24 h. Changes in the expression of osteocyte marker genes were examined with qPCR. Secreted sclerostin in conditioned media was measured by ELISA. The presence of bone cells was determined histologically.

Histological analysis, and the abundant expression of osteocyte-specific markers, including *Sost* and *Dmp1*, confirmed our culture was osteocyte-rich. Treatment with PTH increased the expression of the *Tnfsf11* (encodes RANKL) by 1.6-fold in comparison to untreated control, while *Sost* (encodes Sclerostin) was 6.5-fold lower in the treated cells. Similar effects of PTH on gene expression in osteocytes have previously been shown in the literature. Osteocytes within the bone secreted sclerostin (584 pg/mg bone) into the culture media during the 24 h. PTH treatment did not alter the amount of sclerostin secretion.

This ex vivo culture system retains osteocytes within the bone matrix. The response of osteocytes to PTH was similar to those that had been previously shown in the literature. In future studies, this experimental model will be used to determine osteocyte responses to novel bone therapeutics.

Keywords: Osteocyte, ex vivo model, PTH, sclerostin, therapeutics

P122

Correlations between the osteocyte network and its surrounding matrix in newly formed bone

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The structure of bone is adapted at every hierarchical level to its mechanical needs, i.e. the extra cellular matrix (ECM) is subjected to a lifelong interplay between bone resorption by osteoclasts and bone formation by osteoblasts. Osteocytes are embedded in the bone matrix and orchestrate the remodeling process via fluid flow in the lacuno-canalicular network (LCN) and likely contribute directly to mineral homeostasis. Since the repair of bone fractures recapitulates skeletal development, healing mouse bone is an ideal model system to study correlations between the architecture of the LCN and the surrounding bone matrix in different tissue types.

To visualize correlations between LCN and ECM within the same bone volume we are using a combination of experimental techniques. Confocal laser scanning microscopy and high resolution μCT measurements are used to characterize the LCN, while the mineral content of the sample surfaces is measured by quantitative backscattered electron imaging (qBEI) and the mineral particle characteristics by scanning small/wide angle X-ray scattering (SAXS/WAXS).

Data about the LCN and ECM in healing bone does not only allow a distinction between cortex, cartilage and callus, but also between different bone types within the callus. Callus woven bone exhibited thicker and less organized mineral particles and a less dense LCN compared to newly formed callus lamellar bone. Overall, we identified correlations between osteocyte network architecture and mineral particle characteristics in all tissue types, which supports our hypothesis that osteocytes directly influence the mineralization process.

Keywords: Osteocyte, lacuno-canalicular network, mineral particles, healing bone, x-ray scattering

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Mineralization and autophagy in bone is suppressed by EphrinB2 through the RhoA/ROCK pathway

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Controlled mineralization of the collagen matrix is essential to maintain optimum bone strength. Previous work has shown that mice with EphrinB2 (*Efnb2*) knockdown in osteocytes develop brittle bones, and that this is associated, not with any change in bone mass, but with a high level of mineralization and abundant autophagosome formation within osteocytes. In this study, we sought to determine the mechanisms by which EphrinB2 acts in osteocytes to restrain the bone mineralization process.

To determine whether increased autophagy leads to increased mineralization, two osteocyte-like cell lines (OCY454 and Kusa 4b10) were treated under mineralizing conditions with 0.05, 0.1 and 1 nM of the autophagy inducer Rapamycin when first mineral crystals were detected. After 14 (OCY454) and 19 (Kusa4b10) days, when these cells express osteocyte markers, cells were fixed and mineral deposition was detected with Alizarin Red Staining (ARS). Both cell lines showed greater mineral deposition when autophagy was induced with Rapamycin. This suggests that stimulation of autophagy in osteocytes stimulates their mineral release.

We next sought to determine whether EphrinB2 inhibits autophagy, and, since EphrinB2 has been shown to signal through the RhoA/ROCK pathway, we tested whether the action was mediated by this pathway. We stimulated OCY454 cells with clustered EphrinB2-Fc to initiate intracellular signaling for 1 h, in the presence of Bafilomycin A1 to determine whether it modifies autophagosome formation. Cells were fixed and stained with anti- microtubule-associated protein 1 light chain 3 (LC3), and LC3 punctae were counted to determine changes in autophagy levels. EphrinB2-Fc treatment significantly suppressed Bafilomycin-induced accumulation of autophagosomes. Addition of the RhoA/ROCK inhibitor H1152 prevented this decrease, indicating that EphrinB2 reduces autophagosome formation, at least in part, through RhoA/ROCK signalling.

This reveals a novel pathway explaining how EphrinB2 signaling in osteocytes limits mineralization and maintains bone strength.

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Trichloroethylene-related compound *S*-(1, 2-dichlorovinyl)-L-cysteine localized on mouse cartilage

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