Osteogenesis Imperfecta: the Effect of a Single Amino Acid Substitution on the Hierarchical Organization of Bone

Contact:

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Project Summary and Goals

Osteogenesis imperfecta (OI), also known as the brittle bone disease, is a genetic disorder that causes severe mutations in bone structure. Depending on the specific genetic mutation, patients can die already during childhood or even birth. In many other cases, applying little force on the bone will lead to bone fracture. The basis of this disease is a genetic mutation that hampers the correct assembly of collagen molecules into fibrils (~80 nm), which form the main organic matrix in our bones. As a result of this faulty organization, the collagen does not mineralize properly and a brittle materials is formed.

Hence, this genetic defect changing only one amino acid in the collagen molecules, has an impact on bone structure from the nanoscale all the way to the millimetre level. We therefore will analyse the 3D organization of collagen in term of local density, orientation and alignment in a multiscale approach. This research is the first step towards comprehensive understanding of OI and will open the door for a new approaches for OI treatment.

In this study we aim to understand the collagen organization in OI bone samples in the nano to micro metre scale and to elucidate the structure-function relationship as it comes to expression in OI bones. To achieve that, three dimensional focused ion beam scanning electron microscopy (3D FIB/SEM) is used to image the 3D structure of bone from the micrometre down to the nanometre level using block face imaging in *"slice and view mode" or "serial slice view (SSV)"* (Fig. 1).

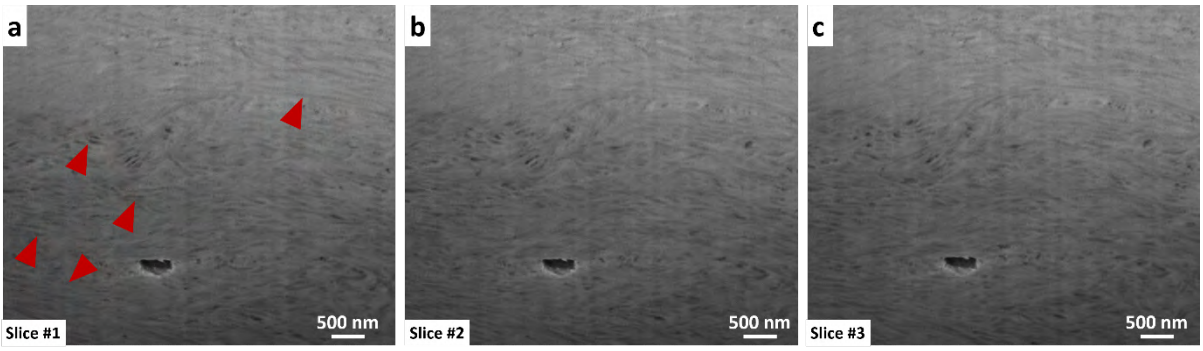

Figure 1: Representative image from the serial slice and view procedure. Individual collagen fibrils are indicated by the red arrows

We use Avizo software for the 3D volume reconstruction of collagen (Fig. 2). To determine the network of collagen fibrils in OI bone, they were labelled with a yellow color.

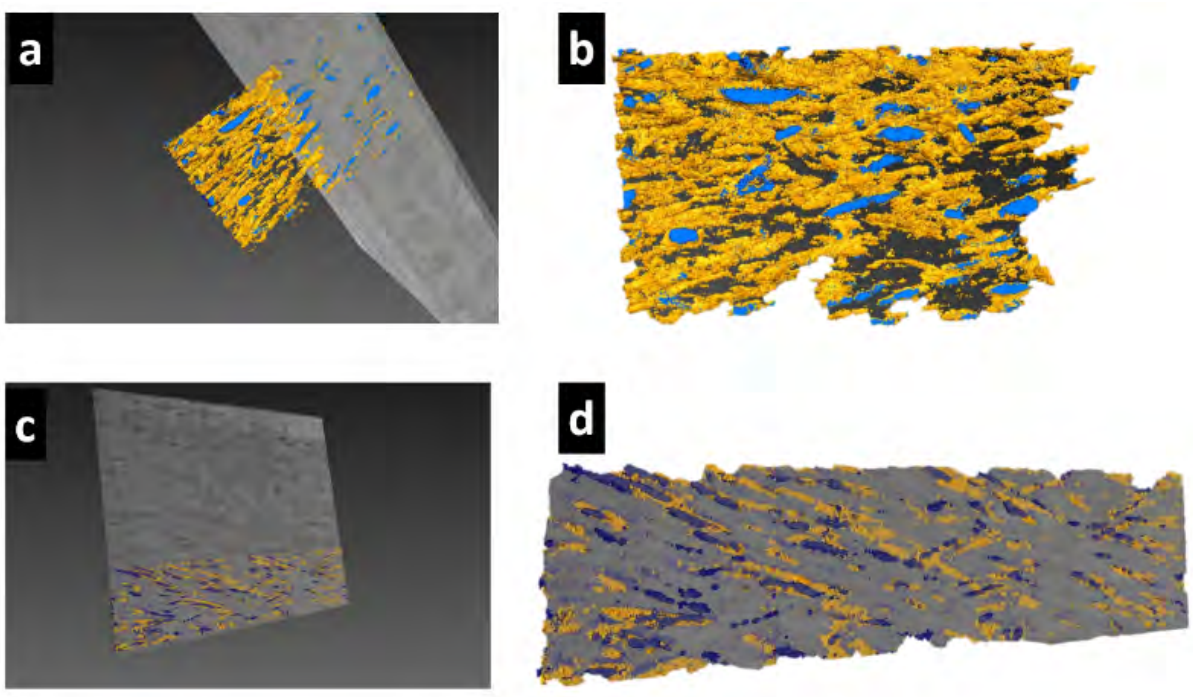

Figure 2: 3D reconstruction of collagen fibrils in OI bone. Individual collagen fibrils are labelled with yellow colour**.**

Currently, there is a gap in understanding the 3D structure of OI bones. This lack of knowledge limits progress in understanding pathological tissue formation. Unraveling this 3D structure and analyzing density maps and collagen orientation maps can bring us one step closer toward understanding structure-function relation in OI bone and hopefully will help to design better OI treatments.

Recent literature studies showing collagen orientation and organization in 3D

Two recently published articles show approaches to analyse collagen orientation in 3D. The segmentation of collagen fibrils in a given volume can help us to describe the network of the fibrils inside the bone tissue.

The first example is from a demineralized human cortical bone. Reznikov *et al*. employed 3D FIB/SEM to collect a volume of images and label the collagen fibrils manually to segment them (1). Collagen fibrils imaged by FIB scanning electron microscopy can be seen in Figure 3A. In order to determine the length of individual collagen fibril segments, Reznikov et al. performed ***manual*** labelling of collagen fibrils. Individual collagen fibrils were color-labeled where they can be continuously traced. Each of the 100 labels is shown in a different colour visualizing the distribution of segment lengths (Fig. 3B).

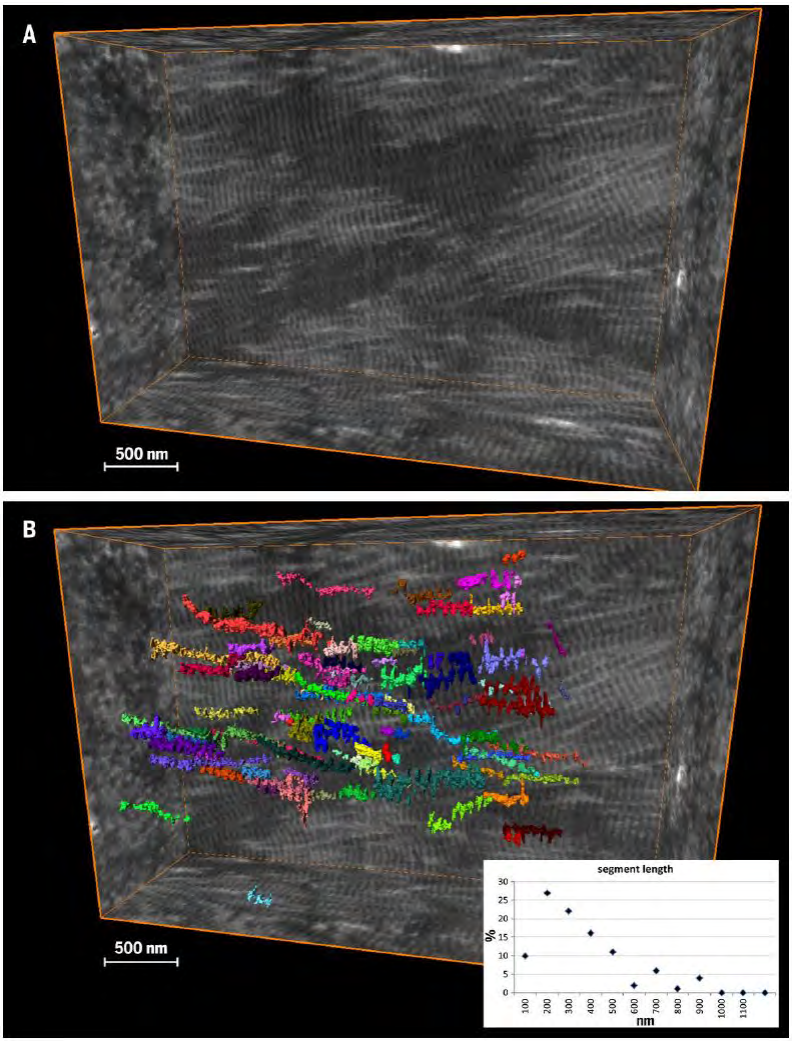

Figure 3: 3D Reconstruction of collagen fibrils in the ECM of healthy bone.

The second example focuses on periodontal ligament (PDL) which connects the tooth to the bone. In this paper Naveh et al. (2) employ microCT to demonstrate the fibrous network within the PDL. Later they use Avizo software to carry out the 3D image processing and analysis of the collagen matrix.

Naveh *et al*. visualize the 3D structure (distribution and direction) of collagen networks and show the uniformity of the fibers in this network (Fig. 4). Volume rendering (Avizo software) show the collagen fibrils (Fig. 4B), while density maps of the collagen fibres evaluate the locations of dense and sparse collagen networks (Fig. 4C, D). The density maps show a difference between fiber organizations at different parts of the sample. While some parts show uniform dense network of collagen fibril, other parts contain sparse network of collagen. In addition they conduct directionality analysis (Fig. 4E) where they show differences between directions of the collagen fibrils and preferred orientation.

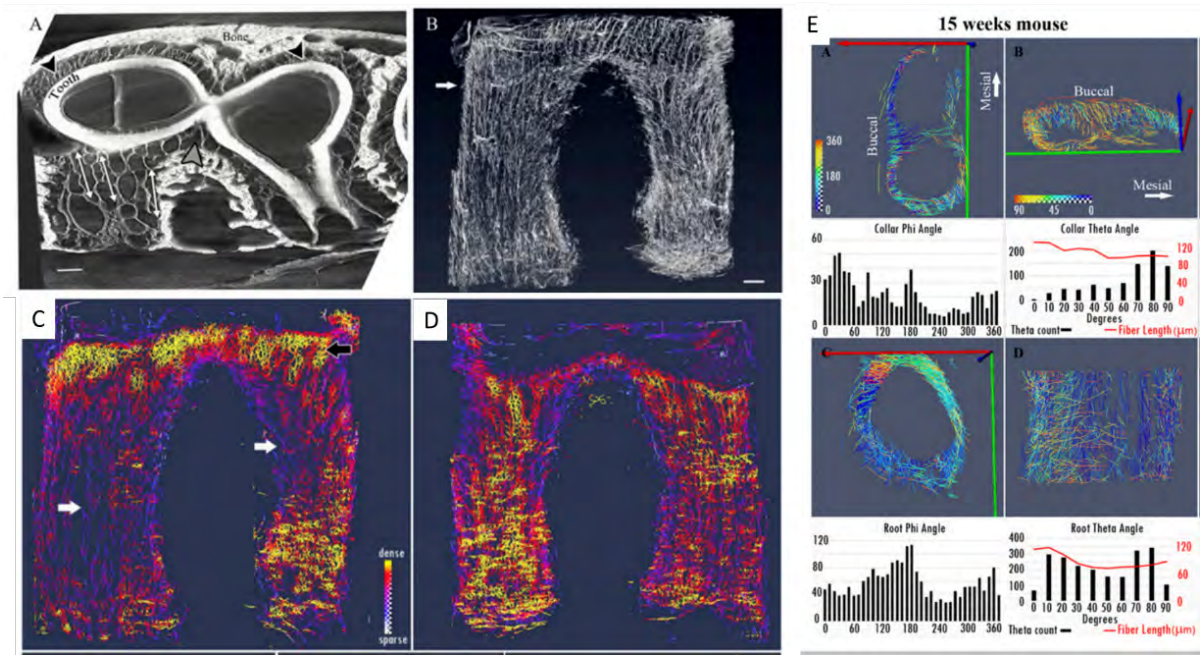

Figure 4: A) Reconstructed volume showing the first molar tooth of a mouse inside the bone and the collagenous component of the B) 3D view of only the collagen network of the PDL. C) and D) Relative distribution maps of collagen network; cooler colours toward blue are low-density fibers, hot colours toward yellow are high-density fibers. E) Fiber directionality analysis.

Project aim:

Post processing analysis of the 3D FIB/SEM of OI bones is required to determine the collagen distribution, direction and density in a giving volume as it. These results will help to estimate the structure –function relationship of affected OI bones.

References

1. N Reznikov, M Bilton, L Lari, MM Stevens, R Kröger et al. Fractal-like hierarchical organization of bone begins at the nanoscale Science 360, 6388 (2018)
2. G Naveh, J Foster, T Santisteban, X Yang, B Olsen et al. Nonuniformity in ligaments is a structural strategy for optimizing functionality PNAS 115 (36) 9008-9013 (2018)