QUANTITATIVE ASSESSMENT OF GROWTH FACTORS IN REAMING ASPIRATE, ILIAC CREST, PRP AND PPP

Schmidmaier G. 1, Herrmann S. 1, Green J. 2, Weber T. 3, Haas N.P. 1 and Wildemann B. 1
1Center for Musculoskeletal Surgery, Charité-Universitätsmedizin Berlin, Free and Humboldt-University, Germany
2Synthes, USA, 3Methodist Hospital, Indianapolis, USA
Gerhard.Schmidmaier@charite.de

Introduction
Large bony defects and non-unions are still a feared complication in trauma and orthopaedic surgery. Treatment strategies include the use of autogenous materials (iliac crest), allogeneic bone, bone substitutes, and currently stimulation with growth factors such as BMP-2, BMP-7 or platelet rich plasma (PRP). Another source of bone graft material, with high osteoinductive potency, might be the cuttings produced during intramedullary reaming. The aim of this study was to compare the quantity of various growth factors found within iliac crest, reaming aspirate, reaming irrigation fluid, platelet rich and platelet poor plasma.

Material and Methods
Seven patients, five male and two female (average age 41.1±13.1 years) donated reaming debris and 30 ml of irrigate fluid during bone grafting procedures utilizing the Reamer Irrigator Aspirator (RIA)-system (Synthes, USA). Briefly, a 13 mm cannulated drill bit was utilized to open the entry site after which a 2.5 mm ball tip guide rod was advanced down the canal of the femur. The RIA-system was then prepared to harvest bone graft (Figure 1). This included attaching a 3000 or 5000 cc saline bag to the irrigation port, suction to the aspiration port and a screen trap in line with the suction tubing. The screen trap (Biomet, USA) has a pore size of approximately 500 µm. The femur was then reamed with an appropriate size reamer head. The harvested femur received 2–4 passes of the RIA.

Age and sex matched iliac crest samples were obtained from seven other patients. Volunteers (n=8, age and sex matched) of the Center for Musculoskeletal Surgery, Berlin, Germany donated 60 ml of blood drawn into a syringe with EDTA-K+. Samples were gently agitated to mix the anticoagulant with the blood.

Sample preparation
Reaming debris and iliac crest were harvested during surgery (Methodist Hospital, Indianapolis, USA) and stored in PBS plus Proteinase inhibitor (Complete, Roche, Germany) at –80°C until examined. The iliac crest samples were pulverized using a cooled mill (Retsch, Haan, Germany). The reaming aspirate was homogenized with an ultra turrax (IKALabortechnik, Germany). The homogenized bony material was then diluted for 2 hours in PBS plus Proteinase inhibitor at 4°C. After extraction the samples were centrifuged at 6000 rpm and the supernatant was stored at –80°C.
Platelet rich plasma was prepared using the Gravitational Platelet Separation System (GPS) in accordance with the instruction of the manufacturer (Biomet Merck Biomaterials, Germany). Sixty cc of blood was injected into the GPS disposable unit, centrifuged for 12 minutes at 3200 rpm (GPS-System, Thermo IEC, Needham Heights, USA), the PRP and platelet poor plasma (PPP) were then collected and stored at –80°C until analyzed.

Protein and growth factor quantification
To quantify the total protein concentration a Coomassie Plus Protein Assay was used. IGF-I, TGF-β1, BMP-2, BMP-4, PDGFbb, FGFα, FGFβ and VEGF concentration in the extracts was quantified using human ELISA-methods (R&D-Systems, Germany). The analyses were then performed as instructed by the manufacturer.

Results
Comparison of iliac crest and bony reaming aspirate
BMP-4 was not detectable in iliac crest or RIA specimens. The quantity of five of seven growth factors (FGFa, PDGFβb, IGF-1, BMP-2 and TGF-β1) were found to be in higher concentrations in samples obtained from intramedullary reaming aspirate than those from the iliac crest. FGFα was 2.1 times more abundant in the reaming aspirate when compared to iliac crest. PDGFβb was 2.9 fold higher, IGF-I 1.6 fold, TGF-β1 3.5 fold and BMP-2 three times higher in the reamer cuttings than in the curettages from to iliac crest. The quantity of FGFβ (0.5 fold) and VEGF (0.5 fold) was significantly higher in the iliac crest compared to the reaming debris.

Comparison of PRP, PPP and reaming irrigation fluid
BMP-4 was also not measurable in the liquid samples.
Comparing platelet poor plasma and platelet rich plasma an enrichment of growth factors was detectable in the PRP. VEGF was significantly increased by 52.7 fold and showed the highest degree of enrichment which was followed by PDGFβb (23 fold, significant), TGF-β1 (14 fold, significant) and BMP-2 (2 fold). The level of IGF-1 was comparable between the PRP and PPP. FGFα was not measurable in the PRP or PPP. FGFβ could not be detected in PPP but was found in PRP. The analysis of the reaming irrigation fluid revealed that FGFα was abundant. A significantly higher level of FGFβ (8.3 fold increase) was measurable in the RIA suction irrigate compared to PRP. A significantly lower amount of VEGF (0.1 fold) and PDGF (0.1 fold) were present in the irrigation liquid compared to PRP. The differences for IGF-I (0.4 fold), TGF-β1 (0.6 fold) and BMP-2 (0.3 fold) were not significant.

Discussion
The present study quantified the growth factors FGFα, FGFβ, PDGF, VEGF, TGF-β1, IGF-1, BMP-2 and BMP-4 in iliac crest, reaming aspirate, reaming irrigation fluid, PRP and PPP. Comparison of growth factor content in the bony material revealed elevated levels of FGFα, PDGF, IGF-1, BMP-2 and TGF-β1 in the reaming aspirate when compared to the iliac crest. BMP-4 was not detectable in any sample. Two (FGFβ, VEGF) of seven growth factors were in higher concentrations in the iliac crest compared to bony reaming aspirate. Comparing the reaming irrigation and the PRP, two of seven growth factors were higher in the irrigation fluid (FGFα, FGFβ). These results demonstrate the high potency of reaming aspirate and irrigation fluid in terms of growth factor content. Several studies have shown enhanced bony healing after reamed nailing compared to unreamed nailing {Larsen, Madsen, et al. 2004, Court-Brown CM, Will, et al. 1996, Bhandari, Guyatt, et al. 2000}. This might be explained by the existence of viable cells with osteoblastic potential in the reaming aspirate {Frolke, Nulend, et al. 2004, Hoegel, Mueller, et al. 2004, Wenisch, Trinkaus, et al. 2005}. The osteoinductivity could also be explained by growth factors such as those found in the reaming aspirate as was demonstrated in the present study.
This study also demonstrated an growth factor enrichment in the PRP compared to PPP. Epperly et al. used the same enrichment system and found comparable results without differences in the IGF-1 content {Eppley, Woodell, et al. 2004}. The RIA systems is now being used in a prospective clinical trial for bone graft harvesting, an application for which it is FDA approved. The treated patients have large bony defects and some have had several prior surgeries for grafting. Anecdotally, patients treated previously with iliac crest grafts report lower pain and less discomfort after the acquired IM graft. At this time, no complications have occurred referent to the RIA technique. However, further results are necessary.