Report for experiment MD-672, 27/07-1/08/12, 15 shifts

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Background

Under healthy conditions human bone undergoes permanent remodeling in order to adjust to mechanical demands, repair micro-fractures and maintain mineral homeostasis. This process is called remodeling, which is performed by osteoblasts and osteoclasts, the bone-forming and bone-resorbing cells, respectively. The activity of these cells is believed to be orchestrated by osteocytes, the most frequent bone cells, housed in cavities of the bone matrix, called lacunae. Osteocytes are interconnected through slender canals called canaliculi each of only several hundred nm in diameter. The osteocyte network plays a role in sensing the mechanical signals (mechanosensation). The morphology of the lacuno-canalicular network (LCN) is believed to be related to the mechanosensation and mechanotransduction processes of osteocytes (Schneider *et al.*, 2010, Lanyon, 1993, Burger & Klein-Nulend, 1999, Zhou *et al.*, 2009, Wang *et al.*, 1993, Weinbaum *et al.*, 1994, McCreadie *et al.*, 2004, Vatsa *et al.*, 2008, van Hove *et al.*, 2009, Currey, 2003, Mullins *et al.*, 2007). Furthermore, the LCN ensures the transport of cellular waste and nutrients (Burger & Klein-Nulend, 1999). Additionally, the LCN has been reported to be essential for micro-crack repair by triggering bone remodeling (Currey, 1984).
In addition to their mechanosensive function the osteocytes are hypothesized to regulate the metabolism of bone mineral, e.g. phosphate (Westbroek *et al.*, 2002, Nakashima *et al.*, 2011). The well-adjusted osteoblast vs. osteoclast activity is crucial in healthy bone tissue and is altered in bone diseases such as osteoporosis. In the course of osteoporosis or development of bone metastasis the treatment with bisphosphonates (BP) is one common intervention to suppress bone resorption by inhibiting osteoclast activity (Dhillon & Lyseng-Williamson, 2008, Liberman *et al.*, 1995). The action of BP relies on the reduction of bone resorption by inhibiting osteoclast activity. A severe and most often irreversible adverse effect of high-dosed BP treatment is the potential occurrence of osteonecrosis of the jaw (Mercer *et al.*, 2013, Allen & Ruggiero, 2009). Although multiple hypotheses have been formulated recently, the underlying pathophysiological mechanisms of bisphosphonate related osteonecrosis of the jaw (BRONJ) are still not completely understood (Otto *et al.*, 2010, Bertoldo *et al.*, 2007). In animal models it could be shown that the bone turnover of jaw bone is higher compared to other sites (Huja *et al.*, 2006, Vignery & Baron, 1980), which is potentially explained by high stress and tooth movement (Bertoldo et al., 2007).

Material and Methods

The experiment was performed on beamline ID22. The imaging conditions were similar to that fixed in a previous work (Langer *et al.*, 2012). The spatial resolution was 50 nm, the energy was 17 keV and the scintillator was a LSO-20µm. First, a short scan (1199 projections, counting time in each projection: 0.2 sec) was performed to get an overview of the sample (voxel size 350nm). This overview image was used to position the sample for the high

resolution scans (voxel size 50 nm, FOV=100x100x100 μm^3) to avoid the Haversian canals. Then the high resolution data acquisition consisted in four scans at distances (focus-detector distance: 526,2 mm, distances focus-sample: 36.1, 37.1, 41.1, 51.1 mm). The number of projections at each distance was 2999 at counting time = 0.2s.
The imaged samples included 19 human jaw-bone specimens, 7 human femoral, 3 tibial specimens, as well as one phantom at 50 nm spatial resolution and one phantom at 350 nm resolution. Out of the jaw bone specimens, 10 samples originate from donors suffering BRONJ.
The experiment provided a very large amount of data (about 4 Terabytes). The overview scan were reconstructed by using the Paganin approach (delta/beta=199) followed by Filtered Back Projection. The high resolution images were reconstructed after performing the four distances phase retrieval based on the mixed approach with a subsequent iterative non linear refinement approach. Up to now, 3D reconstructions have been performed on all scans.

Results and Discussion

Figure 1 illustrates an overview image, the high resolution image and a maximum intensity projection (MIP) image on 200 slices showing clearly the lacunae and the connecting canaliculi.

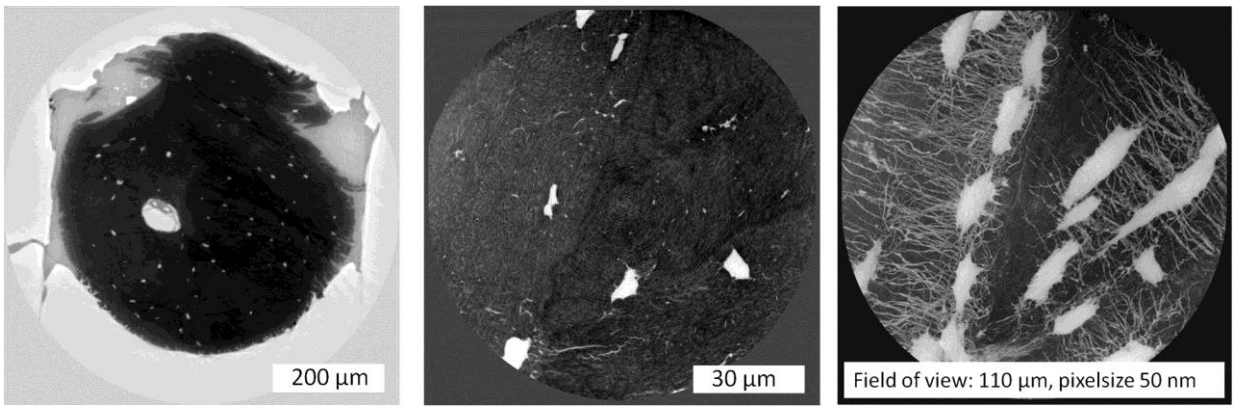

Figure 1: a) slice in an overview image (voxel size 350nm), b) high resolution image (voxel size 50nm), c) Maximum Intensity Projection (MIP) on 200 slices

The processing of the overview-scans is finished resulting in one publication (Hesse *et al.*, 2014, in press). There, the volume distributions of osteocyte lacunae of human cortical jaw bone have been investigated, and a comparison between healthy subjects and patients treated with bisphosphonates was carried out. We hypothesized that bisphosphonate-treated patients would exhibit larger osteocyte lacunae volumes, since tissue at the surface of the peri-lacunar region has been resolved, a process called osteocyte osteolysis, to maintain mineral homeostasis since the mineral available by the remodeling process is decreased. Interestingly, we could not confirm this hypothesis. It remains unclear if the potential process of osteocytic osteolysis results in a decreased peri-lacunar mineralization as opposed to enlarged osteocyte lacunae. We observed a decreased degree of mineralization of jaw bone compared to the

femoral or tibial site, indicating a higher bone turnover of jaw bone. In a comparison between volume distribution of the jaw bone and tibial/femoral site, we observed a higher higher value of large osteocyte lacunae volumes in jaw. We concluded that bisphosphonates are deposited into jaw bone at a higher rate, due to higher bone remodeling rate. Moreover, if deposited into osteocyte lacuane during mineralization of osteocyte lacunae (micro-petrosis), a toxic concentration of bisphosphonates may be reached after de-solution of mineral caused by e.g. decreased pH level in the course of infections. This process potentially explains a serious side-effect of bisphosphonate treatment: Osteonecrosis of the jaw. However, more in-depth studies are necessary to support this hypothesis

The next step was to quantify properties of the LCN and the perilacunae and pericanalicular bone matrix based on the high resolution data. We hypothesized that the secondary mineralization process takes place by a diffusion process through both, the interface of the extra-cellular fluid of the lacunar and the canalicular surface. With respect to the diffusion constant of mineral through the mineralized bone matrix the proposed diffusion process should result in mass density gradients with respect to the distance to the pore boundary.
We observed that the hypothesized mass density gradients indeed exist at both, lacunar and canalicular interface. However, based on our finding that the diffusion occurs at the lacunar as well as at the canalicular surface, we further hypothesized that the process of mineral exchange between the extra-cellular fluid and the mineralized matrix occurs at all bone surfaces, including the canalicular network. Our data suggested that the capacity of the matrix to exchange mineral with the extra-cellular mineralized bone matrix is increased by one order of magnitude if the canalicular surface is taken into account. Based on the morphology of the lacuna-canalicular network, we could show that the mass density gradients are likely unaffected by short-term fluctuations of mineral in the fluid but solely by secondary mineralization processes. We could also put these findings into the context of BP treatment. A publication on these data is ready for submission to JBMR (Hesse *et al.*, 2014, in preparation).

Aiming to quantify the role of the LCN-morphology on mechanosensation of osteocytes, a pilot study investigating femur specimens by means of finite element analysis of the in situ strains that osteocytes experience was performed (Varga *et al.*, 2014, in preparation). One of the findings of this analysis was that the detailed LCN geometry predicted that the externally applied strain can be amplified by a factor up to 50-70.
We speculated that the ability of mechanosensation is altered in BRONJ. This will be addressed in a next step by applying a finite element analysis on healthy jaw and BRONJ high resolution data.

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