COMPOSITION AND MINERAL STRUCTURE OF CALLUS TISSUE DURING FRACTURE HEALING

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Introduction

Callus formation is critical for successful fracture healing. Bone morphogenetic proteins (BMPs) alone or combined with bisphosphonates [Bosemark, 2013] can be valuable in treatment of fracture non-unions.

Fourier transform infrared (FTIR) microspectroscopy and small angle X-ray scattering (SAXS) can be used to evaluate the composition and mineral structure of bone [Camacho, 1999]. However, the composition and mineral structure of the callus tissue during fracture healing has received very little attention [Yang, 2007].

The aims were to analyse the feasibility of FTIR and SAXS for evaluating the composition and mineral structure of callus tissue and to study these differences between fracture callus and cortex, and intact (control) cortex.

Methods

Right femurs of 12 male Sprague-Dawley rats (9 weeks old) were osteotomized and fixed with an intramedullary 1.1 mm K-wire. BMP-7 was placed locally around the fracture and zoledronate (ZO) or saline (NaCl) was injected after 2 weeks (4 groups: NaCl, BMP-7+NaCl, BMP-7+ZO, ZO). Rats were sacrificed after 6 weeks and both femurs were harvested, dehydrated and embedded in PMMA.

SAXS: 300 µm sections were sawed and mapped (Fig 1) with 200 µm step size using I911-4 beamline at Maxlab [Labrador, 2013]. From 2-D SAXS intensity pattern the mineral plate thickness (T), orientation of the mineral crystals and degree of orientation (DoO) were calculated [Fratzl, 1996].

FTIR: 3 µm thick sections were cut and mapped (Fig 1). Mineral/matrix (M/M, *i.e.* mineralization) and carbonate/phosphate (C/P, *i.e.* carbonate substitution) ratios were calculated from the linearly baseline corrected IR absorbance peaks [Boskey, 2007]. Collagen maturity (XLR) [Paschalis, 2001] and crystallinity [Pleshko, 1991] were evaluated through second derivative peak fitting.

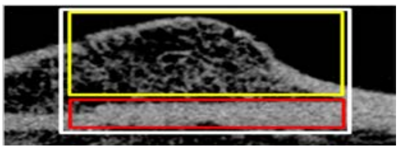

Figure 1. Typical measurement areas (white, 20-30 mm^2) containing callus (yellow) and cortex (red).

Results

Results on pooled SAXS and FTIR data is presented in Table 1. M/M and XLR were lower in fracture calluses than in cortices. Crystallinity was higher in fracture callus and cortex than in control cortex. No differences were observed in C/P or T between different locations (means 0.01 and 2.14, respectively). Orientation was higher in calluses compared to cortices. DoO was higher in control cortex than in fracture callus and cortex.

Parameter	Control cortex	FX cortex	Callus
M/M	6.7±0.8†	6.4±0.5##	5.1±1.0
XLR	2.4±0.1††	2.4±0.1#	2.2±0.1
Crystallinity	0.21±0.08**††	0.37±0.16	0.39±0.16
Orientation	184±6†	182±7#	214±24
DoO	0.65±0.01*†	0.60±0.03	0.57±0.02

*Table 1: Composition and mineral structure in control cortex, fracture (FX) cortex and callus (mean±SD). ** $p<0.01$, * $p<0.05$ Wilcoxon signed rank test, * between control and FX cortex, † between control cortex and callus, # between FX cortex and callus.*

Discussion

Significant differences were found in composition and mineral structure between the fracture callus and cortex, and control cortex. The results support the general idea of new bone formation and indicate the feasibility of FTIR and SAXS to evaluate the composition and mineral structure of bone fracture callus.

References

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