Healing of Long Bone Critical Size Defects

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Introduction

In an aging society, osteoporosis is diagnosed in around 40% of postmenopausal women. Bone loss, lack of coordination, and muscle weakness result in being prone to fractures from normally inadequate trauma. Osteoporosis is a chronic disease that interrupts the equilibrium of natural bone formation and resorption process, causing the bone to thin and weaken. Treatment is possible using anti-resorptives like bisphosphonates (BP), anabolic treatment, and Denusomab. Treatment with BP results in Osteoclasts activity being arrested upon uptake of BP. Little to no resorption in the bone is causing a “frozen” state. In this state, the bone accumulates microfractures and is susceptible to atypical fractures over time. The mousefix project aims to better understand the healing of critical size defects in long bones of mice and the influence of BMP2 and L51P growth factors on bone healing and beta TCP implant resorption. The animals were either subjected to ovariectomy or sham surgery. They are either treated with ALN or not and within these groups have different implant coatings of BMP and L51P. Further investigated is whether MicroCT or image analysis can be used to distinguish between different tissues, including old bone, new bone, and implant material.

Materials and Methods

120 mouse femora are embedded in MMA, and histological sections are produced. These sections are stained, and stitched images are made. Matlab's image processing toolbox analyzes these stitches according to three regions of interest (ROI). The first ROI is the defect site, and the second and third ROI reach from the defect site to the most proximal and distal screw. Further, the femora are scanned using micro-computed tomography (MicroCT) and are analyzed for bone volume and density.

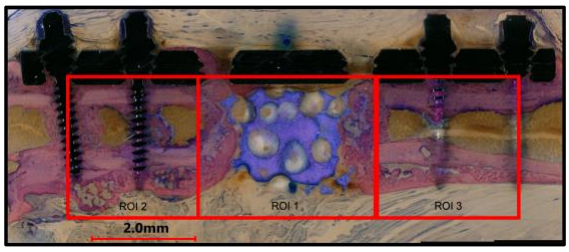

Fig. 1 Stitched image of a mousefix sample with a beta TCP implant in the middle and illustrated ROI.

Results

Comparing the evaluated total bone after six and twelve weeks shows that the mice treated with alendronate (ALN) show more bone in total than untreated animals. Coating with BMP2 and or L51P did not cause a significant change in bone formation. In the density of the defect site, one can observe a decreasing trend in treatment with ALN. No difference in BV or new bone formation is found in animals not treated with ALN. A decreasing trend is expressed in the residual implant area when increasing BMP concentrations for the OVX and OVX+ALN groups.

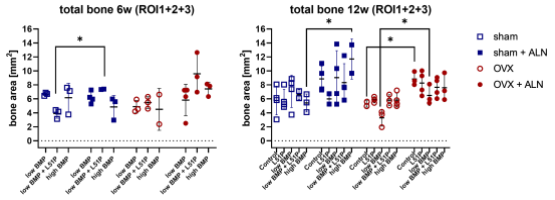

Fig. 2 Image analysis results for six- and twelve-week-old mice total bone in all three ROI.

Discussion

Direct bone formation can be observed in the histology images. An effect of treatment with growth factors was not found. However, the treatment with ALN shows a positive impact after 12 weeks on the total bone area. In histology, one can distinguish between the implant material, old bone, new bone, and soft tissues. Using MicroCT, one can only distinguish between hard and soft tissues, so mineralized tissues cannot be separated. With Mathlab’s toolbox, one can distinguish between bone and CaP material but not between old and new bone. Therefore, only classical histomorphometry can provide us with all the data seen in a histological section.

References

Rachner, Tilman D et al. “Osteoporosis: now and the future.” Lancet (London, England) vol. 377,9773 (2011): 1276-87. doi:10.1016/S0140-6736(10)62349-5

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