X-ray Analysis of the zebrafish tail bone formation

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We investigated the mineral phases involved in the pathway of bone mineralization in larval zebrafish tail. The zebrafish is a unique model system which is advantageous for investigating bone formation mechanisms – in particular the initial stages of mineral aggregation and transport from the blood vessels and/or the cells towards the bone. To date, the mineral phases and structures present in these initial stages are still poorly described (1-3). The larval tail bones have several advantageous characteristics, among which the rapid growth, the well-defined geometry of the vascular network relative to the forming bone in the thin tissue, and the absence of other organelles make them particularly appropriate for this study (4).

The accepted concept of the bone formation process involves the transport of ions to osteoblasts, which are responsible for mineral formation and mineral deposition. We have observed cells juxtaposed to the bone tissue, containing intracellular vesicles (0.5-1 µm) filled with a poorly characterized disordered calcium phosphate mineral phase. The mineral-bearing intracellular vesicles release their content into the extracellular collagen fibrils, where the mineral subsequently crystallizes to carbonated hydroxyapatite (2). Still, little is known about the mineral pathway and the mechanisms by which the mineral is stabilized and destabilized on its way to the bone (4).

At the MINAXS Nanofocus Endstation we utilized a combination of high-resolution small and wide angle X-ray scattering (SAXS, WAXS) together with X-ray fluorescence to map structural formation from mineral particles in developing zebrafish tail bone with a monochromatic beam (13keV) with the size of 1 µm. We characterized the bone mineral concerning its mineral structure, shape, size and composition. By this approach we obtained valuable structural information on local mineral properties of the zebrafish bone mineralization. The measured tail was taken from a 25-28 days old fish that was sacrificed and placed on kapton foil. To prevent in situ crystallization, the fish tail was washed by doing fast dehydration with few droplets of acetone which were immediately removed. (Fig 1a). We scanned along the thin long sections using a scanning stage (xy). To assure statistical significance, we measured several tail bones. Scanning areas had side lengths from 100 µm up to 300 µm.

Results and preliminary analysis

Using X-ray fluorescence together with wide angle X-ray scattering we could identify mineral bearing vesicles and/or aggregates in the vicinity of individual zebrafish tail bones which may have emerged from cells and/or blood vessels (Fig 1b). We also measured a transition from a calcium phosphate precursor phase to a more stable hydroxyapatite phase at the border of the growing bone structures (Fig 1c). Due to the large amount of data collected during the beamtime (approximately 5000 diffraction patterns per sample) we are still characterizing the mineral phases and particle orientation, size and shape.

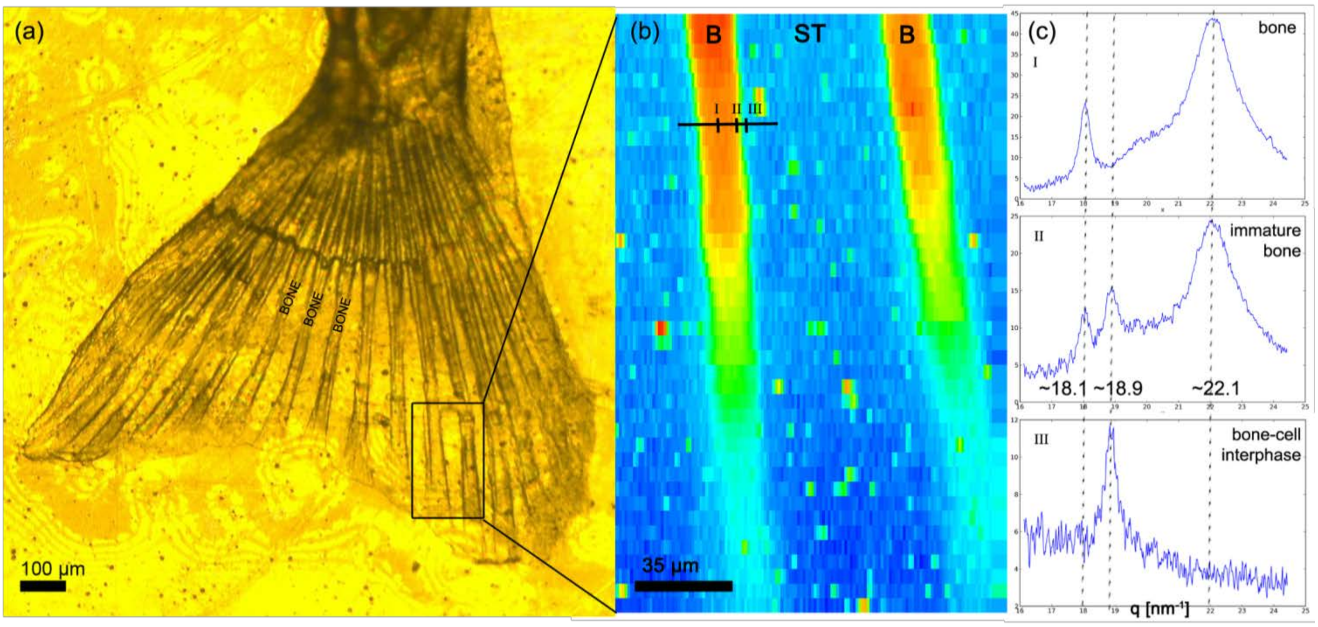

Figure 1: Zebrafish caudal fin measured at the MINAX Nanofocus Endstation (a) light microscopy picture of a representative zebrafish tail mounted on kapton foil. (b) X-ray fluorescence (XRF) depicting the calcium distribution in the caudal fin showing the mineralized zebrafish tail bones (B) embedded in the soft tissue (ST). Note the presence of calcium-rich aggregates (red) between bone structures. (c) wide angle X-ray diffraction measured at different positions of a tail bone (indicated by I-III in the XRF map) showing mature bone (I), immature bone (II) and the bone-cell interphase (III). Peaks at q = 18.1 nm^{-1} and 22.1 nm^{-1} are indicative of a hydroxyapatite mineral phase present in mature bone mineral. Note the peak appearing at around q = 18.9 nm^{-1} in the transition zone between the bone structure and the soft tissue, which may represent a calcium phosphate precursor phase before crystallization to the hydroxyapatite phase.

References

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