

# Bone Marrow Concentrate: A Novel Strategy for Bone Defect Treatment

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**Abstract:** *Background:* Although strong efforts have been made over the last decade to introduce stem cell and tissue engineering treatment strategies to the field of orthopaedics, only few clinical applications are currently available.

*Materials and Methods:* The clinical outcomes of ten patients with volumetric bone deficiencies treated with mesenchymal stem cells and bone marrow aspirate are presented in this case series. Results were evaluated with radiographs. In addition to the *in vivo* data, we also presented *in vitro* data of BMC cultivated onto a porous collagen I scaffold and the technique of bone marrow aspiration *via* a commercially available system.

*Results:* Our results demonstrated that there is a rationale for a clinical application of BMC / bone aspirate in the treatment of osseous defects. The intraoperative harvest procedure is a safe method and does not significantly prolong the time of surgery. In addition, MSC isolated from the aspirate was able to adhere and proliferate onto a collagen scaffold in significant numbers after a 15 min incubation period. These cells were then able to allow osteogenic differentiation *in vitro* without any osteogenic stimuli.

*Conclusions:* The local application of BMC / bone aspirate in the treatment of bone deficiencies may be a promising alternative to autogenous bone grafting and help reduce donor site morbidity.

**Keywords:** Bone marrow, mesenchymal stem cell, bone defect, osteoblast.

## INTRODUCTION

There is considerable evidence that the human bone marrow supplies the body with not only hematopoietic but also a significant number of mesenchymal stem cells (MSCs) [1]. However controversy exists in the literature in regards to the definition of "Mesenchymal Stem Cells" and scientists have utilized several markers to help identify them (Table 1a) [2-6]. More recently, tissue engineering and cell based therapy have provided clinicians with alternative ways of harvesting autologous bone marrow concentrates to treat local bone defects [7-10]. However, there are currently very few clinical publications / trials reporting the use of MSC from the bone marrow harvest for treating bone deficiencies.

There are several advantages in utilizing MSC and autologous bone marrow aspiration to treat bone defects. From a basic science perspective, osteogenic differentiation from MSCs has several advantages when compared to the differentiation of other mesenchyme tissues. First of all, osteoblast differentiation from MSCs is very well described and standardized in many protocols. Secondly, the MSCs harvested from the bone marrow aspirate are able to differentiate spontaneously into osteoblasts *in vitro*. Furthermore, several biomaterials such as collagen I, tricalciumphosphate (TCP) or hydroxyapatite (HA) are currently available clinically as bone substitutes and can be used as scaffolds in combination with the MSCs and aspirate to expedite bone healing. These scaffolds may be used in combination with

the harvested MSC to facilitate healing. Clinically, the harvesting process of bone marrow cells *via* a Jamshidi vacuum aspiration can help to reduce donor-associated co morbidity and currently, there are no reported incidences of malignant transformation of autologous bone marrow cells in the literature. Lastly, a newly formed bone does not require the complex cytoarchitecture compared to other tissues like cartilage, thus making bone marrow harvesting / MSC a feasible alternative in the treatment of bone defects.

From a surgeon's perspective, there are several advantages to a one step clinical application of bone marrow concentrate. An immediate autologous transplantation of bone marrow concentrate can prevent complications related to the reduced quality of the transplanted cells such as pre-aging (telomere shortening), reduced viability, or dedifferentiation / reprogramming that is associated with *in vitro*-cultivation [11]. In addition, the risk for infection is reduced by decreasing the *ex vivo* time period. Our study reports a case series on the clinical outcomes after autologous MSC in combination with bone marrow aspirate transplantation utilizing a commercially available system in treating volumetric bone deficiencies.

## Clinical Results After Cell Transplantation for Bone Regeneration

There is a wide discrepancy in the literature when it comes to the number of publications dealing with basic science research versus clinical research involving mesenchymal stem cells (MSC) and osteoblast cells. Based on a Medline database review in September 2008, [http://www.pubmed.de] we found 847 articles by using the key words "mesenchymal", "stem cell", and "osteoblast", however, when the word "patient" was added, only 27 papers were found [12-

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**Table 1. a) Consent Common Biomarkers and Characteristics of Mesenchymal Stem Cells Considering Recent Data from Scientific Literature. b) Patients who Underwent Local Autologous Cell Therapy by Bone Marrow Concentrate**

a)

Biological Competence	Positive Markers	Negative Markers
ability to adhere on plastic <i>in vitro</i>	CD44+	
ability to form colony forming fibroblasts (CFU)	CD73+	CD34-
ability to differentiate into mesenchyme lineages (adipocytes, chondrocytes, osteocytes, myocytes, tenocytes)	CD90+ CD105+	CD45- CD133-
promotion of hematopoiesis	CD166+	
self renewal potential	HLA-ABC	
high proliferation potency		
suppressing alloresponse after transplantation		

b)

ID	Age at Surgery	Sex	Affected Area	Diagnosis	Defect Size [cm <sup>3</sup> ]
1	64	f	proximal femur	aseptic loosening of a cemented stem	10.0
2	39	f	ilium	pseudarthrosis after pelvic osteotomy	1.8
3	20	f	ilium	pseudarthrosis after pelvic osteotomy	1.3
4	29	f	proximal femur	enchondroma	17.4
5	10	m	humerus	bone cyst	13.0
6	22	f	phalanx D II	enchondroma	0.5
7	4	m	proximal femur	bone cyst	16.7
8	45	m	ilium	bone cysts	69.4
9	15	f	proximal fibula	bone cysts	52.9
10	27	m	proximal femur	bone cyst	24.8

38]. Excluding the articles in Chinese and Japanese, none of the remaining 25 papers in English presented any clinical data showing that MSC application influences bone healing. When using the key words “bone marrow”, “concentrate”, “osteoblast”, and “mesenchymal stem cell”, no items were found in the Medline database. Furthermore, the phrases, “bone marrow”, “concentrate”, and “osteoblast” resulted only in two articles, both of which were *in vitro* studies [39,40]. Although using the terms “mesenchymal”, “stem cell”, “bone”, “healing”, and “patient” presented with 11 hits in *pubmed*, [14,27,41-49] only one of these articles (case report) reported about successful bone healing *via* MSC derived from the periosteum in an atrophic femoral non-union [44]. All of the other studies were either *in vitro* [49], review of the literature [41-43,45,46], or research dealing with non-osseous tissue defects such as diabetic ulcer [48] and skin defects [43, 47].

Our extensive literature search yield only two studies that present preliminary clinical data showing successful healing of local bone defects by bone marrow derived MSC. Kitho *et al.* [27] showed that cultivated MSC from the posterior iliac crest combined with platelet-rich plasma (PRP) facilitated bone healing in two patients (3 femora) with achondroplasia and one patient with congenital pseudarthrosis of the tibia (CPT). These results correspond to Cadedda *et al.* [14]

who showed osseous healing of long bone defects by MSCs in combination with a HA carrier that was stabilized with an external fixator.

Positive effects of MSC could also be demonstrated for spinal fusions. Here, Gan *et al.* [50] used enriched bone-marrow-derived MSC combined with porous beta-tricalcium phosphate (beta-TCP) for spinal fusion in 41 patients. A solid fusion was achieved in more than 95% after 34.5 months and only 4 patients showed prolonged exudation and local swelling. Another indication for MSC application is the treatment of bone cysts [51,52]. It was shown in 18 patients that local injection of autogenous bone marrow cells is a safe and effective treatment method for simple bone cysts, but sometimes repeated injections are necessary [53].

Although Kasten *et al.* [54,55] reported only week osteogenic properties of platelet-rich plasma on calcium-deficient hydroxyapatite (CDHA) and tricalcium phosphate (TCP) scaffolds, they demonstrated that platelet rich plasma is able to stimulate MSC proliferation. However, Kasten *et al.* [56] showed in their experiments on rabbits that boths, platelet-rich plasma and MSC are potent to heal critical-size long-bone defects. The osteogenic potency of platelet rich plasma was also confirmed for the treatment of delayed unions [57].

We can assume that although strong efforts have been made in the scientific world to develop cell therapeutic strategies in bone regeneration, there are still currently no reliable publications / data in the literature supporting its application. Therefore, our research group has initiated a clinical study to investigate the effects of treating volumetric bone defects with local application of MSC derived autologous bone marrow concentrate.

## MATERIAL AND METHODS

### *In Vivo* Study

**Patients and parameters:** We evaluated the handling, clinical and roentgenological effects of a bone marrow concentrating system (BMAC™, Harvest Technologies GmbH) applied to induce and / or stimulate bone formation in patients with pseudarthrosis, bone cysts or revision endoprosthetic bone defects. Human bone marrow aspirates were obtained from the posterior iliac crest of 10 different donors (6 females and 4 men, mean age: 28.4 years, SD: 19.04). All volunteer donors were informed about a healing attempt and had given written consent according to the Declaration of Helsinki in its present form.

The average volume of biliary marrow which was vacuum-aspirated from the posterior iliac crest was 67.5 mL (SD: 21.76). The average volume of the applied bone marrow concentrate (cell suspension) was 8.8 mL (SD: 3.36). Standard x-rays in two planes were made before surgery and after 2, 6 and 12 weeks and after 6 months postoperatively. The mean clinical and roentgenological follow-up was 8.3 month (SD: 4.24). Clinical success was defined by reduced or absence in pain combined with new bone formation at the transplantation site. Newly formed bone within the transplantation area was evaluated by x-rays in two standard planes. Before surgery, the volume of the osseous defect was calculated approximately by x-rays in 2 planes. Table 2b gives an overview about the treated patients. In addition, the ability to return to profession and activities prior to the causative disease was documented.

### *Device Description and Preparation*

This study utilized the SmartPREP2 Bone Marrow Aspirate Concentrate (BMAC™) System (Harvest Technologies GmbH, Munich, Germany); a dedicated microprocessor controlled decanting centrifuge system, designed for and cleared by the FDA and CE-marked for the production of autologous Bone Marrow Aspirate Concentrate (BMAC). The device is a table top centrifuge system and can use up to four dual chamber disposables. The first chamber contains a floating shelf of a specific density. During the initial centrifugation phase the heavy red blood cells (RBC's) are separated from the nucleated cells, platelets and plasma. The cellular elements and plasma are automatically decanted into the second chamber and concentrated by centrifugation. A portion of the supernatant plasma is removed and the cellular elements are resuspended in the remaining plasma. The system can utilize an initial volume as small as 60 – 120 cc's of bone marrow aspirate. This process was accomplished in less than 15 minutes and required a minimum of (non-specialized) operator intervention. As demonstrated by Hermann *et al.* [58] the

bone marrow concentrate isolated by the Harvest System contains identical numbers of myelocytes, granulocytes, lymphocytes, monocytes, proerythroblasts and erythroblasts compared to the initial bone marrow aspirate.

Bone Marrow Aspirate (BMA) was obtained in accordance to manufacture's instructions (Table 2a), by aspiration technique, from the posterior iliac crest using a 6 lumen Jamshidi type trocar needle and 20 mL syringes, pre-flushed with heparin (concentration of 1000 units / mL) (Liquemin, Roche, Grenzach-Wyhlen, Germany). The trocar was initially directed at 30° to the horizontal, parallel to the plane of the crest. It was then inserted to a depth of ~ 5 cm toward the anterior cortex and either 60 mL or 120 mL of BMA was extracted while rotating and slowly withdrawing the needle toward the cortex.

The BMA was then concentrated using the SmartPREP™ 2 Centrifuge System in the operating suite. Between 7 – 10 mL of the Bone Marrow Aspirate Concentrate (BMAC) were obtained per BMAC Process Disposable (PD) and transferred by sterile technique back to the sterile field. The resulting BMAC volume (7 - 20 mL) was then applied to a collagen matrix material (Gelaspon®, description please see below) allowing a cells distribution and adherence throughout the matrix in a uniform manner. All adverse events experienced by the subject during the course of the study were documented. Additionally, BMAC were reserved for laboratory *in vitro* seeding analyses. Table 2a gives an overview about the intraoperative procedure.

### *In Vitro*-Experiments

#### *Scaffold*

A porous collagen I carrier qualified for local hemostasis and tamponade of surgical wounds [59] was used as a bio-carrier for cell transplantation. The matrix is a 3-dimensional, 8.5 x 4 x 1 cm denaturated gelatine sponge (Gelaspon®, Chauvin Ankerpharm, Germany). According to manufacturers information the porcine derived biomaterial consists of more than 80% proteins, has a neutral pH and will be resorbed *in vivo* within 2-3 weeks when applied within the tissue but much faster (in 2-3 days) when used to seal or cover wounds. In addition, it was shown previously that collagen carriers allow for an *in vitro* and *in vivo* osteogenic differentiation from bone marrow derived progenitor cells [60-62].

#### *Cellular Culture*

To show the adherence of mononuclear bone marrow cells onto the collagen scaffolds after an incubation period of 15 minutes, samples of the biomaterial were fixed with formaldehyde. In addition to conventional staining such as H.E., cellular adherence onto the scaffold was evaluated by fluorescence microscopy using DAPI nuclei staining.

In order to evaluate the osteoblast differentiation potential of the bone marrow cells isolated by the centrifugation system, cells of a 20 year-old female donor were incubated onto a collagen scaffold for 15 min at RT and cultivated in DMEM-low glucose medium. One portion of the samples was stimulated with osteogenic mixture (dexamethasone, ascorbic acid, β-glycerolphosphate, DAG) over 28 days

**Table 2.** a) Intraoperative Management and Structured Work-Flow of Autologous Cell Transplantation for the Treatment of Local Osseous Defects. Although the Whole Procedure Lasts About 45 min. There is no Prolonged Operation Time Because the Bone Marrow Concentration Process and, also the Scaffold Seeding Run Parallel to the Surgery by an Additional, Trained Person. Considering this, the Surgeon has Only to Aspirate the Bone Marrow and Implant the Scaffold-Cell-Composit into the Osseous Defect which is Much Faster than to Perform an Autologous Bone Grafting from Pelvis and may Help to Reduce Operating Time. b) Outcome in Patients you Underwent Local Autologous Cell Therapy by Bone Marrow Concentrate. y: yes, n: no

a)

Bone Marrow Aspiration and Processing Protocol		Time Line
Principle	Comment	
Vacuum aspiration of autologous bone marrow	Posterior ilium High vacuum Small volume per donor site Sufficient anticoagulation of the aspirate (e.g. heparin, ACDA; be sure anticoagulant has coated the entire inner surface of the syringe) Total volume approximately 60 – 120 mL	0 min. 
Isolation of bone marrow concentrate by centrifugation	Sterility Application at the OR by trained staff only	20 min. 
Seeding of a scaffold followed by an incubation period to allow for cellular adherence phase	Bioresorbable biomaterials preferred, characterized by ... A high degree of porosity / large surface Low toxicity (e. g. collagen sponges)	30 min 
<i>In vivo</i> implantation	No local drains close to the transplantation area	45 min.

b)

ID	Aspirated Volume (Bone Marrow)	Applied Volume (Mononuclear Cells)	Latest FU	Complications	Roentgenological Bone Formation	Bone Healing
1	70	7	18	n	y	y
2	60	7	7	prolonged hematoma	y	y
3	90	7	14	n	y	y
4	60	7	7	n	y	y
5	60	8	7	n	y	n
6	50	9	6	n	y	y
7	50	7	7	n	y	y
8	120	18	7	n	y	y
9	60	9	5	n	y	n
10	55	9	5	n	y	n

whereas unstimulated samples served as controls. Before and 28 days after *in vitro*-stimulation cells adhered onto the scaffolds were stained against factors and antigens as described previously [61]: Alkaline Phosphatase (ALP, Blue Alkaline Phosphatase Substrate Kit III, # SK-5300, Vector), Receptor-activator of NFκB-ligand (RANKL, rabbit-anti, 1:100/-PBSTA (10 μL / mL), Santa Cruz SC-9073), signals were enhanced by an anti-rabbit-IgG / biotin secondary antibody

system (anti-rabbit-IgG/biotinylated, avidin-biotin-complex, Vector, + DAB, Sigma). In addition von-Kossa staining served to detect any calcium-phosphat deposition as a signs of osteoblastic differentiation. After incubation, bone marrow cells were morphologically analyzed using phase-contrast microscopy (Axiovert 200, Zeiss, Germany) supported by a computer picture analysis system (Axiovision, Zeiss).

### Statistics

The average values (X) and standard deviations (SD) served as descriptive parameters.

## RESULTS

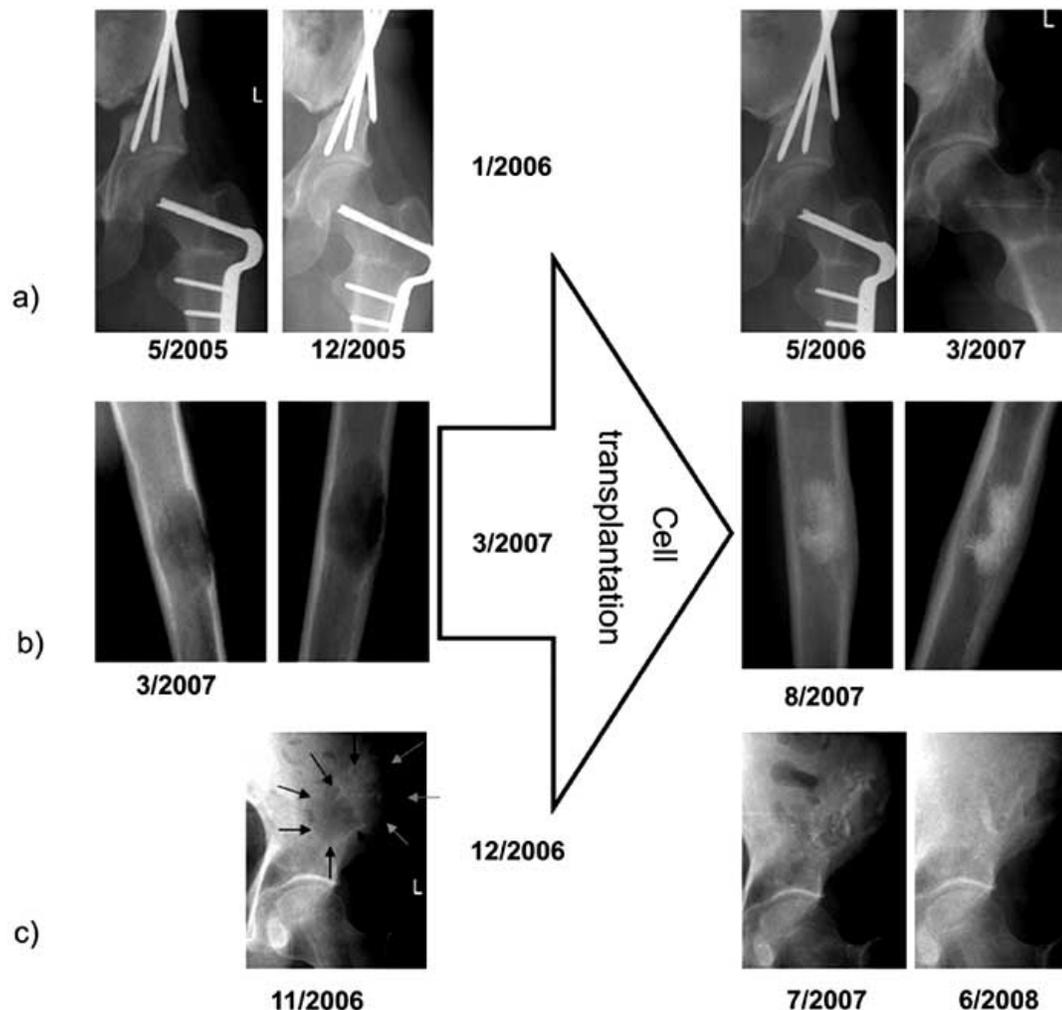
### *In Vivo* Results

For vacuum aspiration the average time was 15 min, followed by a cell concentration procedure which lasts about 20 min. The cell transplant was ready for transplantation when the surgical approach was done and the bone defect was prepared sufficiently (resection of fibrous tissue in pseudarthrosis, curettage in bone cysts, debridements in revision arthroplasties). Therefore, no extra surgical time was needed. The seeding procedure was easy to handle. To promote cellular adherence and migration within the scaffold, we per-

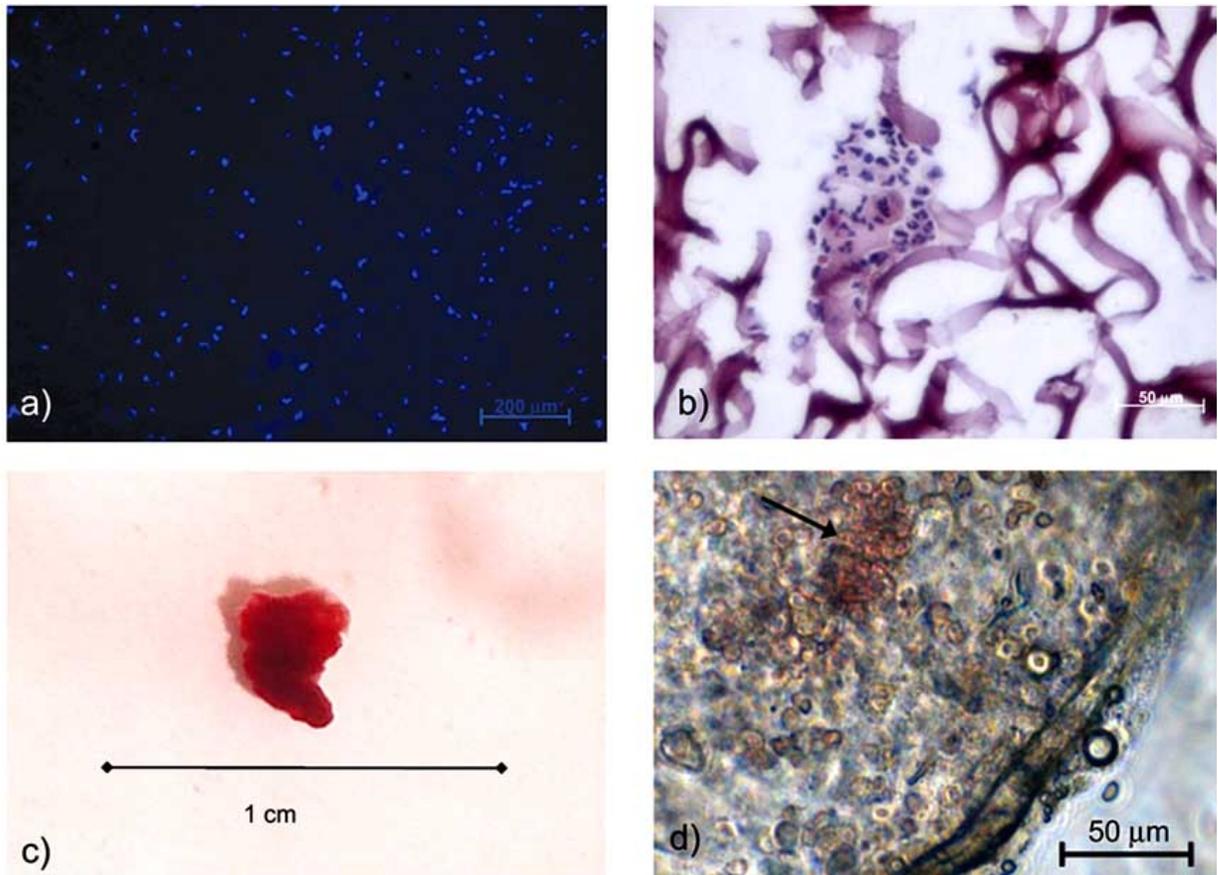
formed a standard 20 G (0.9 x 40 mm) needle (Braun, Melsungen, Germany) to inject the cell suspension within the collagenous sponges prior to transplantation. We found no perioperative complications with the exception of one persisting pelvis hematoma, which indicate the safety of the system. All patients showed bony healing and / or sufficient new bone formation within follow-up. There were no restrictions to any physical activities prior to the causative disease at latest follow-up. All patients returned to their profession after treatment. Table 2b gives detailed information about the outcome in patients who received concentrated marrow. The follow-up x-rays of one of the adult female, one male child and one male adult are shown in Fig. (1a-c).

### *In Vitro* Results

There were notable cells numbers after the 15 min incubation process present at the biomaterial's surface. These



**Fig. (1).** **a)** Follow-up x-rays showed a bone healing of a pelvic pseudarthrosis of an 18 year old female after cell transplantation who underwent Salter osteotomy before. Four months after cell therapy we found solid bone healing of the pseudarthrosis. **b)** The figure shows the roentgenological results in two planes of an 11-year old male patient with the diagnosis aneurysmal bone cyste of the left humerus (approved by histopathological findings). Before surgery the cortical bone is destroyed by the tumor-like lesion. Four months after curettage and cell transplantation not only the cystic lesion showed a healing but also the cortical bone is remodeled. In this case, the transplant was augmented by porous beta-tricalciumphosphate (TCP) granules. **c)** Follow-up pelvis x-rays of a large bone cyst (arrows) in a 53 year-old male before and after curettage and bone marrow concentrate transplantation. Eight and 19 months after treatment significant new bone formation is detected on x-rays.



**Fig. (2).** **a)** and **b)** Collagen I scaffold (Gelaspon<sup>®</sup>) 15 min after incubation with cells from a bone marrow concentrate of a 23 year old female donor. The cells were isolated by density centrifugation (Harvest System) and pipetted onto the porous membrane. **a)** DAPI staining showed a good adherence of the cells only 15 minutes after incubation. **b)** H.E. staining showed mononuclear cells adherent at the collagen fibres within the 3-dimensional network of the scaffold. **c)** Macroscopic view of the 10 x 10 mm scaffold after *in vitro* cultivation. Three days after *in vitro*-incubation there was a significant reduction in size and volume of the collagen membrane. The red color indicated the presence of erythrocytes. **d)** The episcopic light microscopy showed still some erythrocytes 6 days after incubation (arrow).

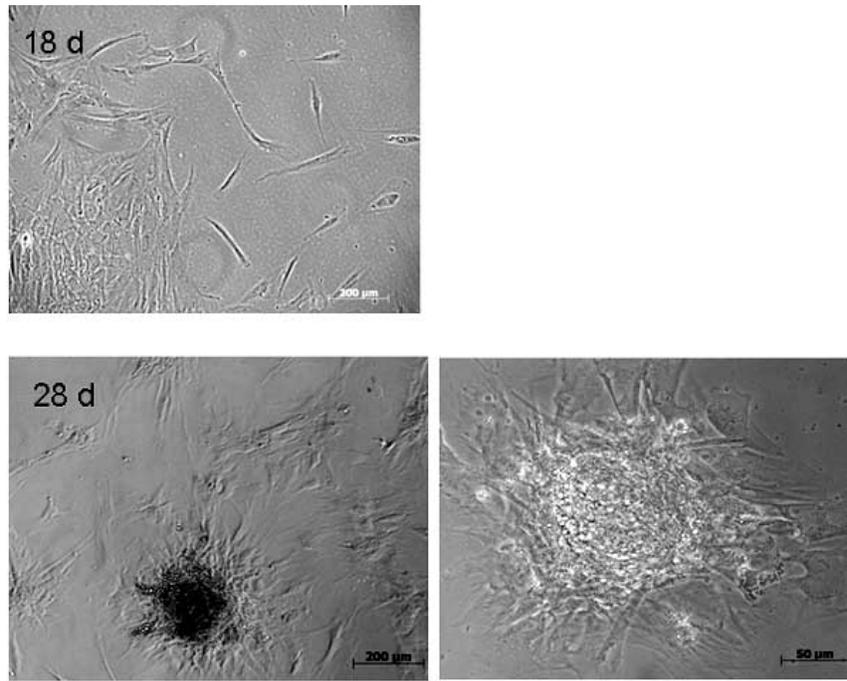
cells adhered within the loose fibrous network of the collagen mesh as demonstrated by DAPI and H. E. staining (Fig. 2a, b). Three days after cultivation we found a notable shrinking of the collagen sponge. Although erythrocytes were removed by the centrifugation process, we found larger numbers of this non-adherent cell type in suspension at this time, as a sign of differentiation of hematopoietic stem cells (Fig. 2c, d). With further incubation erythrocytes disappeared and were not seen as from day 12.

The collagenous scaffold was resorbed and / or degraded completely with further cultivation and was not detectable macroscopically after 10 days *in vitro*, both in controls and in osteogenic stimulated cultures. After 28 days *in vitro* we found signs of spontaneous osteoblastic differentiation which was noticeably increased in those cultures stimulated with an osteogenic mixture. Corresponding to a preliminary stage of biomineralization, we found cellular clusters with morphological characteristics of bone nodules (Fig. 3). These findings were supported by a positive *von Kossa*-staining which showed a weak signal in non-osteogenic stimulated groups whereas DAG-treated cultures present a strong staining reaction. In addition, the antigen expression of various antigens confirmed an osteoblastic differentiation (Fig. 4).

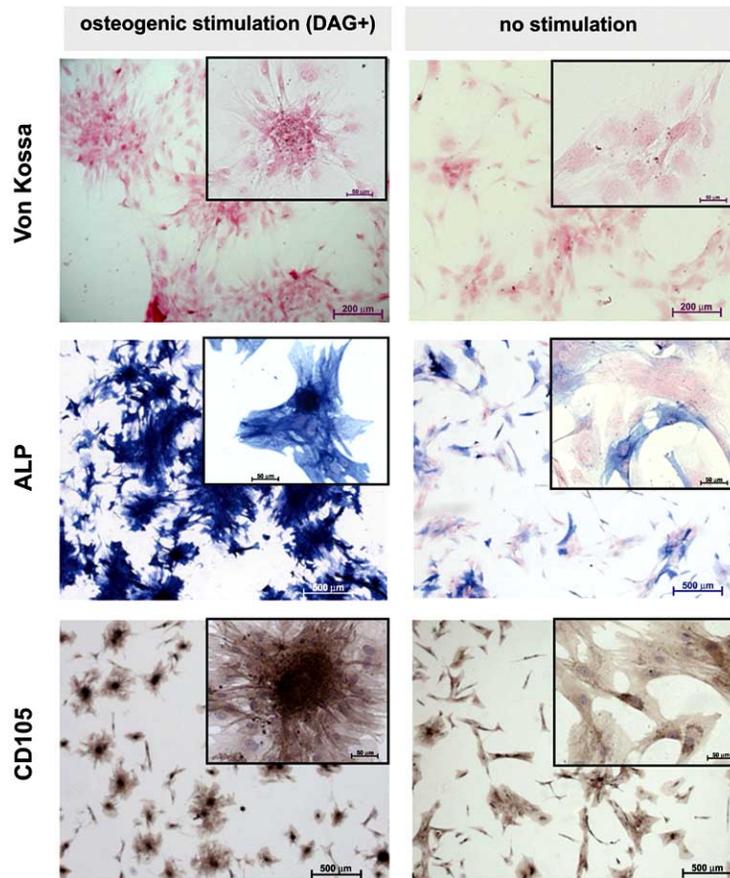
## DISCUSSION

Recent research has shown the autologous mononuclear cells from bone marrow in combination with different scaffold are effective in promoting bone formation in various animal models such as sheep [63], dogs [64], rabbits [65,66] and mice [14]. Encouraged by the animal data and also by clinical results seen in the treatment of osteonecrosis [67,68], the application of autologous mononuclear bone marrow cells obtained by vacuum aspiration may provide orthopaedic surgeons with an alternative way in treating volumetric bone deficiencies while minimizing the morbidity associated with autologous bone transplantation [69]. Our study reports on a series of patients (n=10) who have undergone the treatment of volumetric bone defects with autologous bone marrow aspirate with an average follow up of 8.3 months.

Based on the clinical / operative experience of this case series, we found that the collagen scaffold which served as a cell carrier had a hemostatic *in vivo* effects and showed two major disadvantages. First disadvantage of the collagen scaffold is related to its fast *in vitro* resorption and degradation rate which does not allow for further cell guiding functions. The second major disadvantage relates to the visco-elastic properties of the scaffold which make it difficult to fulfil



**Fig. (3).** 18 days after cultivation the mononuclear cells formed fibroblast-like mononuclear layers during the first two weeks *in vitro*. With further cultivation the spindle-type cells but also cuboid shaped cells accumulated locally and formed bone nodules which underwent bioalcification (Fig. 4).



**Fig. (4).** The immunocytochemical stainings with antibodies against typical osteoblast markers indicate that the cultivated cells allow for an osteogenic differentiation which was promoted by DAG stimulation. Under DAG incubation the cells formed cluster formations which have the morphological characteristics of bone nodules. The strong signal in *von Kossa*-staining proves the deposition of calcium and phosphate as a sign of bioalcification.

bone defects with complex geometries and does not allow compression of the matrix.

Another possible complication related to the aspiration procedure is hematoma formation secondary to the bleeding within the transplantation area. During the harvesting procedure, the kit is supplemented by "acidum citricum dextrosus" (ACDA, Baxter) solution to prevent coagulation of the bone marrow aspirate. The suspended bone marrow cells that is ready for implantation may still contains a small amount of this anticoagulant solution when applied to the bone defect causing increase bleeding. Because 4 mmol citric acid can inhibit the coagulation of about one litre of peripheral blood, we found that by supplementing  $\text{Ca}^{2+}$  to the transplant site immediately before cell transplantation, the anticoagulative effect of the ACDA solution maybe reduced, thus resulting in less harvest site bleeding. In addition, the volume of ACDA is also dependant on the aspirate and the total calculated volume of the cell suspension needed for transplantation, thus it is very importation to add the correct amount of ACDA to the aspirate. After applying this method of  $\text{Ca}^{++}$  supplementation in our patient group, only one patient showed a hematoma postoperatively that drained spontaneously three weeks after surgery without any further complications.

Another finding from our study is that although mononuclear progenitor cells are derived from the bone marrow, there is additional data indicating that platelet rich plasma may also provide an additional supportive effect for bone healing *in vivo*. Wojtowicz *et al.* [70] showed the advantages of platelet rich plasma in mandibular bone healing when compared to mononuclear cells without PRP and concluded that cytokines or growth factors may be responsible for the osteoblast stimulation. The importance of these soluble factors (PRP) for bone healing is also supported by our own research data. After xeno-transplantation of stem cells from human cord blood into a rat's bone defect, increased rate of bone healing was observed given that the human cells survived for only 4 weeks. [60]

The commercial aspirate system (BMAC) we utilized in the study as demonstrated by Hermann *et al.* [58] was able yield 2.4 fold higher amount of total numbers of Total Nuclear Cells (TNC) as compared to the conventional Ficoll density centrifugation. However, we also found significant amounts of erythrocytes in the aspirate, which were still apparent after 6 days *in vitro*. This observation indicated that the system isolated not only the mesenchymal progenitor cells but also the hematopoietic stem cells. As demonstrated in recent investigations, hematopoietic stem cells (HSCs) require the support of stromal elements to engraft, self-renew, and progress towards lineage commitment [63,71]. Also there is evidence indicating that the differentiation of osteoblasts from mesenchymal progenitors may be promoted and modified by the presence of HSCs. An example of the above statement is seen in the complex interactions between hematopoietic osteoclasts and mesenchymal osteoblasts [72-74]. Furthermore, primitive adult HSCs can also act as osteoblast precursors [75].

Clinically, it is also not clear from our study that the local application of platelet rich plasma has any significant effects on bone defect healing. Kilian *et al.* [76] showed that on

platelet rich plasma, PDGF stimulates the migration and proliferation of mesenchymal stem cells and endothelial cells but also promotes the differentiation of osteoblasts, especially when  $\text{Ca}^{2+}$  was additionally added. In contrast to these findings, Arpornmaeklong *et al.* [77] report about simulative effects of platelet rich plasma for proliferation but inhibitory effects on osteoblastic differentiation in rat cells cultured onto a collagenous scaffold. Therefore it is difficult for us to conclude whether PRP had any additive or supportive effect in healing the bone defects.

One major limitation of our study is the low number of patients and the number of different presenting diognosis. Although bone healing was seen in the followup radiographs and majority of our patients have symptomatic improvement, we can not conclude from data obtained in this study that bone marrow aspirate and MSC in combination with PRP will be able to heal all volumetric bone losses. However, one conclusion we can draw from this case series is that the system we used for the marrow harvest is easy to handle in a daily practice and does not increase operative time significantly. Furthermore, this system is not associated with any significant or major complications or side effects. It is also possible that the cells transplanted into the bone defect *via* the aspirate may not be the same cells that differentiate into osteoblasts. Another advantage of the one-stage isolation procedure in comparison to the *ex-vivo* expansion of autologous cells from bone marrow is the decrease in cost and infection rates related to the extra personal need and extended time required for the expansion, respectively.

Overall, our case series showed that transplantation of bone marrow aspirate was able to facilitate healing of volumetric bone loss in this particular group of patients. However, future controlled randomized studies needs to be performed in a larger cohort to verify if the application of bone marrow derived cell aspirates can provide the same clinical outcome as the current standard of autologous bone graft transplantation in patients with local bone defects or pseuarthroses. Moreover, a cost-benefit analysis should also be performed to see if the one-stage application of bone marrow aspirate will be able to shorten hospital stay and reduce additional costs such as consumables or the extra staff needed for the *ex-vivo* transplantation.

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