CHARACTERIZATION OF BONE MINERAL COMPOSITION DURING FRACTURE HEALING USING SEM AND EDS

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Introduction

Formation of a bony callus followed by bridging is critical for successful fracture healing. Bone morphogenetic proteins (BMPs) and bisphosphonates can enhance fracture healing in delayed- or non-union [Little, 2007; Bosemark, 2013]. However, little is known if the drugs affect the composition of the mineral in newly formed bone. The aim of this study was to investigate if bone mineralization differs between cortical bone and fracture callus bone by using scanning electron microscopy (SEM) and Energy-dispersive X-ray spectroscopy (EDS). Subsequently, we studied if different treatments affect the mineral composition.

Materials and Methods

Animal model: Femoral osteotomies were performed in 12 Sprague Dawley rats (age 9 weeks) and fixed with a K-wire [Tagil, 2010]. The rats were randomized into (A) Saline, (B) BMP-7, (C) Zoledronate (ZO) and (D) BMP-7+ZO. The experimental protocol was approved by the local animal ethics committee. The rats were sacrificed after 6 weeks and both femurs were extracted, dehydrated, embedded in PMMA, cut, polished and carbon coated.

SEM: A JSM-6700F equipped with an Oxford XEDS system was used.

EDS: Three different areas of interest were chosen for chemical analysis: i) External callus, ii) Inner callus and iii) Cortical bone (Fig. 1). In the case of the non-fractured samples (control) only the cortical bone was analysed. The element-analysis included the weight percentage (wt%) of Ca, P, O, Na, Mg. Differences between callus and cortical bone, and among samples from different groups were investigated.

Results

Groups B-D produced larger calluses with more new bone formation compared to Group A (Fig.1). EDS measurements resulted in a Ca/P ratio of approximately 2 (Table 1), with no statistically significant difference (single factor Anova) between the areas. Na & Mg concentrations were approximately 0.75%wt and 0.64%wt respectively (Table 1).

Discussion

SEM is a powerful tool that allows studying areas associated with the bone-healing process (fracture line, callus). The Ca/P ratios measured with EDS analysis indicate that the mineral in the newly formed bone is also carbonated hydroxyapatite and the Ca/P ratios and the amount of other elements (i.e. Mg, Na) did not change between the locations (Table 1). Considering that the rats were sacrificed at 6 weeks post-fracture, it may be that analyses at earlier time points could reveal differences in the mineralization process [Yang, 2007].

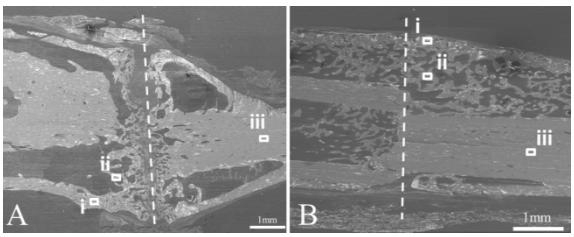

Figure 1: SEM images of samples from a) Group A & b) Group D. The dashed lines indicate the fracture planes. Callus surrounds the cortex. Three areas where chosen for EDS analysis: i) External callus, ii) Inner callus and iii) Cortical bone

Groups	Ca/P i	Ca/P ii	Ca/P iii	Ca/P control	Na	Mg
A mean	2,01	1,98	2,00	2,00	0,76	0,65
SD	0,06	0,03	0,04	0,02	0,04	0,07
B mean	2,04	2,05	2,04	2,02	0,70	0,55
SD	0,05	0,01	0,01	0,01	0,09	0,09
C mean	1,97	2,02	1,99	2,03	0,75	0,69
SD	0,01	0,01	0,01	0,03	0,06	0,04
D mean	2,01	2,00	2,00	2,05	0,80	0,66
SD	0,01	0,01	0,01	0,06	0,07	0,03

Table 1: Ca/P ratios and Na & Mg (Wt%) (mean and stdev) for each group.

References

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