Repair of segmental long-bone defects by stem cell concentrate augmented scaffolds: a clinical and positron emission tomography - computed tomography analysis

Maximilian Petri · Ali Namazian · Florian Wilke · Max Ettinger · Timo Stübiger ·
Stephan Brand · Frank Bengel · Christian Krettek · Georg Berding · Michael Jagodzinski

Received: 1 June 2013 / Accepted: 14 August 2013 / Published online: 8 September 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract

Purpose Treating segmental long-bone defects remains a major challenge. For defects >3 cm, segmental transport represents the gold standard, even though the method is time consuming and afflicted with several complications. The aim of this study was to evaluate healing of such defects after grafting an osteogenic scaffold previously seeded with stem cell concentrate.

Methods We evaluated five patients with segmental long-bone defects (3–14 cm) treated with bone marrow aspirate concentrates (BMAC) seeded onto a bovine xenogenous scaffold. The healing process was monitored by X-rays and positron emission tomography–computed tomography (PET-CT) three months after surgery.

Results Centrifugation led to a concentration of leukocytes by factor 8.1 ± 7.5 . Full weight bearing was achieved 11.3 ± 5.0 weeks after surgery. PET analysis showed an increased influx of fluoride by factor 8.3 ± 6.4 compared with the contralateral side ($p < 0.01$). Bone density in the cortical area was 75 ± 16 % of the contralateral side ($p < 0.03$). The patient with the largest defect sustained an implant failure in the distal

femur and finally accomplished therapy by segmental transport. He also had the lowest uptake of fluoride of the patient collective (2.2-fold increase).

Conclusion Stem cell concentrates can be an alternative to segmental bone transport. Further studies are needed to compare this method with autologous bone grafting and segmental transport.

Keywords Segmental bone defect · Scaffold · Mesenchymal stem cells (MSC) · Stem cell concentrate

Introduction

Treating segmental long-bone defects remains a major challenge in orthopaedic and trauma surgery. Defects can be caused by tumor resection, osteolyses, pseudarthroses and open fractures and infections. Smaller defects, up to 3 cm, are usually treated using autologous bone grafting. Major limitations of this method are donor-site morbidity, limited resources and decreased healing potential in elderly patients [1]. For defects >3 cm, segmental transport represents the gold standard. However, the method is time consuming and afflicted with several complications, such as pin-track infections, pseudarthrosis etc. Novel methods of cell-based therapy are promising in such treatment. Various biological proteins, such as platelet-derived growth factor (PDGF), platelet-rich plasma (PRP) and bone morphogenetic proteins (BMP), seem helpful in restoring critical-size bone defects [1–13]. Stem cell concentrates, such as bone marrow aspirate concentrate (BMAC®, Harvest Technologies Inc.) represent a biological single-step approach to this problem. Human bone marrow mesenchymal stem cells are harvested from the iliac crest, concentrated in the operating theatre and seeded onto an osteogenic scaffold, which is finally implanted into the defect.

M. Petri (✉) · T. Stübiger · S. Brand · C. Krettek · M. Jagodzinski
Trauma Department, Hannover Medical School (MHH),
Carl-Neuberg-Straße 1, 30625 Hannover, Germany
e-mail: petri.maximilian@mh-hannover.de

A. Namazian · F. Bengel · G. Berding
Department of Nuclear Medicine, Hannover Medical School (MHH),
Carl-Neuberg-Straße 1, 30625 Hannover, Germany

F. Wilke
Department of Radiation Protection and Medical Physics, Hannover
Medical School (MHH), Carl-Neuberg-Straße 1, 30625 Hannover,
Germany

M. Ettinger
Orthopaedic Surgery Department, Hannover Medical School
(MHH), Anna-von-Borries-Str. 1-7, 30625 Hannover, Germany

The purpose of this study was to evaluate clinical and positron emission tomography–computed tomography (PET-CT) outcomes of repairs of segmental long-bone defects by grafting an osteogenic scaffold previously seeded with stem cell concentrate. The hypothesis was that BMAC scaffolds induce new bone formation and enable patients to reach full weight bearing earlier than patients with segmental transports.

Fig. 1 Defect after a multifragmentary grade IIIB open fracture of the proximal tibia two years after reconstruction (X-ray)

Fig. 2 Defect after a multifragmentary grade IIIB open fracture of the proximal tibia three months after reconstruction (positron emission tomography–computed tomography)

Patients and methods

Five patients with segmental defects of long bones (6.6 ± 4.4 cm; 3–14 cm) treated between 2009 and 2010 were included in the study and followed for 24 months. The study

Fig. 3 Defect after a grade I open fracture of the proximal femur three months after reconstruction (positron emission tomography–computed tomography)

Fig. 4 Defect after an infected grade IIIB open fracture of the tibia two years after reconstruction (X-ray)

was performed in accordance with the Declaration of Helsinki, and the local Ethical Committee was notified for each patient, all of whom provided informed consent. All were

Fig. 6 Defect after an infected grade II open fracture of the tibia two years after reconstruction (X-ray)

male patients between 13 and 65 (mean 37.8) years of age. Bone defects resulted from a multifragmentary grade IIIB open fracture of the proximal tibia (Figs. 1 and 2), a grade I

Fig. 5 Defect after an infected grade IIIB open fracture of the tibia three months after reconstruction (positron emission tomography–computed tomography)

Fig. 7 Defect after an infected grade II open fracture of the tibia three months after reconstruction (positron emission tomography–computed tomography)

open fracture of the proximal femur (Fig. 3), an infected grade IIIB open fracture of the tibia (Figs. 4 and 5), an infected grade II open fracture of the tibia (Figs. 6 and 7), and a resection of a G3 osteosarcoma of the distal femur (Figs. 8 and 9).

In a single-stage procedure, bone marrow aspirates were processed according to the protocol provided by the manufacturer (Harvest Technologies, Munich, Germany). Briefly, a Jamshidi aspiration syringe was positioned in the middle of the anterior iliac crest and advanced into the intramedullary cavity. For each 3 cc of bone defect, 60 ml of aspirate was transferred into a bag that contained 4 ml of anticoagulant citrate dextrose solution (ACDA). The combined solution was filtered and transferred into a process disposable. The disposable was centrifuged for 15 minutes at 1,200 g in a cell separator. After processing, the plasma was withdrawn, the bone marrow concentrate was resuspended with the remaining plasma and 3 ml of concentrate were obtained; 1 ml was used for analysis of the cell concentration and phenotype, and the

remaining concentrate was transferred into the sterile field and mixed with the bone substitute (Orthoss, Geistlich Pharma AG, Wollhusen, Switzerland). The concentration of stem cells was controlled by fluorescence-activated cell shortening (FACS) analysis, analysing expression of CD13, 15, 29, 34, 44, 45, 73, 90, 105, 106, 146, 166 and 271, cell surface glycosphingolipids SSEA-4 and 3, and anti-human STRO-1 antibody (Fig. 10).

The concentrates were seeded onto a xenogenous bovine scaffold (Chronos®, Geistlich Pharma AG) and implanted into the bone defect. Clinical follow-up emphasised time until pain-free full weight bearing. The healing process was monitored by conventional X-rays every six weeks until bony consolidation. A dynamic [^{18}F] fluorodeoxyglucose PET/CT scan (Siemens, Biograph) was obtained three months after surgery. Blood flow, fluoride uptake, standardised uptake values (SUV) and bone density measured in Hounsfield units (HU) were analysed. Contralateral long bones served as a reference. Additionally, analogous PET data from 11 patients six months after receiving a revascularised fibular transplant served as the control group.

Statistical analysis

Statistical analysis was evaluated between the different groups using Mann–Whitney *U* test and Student's two-tailed *t* test for continuous data. For categorical values, Fischer's exact test and Pearson's chi-square test were used. The SPSS (SPSS 15.0,

Fig. 8 Defect after resection of a G3 osteosarcoma of the distal femur immediately after reconstruction (X-ray)

Fig. 9 Defect after resection of a G3 osteosarcoma of the distal femur three months after reconstruction (positron emission tomography–computed tomography)

Fig. 10 Fluorescence-activated cell sorting (FACS) analysing expression of CD13, 15, 29, 34, 44, 45, 73, 90, 105, 106, 146, 166 and 271, cell surface glycosphingolipids SSEA-4 and 3, and anti-human STRO-1 antibody. The y-axis represents the percentage of cells that demonstrated the individual antigen. Sample 5 was an aspirate taken

from the 14-year-old patient with a malignant tumor after receiving polychemotherapy. His bone marrow showed a different distribution of cells, with more haematopoietic cells (CD34+, CD45+) than the other patients. This may have contributed to the low amount of fluoride uptake and to graft failure

SPSS Inc., Chicago, IL, USA) software package was used. A p value < 0.05 was considered to be statistically significant.

Results

Full weight bearing was achieved 11.3 ± 5.0 weeks after surgery and was maintained up to 24 months of follow-up in four patients. Centrifugation led to a concentration of leukocytes by factor 8.1 ± 7.5 . Mesenchymal stem cell (MSC) count was $62.6 \pm 46.2 \times 10^3$. Results of the FACS analysis of various clusters of differentiation (CD) markers are shown in Fig. 10. Results of PET-CT analysis for blood flow, fluoride influx, (SUV, and HU are shown in Table 1. Bone density in the cortical area was 75 ± 16 % of the contralateral side ($p < 0.03$). Radiologically, the defects had healed in the follow-up in four of five patients (Figs. 1–7).

The patient with the largest defect sustained an implant failure in the distal femur and finally accomplished therapy by segmental transport. He also had the lowest fluoride uptake (2.2-fold increase) and the lowest SUV (7.9 vs. >10 in all others) (Figs. 8 and 9).

Discussion

Treating segmental long-bone defects remains a major challenge. For defects >3 cm, segmental transport represents the gold standard, even though the method is time consuming and afflicted with several complications. Most established methods of regenerative medicine require ex vivo cultivation of stem cells and therefore carry several risks, such as infection, high laboratory costs and the need for at least two operations [14]. As an innovative method of cell-based therapy, using BMAC allows intraoperative enrichment and application of mesenchymal and progenitor stem cells as a single-stage procedure. The first promising clinical results from treatment with BMAC were reported in 2009 by Hendrich et al. [14] and Jäger et al. [15], emphasising the safety of the intraoperative harvest and induction of osteogenic differentiation without further external stimuli. Jäger et al. [1] investigated the clinical follow-up of a heterogeneous patient collective treated with BMAC seeded on either a collagen sponge or bovine hydroxyapatite granula (Orthoss®, Fa Geistlich, Wolhusen, Switzerland). These hydroxyapatite granules are comparable with the Chronos® product used in our study.

Table 1 Results of positron emission tomography–computed tomography (PET-CT) analysis for blood flow, fluoride influx, standardised uptake values and Hounsfield units

	BMAC group	Healthy contralateral bone	Fibular transplant group	P value
Blood flow (ml/min)	0.097 ± 0.052	0.104 ± 0.128	0.082 ± 0.039	Not significant
Fluoride influx (ml/min)	0.089 ± 0.056	0.018 ± 0.010	0.028 ± 0.015	0.0028 (BMAC vs. HCB), 0.0068 (BMAC vs. FTG)
Standardised uptake values	16.5	2.9		0.0001
Hounsfield units	1,232	1,730		0.0002

BMAC bone marrow aspirate concentrates; HCB healthy contralateral bone; FTG fibular transplant group

Those authors found earlier bone formation and healing in the hydroxyapatite group compared with the collagen-sponge group. Both BMAC groups revealed better results than patients treated with bone marrow aspirates without concentration. Gessmann et al. [16] investigated the effect of percutaneous application of BMAC into the regenerated bone section in eight patients with tibial bone defects who underwent segmental transport with a modified Ilizarov external fixation device. They found sufficient bone healing and no adverse events in all patients at a mean follow-up of ten months [16].

As an alternative to segmental transport in long bones, fibula grafts can be used as for bone defects of, for example, the mandible. Schliephake et al. investigated revascularised fibula grafts for mandibular reconstruction by blood flow and fluoride influx by PET [17]. Those authors found a low osteogenic potential and a high rate of nonunion and recommended special care when adapting the graft and the mandible [17]. Guda et al. [18] investigated bone regeneration after filling a long-bone defect in a rabbit model using a hydroxyapatite structural graft covered with a permeable collagen membrane. They found increased bone volume, bone density, bone ingrowth and periosteal remodelling using the membrane, concluding that the membrane may act as a scaffold that fosters early interfacial integration [18]. Reichert et al. [19] used bone defects in a sheep model to compare autografts with biodegradable polycaprolactone and tricalcium phosphate composite scaffolds combined with either autologous bone-marrow-derived MSCs or recombinant human bone morphogenetic protein 7 (rhBMP-7). They found greater bone formation and better biomechanical properties in the scaffolds loaded with rhBMP-7 compared with the autografts. Plain scaffolds and scaffolds loaded with MSC provided less bone formation than the autograft and rhBMP-7 group [19]. Goshima et al. [20] performed a study on segmental bone defect repair in tibial shafts of rabbits using β -tricalcium phosphate (β -TCP) scaffolds loaded with hepatocyte growth factor (HGF). They found that HGF-loaded scaffolds promoted mineralisation, angiogenesis, new bone formation and β -TCP absorption [20]. Sauerbier et al. [21] and Rickert et al. [22] performed randomised controlled clinical trials to investigate formation of new bone in patients with severe atrophy of the maxillary sinus. Patients obtained an augmentation of the sinus from bovine bone material either with BMAC or autologous bone. Those authors found comparable formation of new bone in both groups three to four months after surgery. Zhong et al. [23] investigated immunodeficient mice concerning bone augmentation using β -TCP augmented with either platelet-rich plasma (PRP) or BMAC. Compared with grafting the β -TCP-scaffold alone, both PRP and BMAC increased the number of new bone cells. Those authors found no difference between PRP and BMAC augmentation of scaffolds. With a special emphasis on the problem of bone defects in osteoporotic patients, Cao et al. [24] investigated

osteoporotic bone-defect repair in long-term oestrogen-deficient goats by using porous β -TCP scaffolds loaded with autologous, enriched bone marrow MSCs and found improved bone formation following the enriched MSC therapy [24]. Severe bone loss is also a major problem in revision joint replacement. Buttaro et al. [25] investigated a combination of struts and impacted bone allografts to treat patients with loosening of the hip arthroplasty. Those authors found satisfying survival rates of hip revision and a low incidence of periprosthetic fractures using that method [25].

To our knowledge, this is the first study focussing on critical-size long-bone defects treated with the BMAC method and the first study applying the PET-CT method for surveying the regenerate viability. We found that treating critical-size long-bone defects with stem cell concentrates can yield good results both clinically and radiologically, attaining early full weight bearing and bone healing with sufficiently high bone density.

Conclusion

Stem cell concentrates can be an alternative to segmental bone transport for segmental long-bone defects >3 cm in case of good vascularisation of the implantation site. Measured results for fluoride uptake were comparably high compared with in vitro cultivated constructs and were higher than the results of vascularised allografts. Further studies are needed to define the position of this method compared with autologous bone grafting and segmental transport. For defects >7 cm and for poorly vascularised implant sites, segmental transport remains the gold standard. A multicentric prospective randomised controlled clinical trial is desirable in the future.

Acknowledgments The authors thank Mrs. Luisa Schäck and Prof. Dr. Andrea Hoffmann from the Laboratory for Biology of the Musculoskeletal System, Trauma Department, Hannover Medical School, for their help with FACS analysis.

Conflict of interest None.

References

1. Jager M, Herten M, Fochtmann U et al (2011) Bridging the gap: bone marrow aspiration concentrate reduces autologous bone grafting in osseous defects. *J Orthop Res* 29(2):173–180
2. Al-Zube L, Breitbart EA, O'Connor JP et al (2009) Recombinant human platelet-derived growth factor BB (rhPDGF-BB) and beta-tricalcium phosphate/collagen matrix enhance fracture healing in a diabetic rat model. *J Orthop Res* 27(8):1074–1081
3. Bessa PC, Casal M, Reis RL (2008) Bone morphogenetic proteins in tissue engineering: the road from laboratory to clinic, part II (BMP delivery). *J Tissue Eng Regen Med* 2(2–3):81–96

4. Bessa PC, Casal M, Reis RL (2008) Bone morphogenetic proteins in tissue engineering: the road from the laboratory to the clinic, part I (basic concepts). *J Tissue Eng Regen Med* 2(1):1–13
5. Ghodadra N, Singh K (2008) Recombinant human bone morphogenetic protein-2 in the treatment of bone fractures. *Biogeosciences* 2(3):345–354
6. Giannoudis PV, Tzioupis C (2005) Clinical applications of BMP-7: the UK perspective. *Injury* 36(Suppl 3):S47–S50
7. Dimitriou R, Dahabreh Z, Katsoulis E et al (2005) Application of recombinant BMP-7 on persistent upper and lower limb non-unions. *Injury* 36(Suppl 4):S51–S59
8. Graham S, Leonidou A, Lester M et al (2009) Investigating the role of PDGF as a potential drug therapy in bone formation and fracture healing. *Expert Opin Investig Drugs* 18(11):1633–1654
9. Hollinger JO, Hart CE, Hirsch SN et al (2008) Recombinant human platelet-derived growth factor: biology and clinical applications. *J Bone Joint Surg Am* 90(Suppl 1):48–54
10. Hollinger JO, Onikepe AO, MacKrell J et al (2008) Accelerated fracture healing in the geriatric, osteoporotic rat with recombinant human platelet-derived growth factor-BB and an injectable beta-tricalcium phosphate/collagen matrix. *J Orthop Res* 26(1):83–90
11. Kasten P, Vogel J, Geiger F et al (2008) The effect of platelet-rich plasma on healing in critical-size long-bone defects. *Biomaterials* 29(29):3983–3992
12. Lissenberg-Thunnissen SN, de Gorter DJ, Sier CF, Schipper IB (2011) Use and efficacy of bone morphogenetic proteins in fracture healing. *Int Orthop* 35(9):1271–1280
13. Bishop GB, Einhorn TA (2007) Current and future clinical applications of bone morphogenetic proteins in orthopaedic trauma surgery. *Int Orthop* 31(6):721–727
14. Hendrich C, Franz E, Waertel G et al (2009) Safety of autologous bone marrow aspiration concentrate transplantation: initial experiences in 101 patients. *Orthop Rev (Pavia)* 1(2):e32
15. Jager M, Jelinek EM, Wess KM et al (2009) Bone marrow concentrate: a novel strategy for bone defect treatment. *Curr Stem Cell Res Ther* 4(1):34–43
16. Gessmann J, Köller M, Godry H et al (2012) Regenerate augmentation with bone marrow concentrate after traumatic bone loss. *Orthop Rev (Pavia)* 4(1):e14
17. Schliephake H, Berding G, Knapp WH, Sewilam S (1999) Monitoring of graft perfusion and osteoblast activity in revascularised fibula segments using [18F]-positron emission tomography. *Int J Oral Maxillofac Surg* 28(5):349–355
18. Guda T, Walker JA, Singleton BM et al (2013) Guided bone regeneration in long-bone defects with a structural hydroxyapatite graft and collagen membrane. *Tissue Eng Part A* 19(17–18):1879–1888
19. Reichert JC, Cipitria A, Epari DR et al (2012) A tissue engineering solution for segmental defect regeneration in load-bearing long bones. *Sci Transl Med* 4(141):141ra93
20. Goshima K, Nakase J, Xu Q et al (2012) Repair of segmental bone defects in rabbit tibia promoted by a complex of beta-tricalcium phosphate and hepatocyte growth factor. *J Orthop Sci* 17(5):639–648
21. Sauerbier S, Rickert D, Gutwald R et al (2011) Bone marrow concentrate and bovine bone mineral for sinus floor augmentation: a controlled, randomized, single-blinded clinical and histological trial-per-protocol analysis. *Tissue Eng Part A* 17(17–18):2187–2197
22. Rickert D, Sauerbier S, Nagursky H et al (2011) Maxillary sinus floor elevation with bovine bone mineral combined with either autogenous bone or autogenous stem cells: a prospective randomized clinical trial. *Clin Oral Implants Res* 22(3):251–258
23. Zhong W, Sumita Y, Ohba S et al (2012) In vivo comparison of the bone regeneration capability of human bone marrow concentrates vs. platelet-rich plasma. *PLoS One* 7(7):e40833
24. Cao L, Liu G, Gan Y et al (2012) The use of autologous enriched bone marrow MSCs to enhance osteoporotic bone defect repair in long-term estrogen deficient goats. *Biomaterials* 33(20):5076–5084
25. Buttaro MA, Constantini J, Comba F, Piccaluga F (2012) The use of femoral struts and impacted cancellous bone allograft in patients with severe femoral bone loss who undergo revision total hip replacement: a three- to nine-year follow-up. *J Bone Joint Surg Br* 94(2):167–172