Bone tissue structure and composition of *oim* middle ear ossicles

Maialen Ugarteburu, Luis Cardoso, Claus-Peter Richter, Alessandra Carriero

Hearing loss is a common clinical hallmark of people with osteogenesis imperfecta (OI, or *brittle bone disease*). To date, pathophysiological mechanisms of hearing impairment in OI are uncertain, and structural and compositional properties of the middle ear bones in OI remain poorly studied. In a recent study on the ossicles morphology in the *oim* mouse model of OI, we found consistent fractures of the incus in the *oim* middle ears [1]. To explain the mechanism of these fractures, here we investigate the bone tissue structural and compositional properties of the *oim* middle ear ossicles. We examined bone composition in *oim* and WT mouse (14 w.o.) ossicles using Raman spectroscopy. On the same bones, we quantified collagen content, orientation, continuity, and number of clusters of collagen fibers using second harmonic generation microscopy and a custom-made MATLAB code [2]. On the contralateral ears, we performed synchrotron microtomography to determine ossicular bone tissue porosity at the vascular and cellular level. Bone tissue from the *oim* ossicles showed no differences in the mineral compositional parameters (mineral-to-matrix ratio, carbonate substitution and crystallinity) compared to healthy bone. However, they exhibited a decrease in collagen content, as well as in fibers organization and continuity, with an increased number of fibers clusters, and higher lacunar, but not vascular, density volume. These results suggest that potential causes for bone fragility in the *oim* middle ear bones are to be searched in the collagen organization within the tissue, and in its cellular porosity. These both represent defects within the bone tissue, where a crack can start and easily propagate [3, 4].

Interestingly, while structural changes resemble the ones we found in the long bones [3], but not the compositional properties [4], possibly because the middle ear mineralizes during ossification, with no subsequent remodeling. Furthermore, tissue porosity in the ossicles is in contrast with those found in the otic capsule [5], showing that under pathological conditions, different bones of the same hearing system may have different properties according to their the physiological function.

[1] Ugarteburu, M., et al. ARO 2022
[2] Muñoz A., et al, NEBEC 2022
[3] Carriero A., et al. Bone 2014
[4] Carriero A., et al. JBMR 2014
[5] De Paolis, A., et al JSB 2021