mineralization [8,9] and matrix abnormalities [8,10,11]. These tissue characteristics are also typical of human OI [3,4,12–14].

Several studies have demonstrated reduced mechanical properties in *oim* bone compared to wild type (WT) bone: *oim* bones tested in three-point bending exhibited classic brittle behavior with fracture occurring just after the yield point of the tissue [8,15–17].

The reduced mechanical properties of *oim* bone and its propensity to fracture depend on the material characteristics throughout the hierarchy of bone. At the tissue level bone quality is characterized by factors such as mineral density, tissue matrix fabric (lamellar vs. woven), and

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porosity. Previous work has shown that *oim* bone has high tissue mineral density [18] and more woven bone [5,19] compared to the wild type. Intracortical porosity may also play a role in reduced bone fracture toughness in OI. A few studies have examined the two-dimensional (2D) porosity of human OI bone by light microscopy and scanning electron microscopy, and found higher porosity compared to healthy bones [20–23]. Only one study described the porosity in *oim* bone as increased compared to WT bone, but the techniques used to determine this were not reported [5]. No previous study has investigated three-dimensional (3D) porosity in OI bone from humans or mice, allowing for quantification of intracortical features such as canal architecture and osteocyte lacunar shape. Revealing the 3D porosity and architecture of OI cortical bone is essential for a better understanding of its structure in relation to its inferior mechanical properties, and for developing successful management or treatment strategies to reduce the fracture risk in brittle bone.

The aim of this study was therefore to quantify the 3D cortical tissue porosity and matrix structure in *oim* bone compared to a WT control using SR CT. Further, we assessed if canals are vascular using bright-field microscopy of stained sections and determined the influence of intracortical canal architecture on bone failure risk using micro-architectural finite element (μFE) modeling.

Material and methods

Morphometrical analysis

Three tibiae and four humeri from three WT mice (C57BL/6, Charles River Laboratories, Inc., UK) and three tibiae and three humeri from three *oim* mice (B6C3Fe a/a-Col1a2$^{oim/oim}$ from maintained local colony), all 8 weeks-old and male, were dissected after sacrifice, embedded at both ends in low viscosity poly-methylmethacrylate bone cement (PMMA; Cemex®, Tecres, VR) so that the shaft was aligned vertically. During preparation and before imaging, the samples were wrapped in gauze moistened with phosphate buffered saline (PBS) and stored at −20 °C. All procedures followed local ethical review and Home Office approval.

Bones were oriented with their longitudinal axis parallel to the stage rotation axis and immersed in PBS during scanning at the beamline for tomographic microscopy and coherent radiology (TOMCAT) of the Swiss Light Source [24]. High-resolution 3D representations of the posterior tibial and the lateral humeral mid-diaphysis of the bones were acquired for both mouse strains using SR CT at a nominal resolution of 700 nm. For each 3D data set, a total of 1001 projections were acquired over a range of 180° and at a photon energy of 17.5 keV. Tomograms were reconstructed using filtered back-projection yielding image volumes at the mid-diaphysis of 2048 × 2048 × 2048 voxels (1.43 × 1.43 × 1.43 mm). From this volume only the region occupied by the bone was retained for further analysis.

Intracortical porosity comprising lacunae and canals was segmented as follows (Fig. 1; foreground is black): histogram-based global thresholding was applied to the original image (Fig. 1.a result in b) to segment bone matrix from PBS and soft tissue, using the "Default" iterative intermeans algorithm in ImageJ [25], which selects a threshold that is half-way between the average pixel value of foreground and the average pixel value of background, and which ignores oversaturated and undersaturated pixels [26].

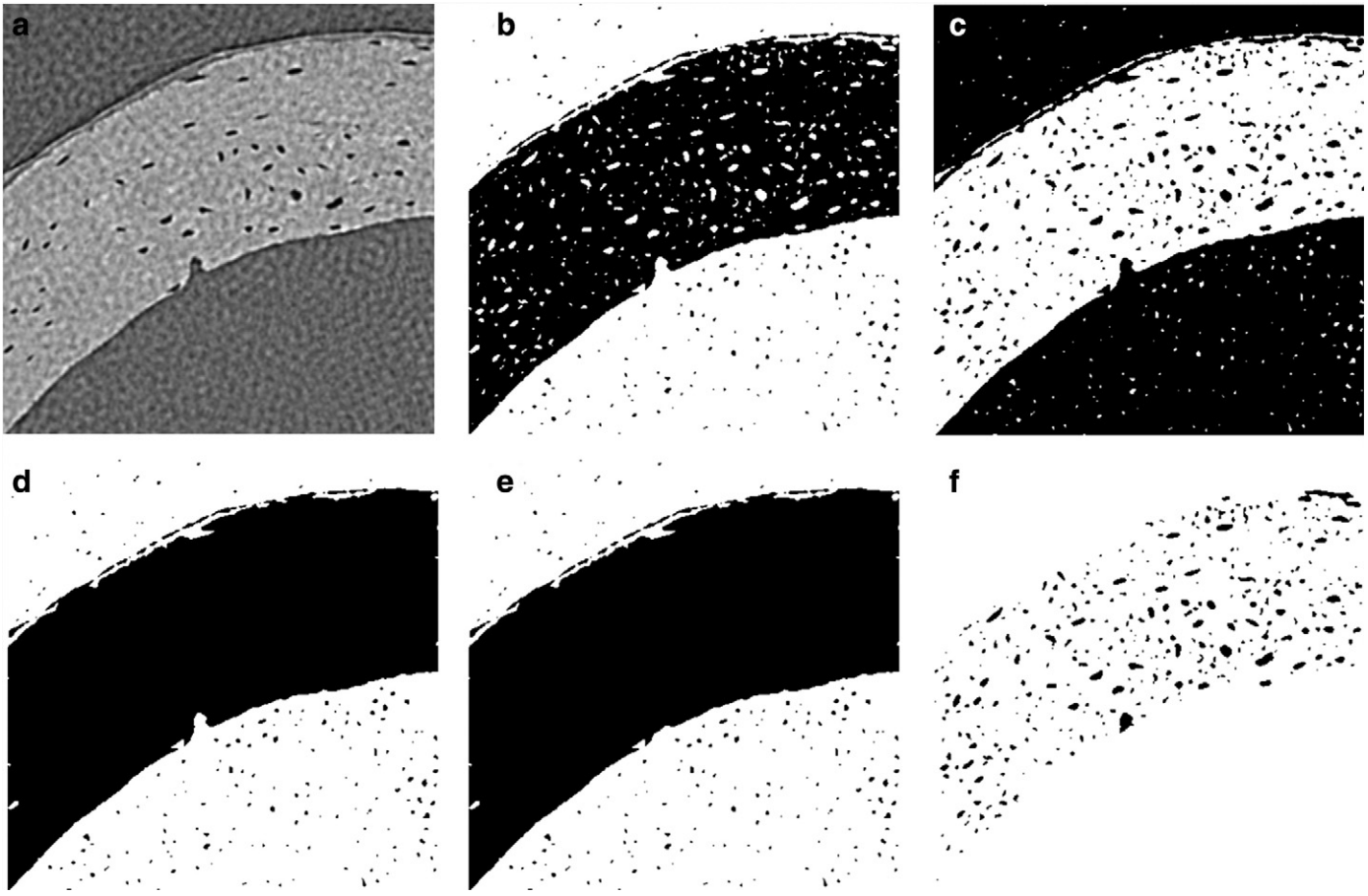

Fig. 1. Segmentation process of the intracortical porosity comprising lacunae and canals. (a) Transverse SR CT of a bone section. (b) The original image resulting in a binary after a histogram-based global thresholding was applied: mineralized tissue is in black and voids in white. (c) Inverted binary image. (d) Outline of the cortex after 2D filling the holes unconnected to the background (lacunae, deep parts of canals) in the binary picture. (e) Outline of the cortex after manually filling the remaining intracortical porosity of the bone continuous with the periosteal and endosteal space. (f) Unmineralized porosity contained within cortical bone after performing logical AND on the inverted original (c) and the filled cortex (e).