POSTER #11

MINERAL DEPOSITION PATHWAYS IN THE ZEBRAFISH CAUDAL FIN BONE

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Vertebrate bone formation has been extensively investigated, both at the cellular and mineral level. Despite this, key aspects remain unclear, such as how the mineral is delivered to site of mineralization and in which form is the transported mineral present. Vertebrate bone is a composite material consisting of collagen (20-30%), carbonated hydroxyapatite mineral (60-70%) and water (10-20%) that together form a well-defined hierarchical structure from the nanometer scale to the centimeter scale [1]. The ions or mineral precursors in different packaging forms have been suggested to be deposited into the bone in a strictly cell mediated fashion [6-7], or directly from supersaturated extracellular fluids [4, 5], controlled by extracellular matrix proteins [10]. In an alternative pathway [3-5], Mahamid et al [9] showed the presence of mineral particles identified as amorphous calcium phosphate (ACP) inside intracellular vesicles, in cells located proximal to the non-mineralized collagen region both in the adult zebrafish tail and in embryonic mice bones.

The zebrafish (*Danio rerio*) caudal fin is a unique model system for vertebrate bone formation study. In the zebrafish caudal fin bone, the time scale for bone labeling by immersion of the larvae in calcein-enriched water changes dramatically from minutes at the age of <~35 dpf (days post fertilization) to hours at juvenile-adult age >~35 dpf. Intracellular and extracellular micrometer sized mineral deposits in the zebrafish caudal fin tissue were found at a distance from the forming bones and in proximity to the vascular system and forming fin bones (Fig. 1a-d). Cumulative observations also indicate a high degree of blood permeability in the larval stage (Fig. 1d). To explain these observations, we suggest the presence of two distinct blood to bone mineral pathways; At the larval stage, it appears that there is a predominant direct pathway of mineral transport through the vasculature and extracellular fluids. At the juvenile-adult stage, the direct mineral pathway appears to be at least partly substituted by a slow cell mediated pathway.

In this study we combine several imaging techniques. We utilize calcein labeling injected directly into the fish circulation in order to monitor the flux from blood to bone in real time. Confocal fluorescence microscopy on transgenically labeled fish of specific cell lineage (Endothelial, Osteoblast), together with calcein labeling, allows observing the relation between mineral–cell–blood vessels interface and tracking of mineral formation and transport in real time at different developmental stages. Higher resolution of the bone-cell-blood interface is achieved by using cryogenic scanning electron microscopy (cryo SEM) on high pressure frozen tail specimens. Block face sequential imaging by cryo-FIB-SEM reveals wide inter-cellular networks, supporting the presence of direct communication between the blood vessels and the growing bone at the larval stage (Fig. 1e-f).

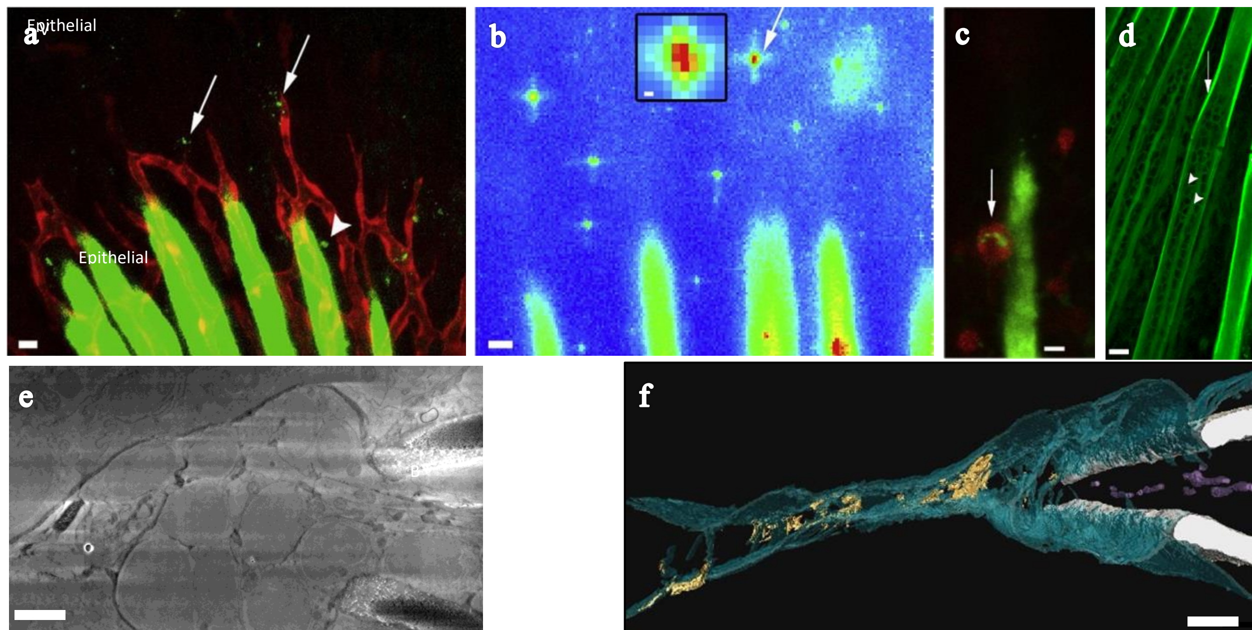

Figure 1: (a,c) In vivo confocal imaging of a *Tg(fli1:DsRED)* larva tail, Endothelial cell (red fluorescence), mineral and bone labeled with green fluorescent calcein. Particles are detected near blood vessel (arrow), in addition to 1µm particles, which are spread throughout the tail (arrowheads) (c) calcein labeled particles inside *fli1:DsRED* positive cell (arrow)[3]. (b) Calcium XRF map of region similar to those in Fig.1b, calcium distribution color scale: Blue-Red, low calcium-high calcium concentration. Inset: a calcium containing deposit located in non-mineralized collagen[3]. (d) Caudal fin show calcein labeled bone (arrows) and the blood vessels and extracellular fluids filled with calcein (arrow heads). (e) Cross-section of caudal fin, blood vessels and bones (middle layer) enclosed within the basement membrane, surrounded by epithelial tissue. The vein is in direct communication with the bone growth region through an inter-cellular network. (B) Bone; (V) vein [10]. (f) Reconstruction and segmentation of (e) sample volume (37.4x23.4x24.4 µm). Color code: Basement membrane (blue), bones (white), intra cellular particles (gold) and mitochondrial network (purple) [10]. Scale bars (a-e): 10µm, (b inset): 1µm. (e-f): 2µm

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