

## Case Report

### Application of a new chair-side method for the harvest of mesenchymal stem cells in a patient with nonunion of a fracture of the atrophic mandible – A case report<sup>☆</sup>

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**SUMMARY.** Purpose: This case report describes a new clinical method for chair-side processing of a cell mixture which contains mesenchymal stem cells (MSCs) which was applied for the first time in the treatment of a nonunion of an atrophic fractured mandible. Methods: Bone marrow was aspirated and a corticocancellous bone graft was harvested from the iliac crest of a 56-year-old woman with medical comorbidities and a fracture of the atrophic mandible. The fracture was stabilized with a reconstruction bone plate, and mononuclear cells including MSCs were concentrated by centrifugation and applied in combination with a particulate bone transplant. A sample of the grafted cells was characterized by flow cytometric analysis and by their ability to differentiate into various cell types. Results: The fracture healed uneventfully. No complications occurred during the 4-month follow-up. Conclusion: Adding MSCs is a feasible alternative to enhance bone healing. This chair-side method requires little training and no cell laboratory support. © 2008 European Association for Cranio-Maxillofacial Surgery

**Keywords:** bone grafting, mandible atrophy, mandibular fracture, mesenchymal stem cells, nonunion, osteosynthesis

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## INTRODUCTION

Atrophic edentulous mandible fractures represent a subset of facial injuries that are more commonly sustained by older victims. Perioperative management of the acutely injured elderly patient is different from the management of younger patients and is more complex. Physiological and anatomical changes that accompany edentulousness in the mandible, and the metabolic effects of aging are factors that appear to influence fracture repair outcome unfavourably. Because fractures of the edentulous mandible occur less often than in dentate patients, it is difficult to study this prospectively.

Despite these difficulties, two multicentre studies of fractures of the edentulous mandible exist (Bruce and Strachan, 1976; Bruce and Ellis, 1993). In the first study, (Bruce and Strachan, 1976) it was shown that older patients had more systemic health problems at the time of injury. They also had thinner mandibles and fracture fragments which were farther apart and required prolonged treatment. Also more systemic complications occurred. In their conclusion, Bruce and Strachan (1976) considered fractures of atrophic edentulous mandibles to be a significant problem with a high risk of nonunion

(20%). They recommended that closed methods should be used because more nonunions occurred when open reduction was applied. However, intraosseous wires were mostly used for “fixation”.

Fifteen years later, Bruce and Ellis (1993) reported another study with a higher incidence of patients treated with open reduction using plate and screw osteosynthesis (81.5%). They found that open reduction with internal fixation by osteosynthesis plates could lead to better results, but they did not examine the efficacy of bone grafting. Even though Bruce and Ellis (1993) mentioned the clinical and biological values of bone grafting, they were discouraged by the fact that older patients with health problems carry a high degree of morbidity, and at that time there was no clear evidence that the rate of fracture union would be improved by this addition.

A recent study by Ellis and Price (2008) showed that atrophic mandibular fractures treated by open reduction, internal plate fixation and immediate bone grafts (in the majority of cases) had an extremely high rate of success. Although they encountered no problems from taking autogenous grafts in their patients, harvesting a bone graft from an elderly patient with comorbidities can be problematic. It would therefore be preferable to provide osteogenic potential in a less invasive manner.

Osteoblasts develop from the proliferation and differentiation of osteoprogenitor cells. These progenitors arise

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from a population of uncommitted mesenchymal stem cells (MSCs) also known as bone marrow stromal cells or multipotential stromal cells (Caplan and Bruder, 2001).

MSCs are a population of cells with multilineage potential, defined by their ability to differentiate into cells of the osteogenic, chondrogenic and adipogenic lineages. MSCs have demonstrated the *in vitro* potential to differentiate into nonhaematopoietic cell types, including cells with neuronal, endothelial or myocyte phenotypes (Kan et al., 2005). The plasticity of MSCs may make these adult stem cells an invaluable resource for the research of cellular therapy of tissue defects and chronic diseases.

Different authors suggest that transplantation of autologous stem cells from aspirated bone marrow can enhance bone healing (Connolly et al., 1989; Healey et al., 1990; Garg et al., 1993; Garg and Gaur, 1995; Connolly, 1998). Connolly et al. (1991) treated 20 patients with tibial nonunion with aspirated bone marrow and followed them for 5 years. They found bone marrow injection as effective as previous open autologous grafting but with considerably fewer disadvantages. They concluded that this technique provides an easily renewable and reliable source of osteogenic stem cells with numerous advantages when compared with standard open-grafting techniques.

The purpose of this case report is to present the treatment of a mandibular fracture nonunion in an atrophic mandible using a new chair-side method of mesenchymal stem cell processing to facilitate healing.

## PATIENT AND METHODS

A 56-year-old woman with a 26-year history of alcohol abuse and 25 years of smoking was referred to the department of maxillofacial surgery of the University Hospital of Freiburg with a pathological fracture of the atrophic mandible.

She suffered from pain over a period of 2 months after she had received new dentures. Her dentist treated her for poorly fitting dentures and administered antibiotics when the patient developed a swelling at the left side of the mandible. Because the swelling persisted, she was referred to a maxillofacial surgeon. He discovered a fracture of the mandibular body on radiographic examination and sent her to the Freiburg University department of maxillofacial surgery.

The past medical history included depression and alcoholic polyneuropathy requiring therapy with selective serotonin reuptake inhibitors and tricyclic antidepressant drugs. Since 1982, she had been admitted to hospital 4 times for alcohol dependency. She reported no allergies or adverse response to any medication. The laboratory results showed no malfunction of the liver. As sign of inflammation the CRP was elevated to 95 mg/l.

A paramandibular swelling on the left side was observed. The lips were pale, dry and showed angular cheilitis. Mouth opening was normal. There was a hypoesthesia of the left 3rd branch of the trigeminal nerve. A left submandibular lymph node was tender and enlarged.

Intraorally a partial prosthesis was found in the maxilla and the atrophic, edentulous mandible supported a full



**Fig. 1** – First preoperative radiograph of the patient with a left mandibular posterior body fracture and sequestrum. The mandible is extremely atrophic and edentulous.

denture. Plaque and generalized gingivitis were present. There was a lesion of the gingiva in the area of the fracture line where the bone was exposed. Radiographs showed a severely atrophic mandible with a displaced fracture (Fig. 1).

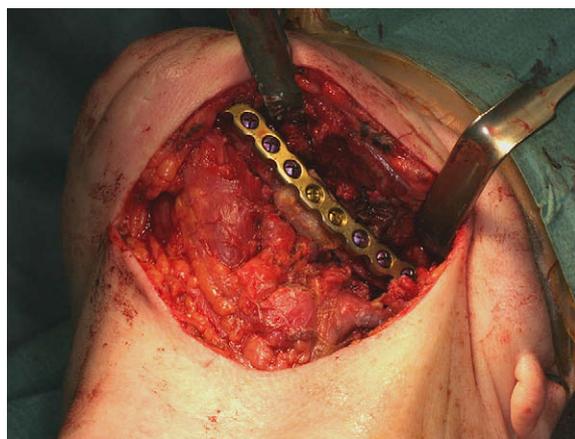
During the first operation, the fracture ends were cleaned by curettage. The fracture was not treated further because the severe atrophy of the mandible did not allow internal fixation. The wound healing was uneventful and the patient was discharged after 1 week of penicillin treatment (Penicillin, Grünenthal, Germany). Two weeks later the patient was readmitted with an increased swelling of the left mandibular region. The X-ray revealed a displaced fracture with sequestrum in the mandibular body region (Fig. 2).

For the second operation, a simultaneous autogenous corticocancellous iliac bone graft was placed between the fracture ends during open reduction after sequestrectomy as removal of the gap was considered too wide for secondary wound healing. Internal fixation with a 2.4 mm locking reconstruction plate (Synthes, Oberdorf, Switzerland) was applied (Fig. 3). Prior to insertion, the cancellous bone was soaked in a suspension of bone marrow concentrate which contained MSCs (see below). A mixture of the cell suspension and autologous thrombin was applied with the conditioned cancellous bone (Fig. 4). To facilitate guided bone regeneration, a collagen membrane (Bio-Gide, Geistlich Biomaterials, Wolhusen, Switzerland) was placed over the graft.

To harvest the stem cells the iliac crest bone was punctured before harvesting the bone graft with a bone marrow biopsy needle (Bone Marrow Aspiration Pack, Harvest Technologies Corporation, Plymouth, MA, USA). With 20 ml syringes (Bone Marrow Aspiration Pack, Harvest Technologies Corporation, Plymouth, MA, USA) each containing 0.3 ml of heparin solution (Heparin-Natrium, 10,000 U/ml, diluted with NaCl to 1000 U/ml, both B. Braun, Melsungen) 60 ml of bone marrow was collected. The aspirate was anticoagulated with 5 ml of heparin solution. Bone marrow cells were isolated using the Bone Marrow Aspirate Concentrate (BMAC) system (Bone Marrow Procedure Pack, Harvest Technologies Corporation, Plymouth, MA, USA) according to the manufacturer's instructions. The system can use up to two disposable containers with two chambers. The first chamber contains a floating shelf of a specific density by which the red blood cells are separated from the nucleated cells, platelets and plasma during

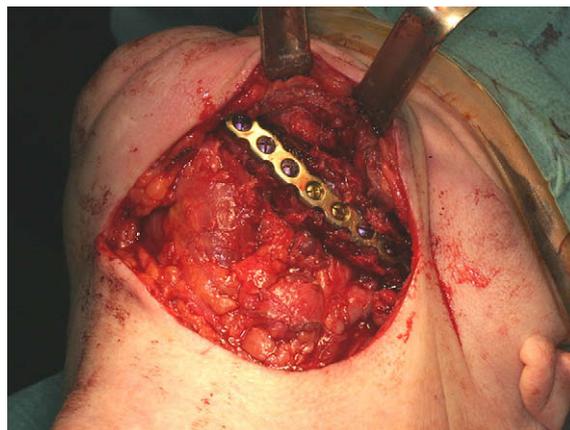


**Fig. 2** – The radiograph which was taken before the second surgical approach shows a fracture of the left mandibular posterior body fracture and a formation of sequestrum.



**Fig. 3** – Open reduction and interposition of a corticocancellous bone graft. The corticocancellous bone graft was placed into the defect because after sequestrectomy the fracture gap was considered too wide for secondary bone healing. The fracture segments and the transplant were fixed with a 2.4 Unilock plate (Synthes, Oberdorf, Switzerland).

the initial centrifugation phase. Cellular elements and plasma are automatically transferred into the second chamber and concentrated by centrifugation (Fig. 5A–D). A portion of the supernatant plasma is removed and the cellular elements are resuspended in the remaining plasma. This concentrate was applied as described above. The concentration process was evaluated by Haemocytometry (Sysmex Analyzer, SYSMEX EUROPE GMBH, Norderstedt, Germany). The mesenchymal stem cell character was proven by flow cytometrical analysis and by the ability of the cell to differentiate into various lineages. Flow cytometry was carried out with the same sample. In one tube, cells were stained simultaneously with – peridinin chlorophyll protein conjugated (Per-Cp) monoclonal antibody to CD 34 and CD 45, allophycocyanin conjugated (APC) monoclonal antibody to CD 105, fluorescein isothiocyanate conjugated (FITC) monoclonal antibody to CD 44 and phycoerythrin conjugated (PE) monoclonal antibody to CD 73 (Becton Dickenson Biosciences, San Jose, CA, USA). After incubation at room temperature for 15 min the specimen was analyzed by a FACScalibur (Becton Dickenson Biosciences, San Jose, CA, USA).



**Fig. 4** – Intraoperative view with a reconstruction plate which stabilizes the fracture. In addition to the corticocancellous bone graft, the defect was filled with mesenchymal stem cell enriched cancellous bone chips to enhance bone regeneration in the patient who has a compromised wound healing.

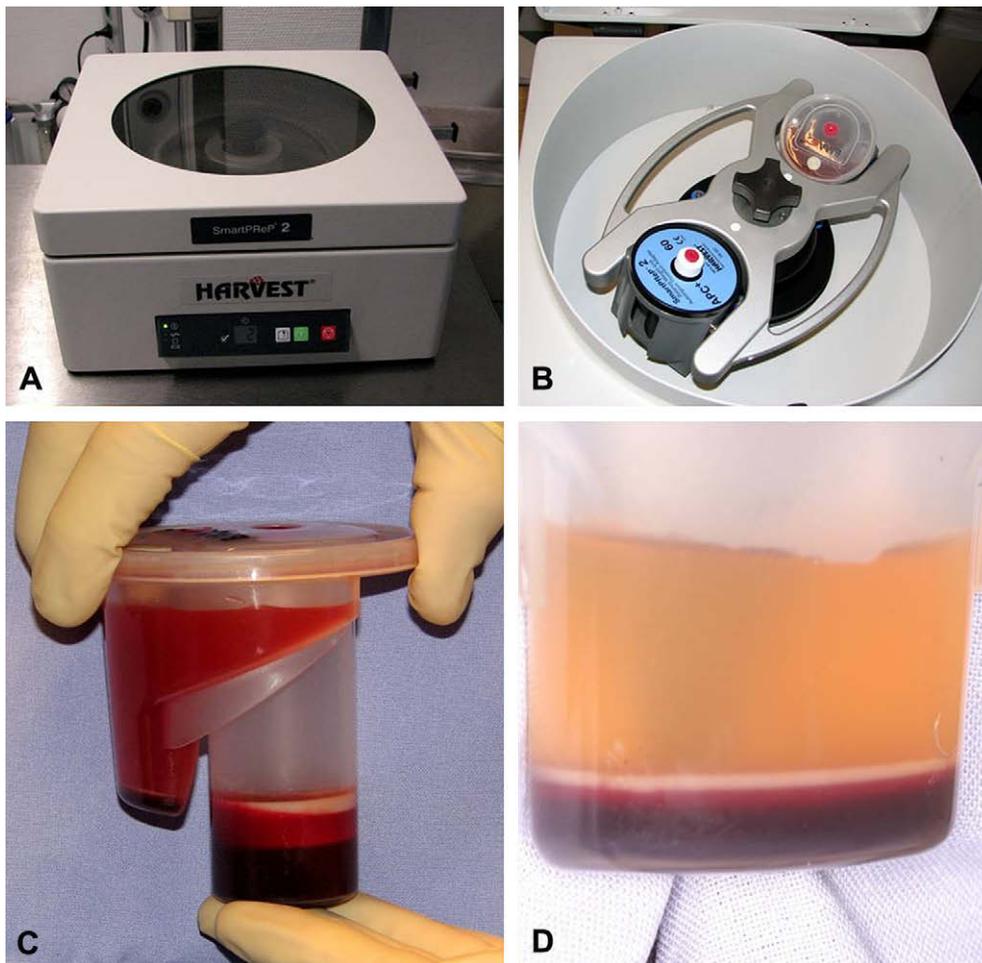
## RESULTS

The patient recovered well from the surgical procedure. Postoperatively there were no complaints about pain or signs of haematoma or infection from the bone harvest and marrow aspiration sites. The postoperative orthopantomogram revealed a good position of the graft and the osteosynthesis plate. The 4-month follow-up visit showed a healed intraoral operation site. Good integration of the bone graft and good plate position could be evident on the panoramic radiograph (Fig. 6). Removal of the plate osteosynthesis hardware was not considered because of the patient's age and her medical compromise.

The BMAC was enriched 5.8 times: We counted  $91.1 \times 10^3/\mu\text{l}$  white blood cells,  $1.53 \times 10^6/\mu\text{l}$  red blood cells and  $407 \times 10^3/\mu\text{l}$  platelets. For proof of pluripotency, the cells from the bone marrow concentrate were amplified and differentiated into three cell lineages. The lines consisted of the chondrogenic, adipogenic and osteogenic phenotype, proving that the transplanted cells were actually pluripotent stem cells. Adipocytes were stained with oil red O, a lipophilic red dye. The cultured MSCs (Fig. 7A) could be differentiated successfully into adipocytes as shown by oil red O staining (Fig. 7B), chondrocytes as shown by aggrecan immunostaining (Fig. 7C) and osteoblasts as shown by calcification, alkaline phosphatase and collagen type I activity (Fig. 7D–F). The FACS analysis showed a distinct population of CD 34 and CD 45 negative cells which were positive for CD 44, CD 73 and CD 105 (Fig. 8A–D). The results of the FACS analysis together with the results of the differentiation assay prove that actual MSCs were transplanted according to *Pittenger et al. (1999)*.

## DISCUSSION

There are no prospective, statistically validated, long-term outcome studies about the treatment of infected fractures of the atrophic edentulous mandible. Whether more invasively treated fractures have a greater



**Fig. 5** – The specially developed and CE-certified centrifuge can be used inside the operating facility (A). The tube for the processing of autologous thrombin is placed in the blue counter weight and the two-chamber container opposite (B). Red blood cells are kept under the floating shelf in the large chamber while the mononuclear cells and thrombocytes settle in the small chamber (left side of C). Close up of the small chamber after most of the supernatant has been removed (D). The amber coloured fluid is the supernatant. The white line is composed of mononuclear cells. This layer floats on top of the thrombocytes.

likelihood of complications and increased general morbidity is discussed controversially in the literature (*Barber, 2001; Marciani, 2001*).

Surgical techniques to repair mandibular fractures have improved considerably since the study of *Bruce and Strachan (1976)*. Nevertheless, open reduction and internal fixation are a preferred option for the treatment of mandibular fractures (*Sauerbier et al., 2008*), if not the gold-standard. Fractures of the severely atrophic mandible must be regarded separately. Open reduction is the procedure of choice (*Ellis and Price, 2008*). Rigid internal fixation devices should be used that will permit immediate function and long-term resistance to hardware fracture (*Gutwald et al., 2003; Schupp et al., 2007*).

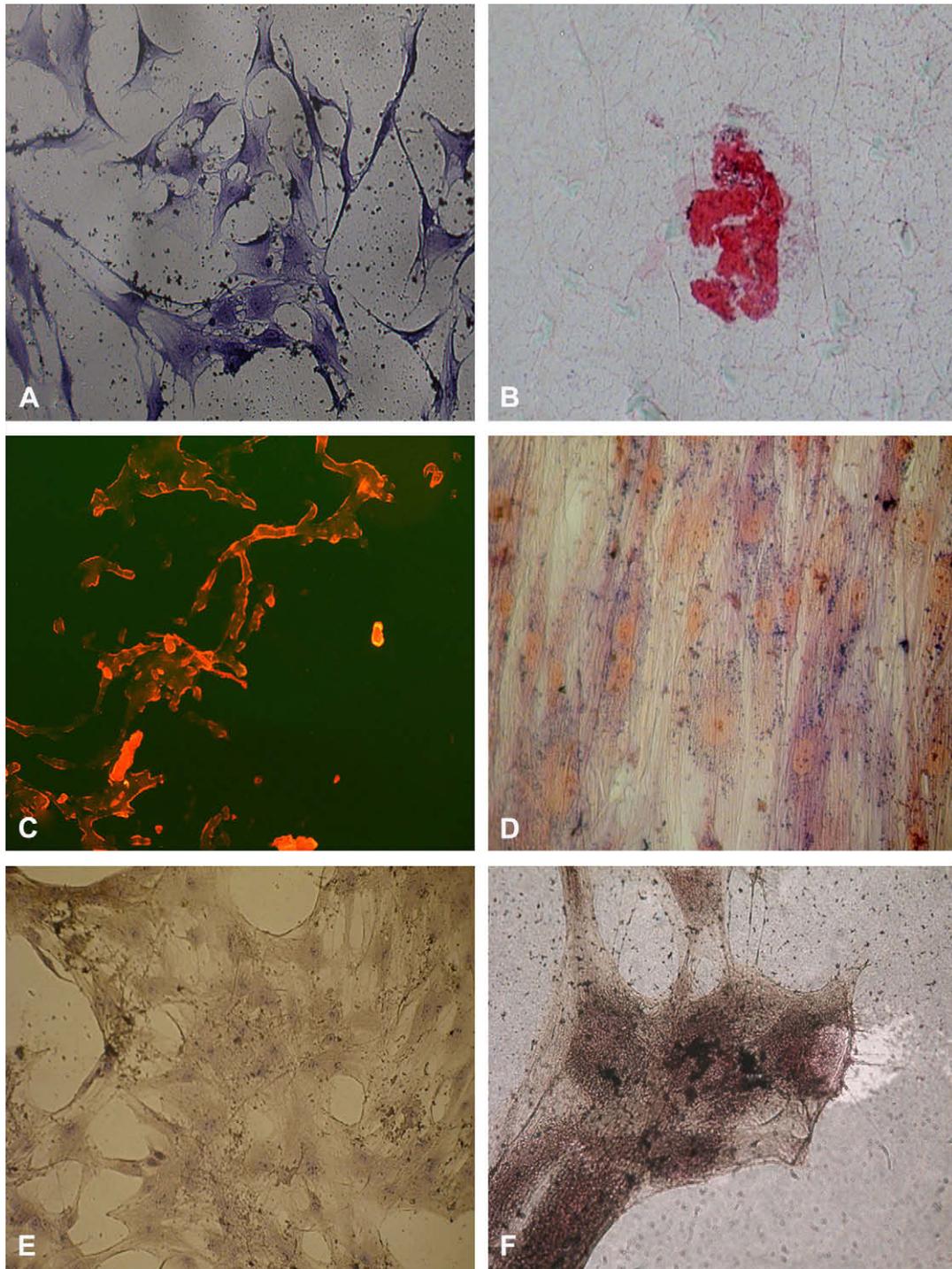
Invasive techniques for the treatment of the atrophic mandibular body fracture should be considered when conservative techniques have a high likelihood of non-union. One advantage of open techniques is the possibility of adding autogenous bone grafts, growth factors or cell therapy with minimally increased morbidity.

New bone formation in defects is a multistep process and depends on the biological capacity of the cells (*Muschler and Midura, 2002*). Stem cells or progenitor



**Fig. 6** – Four months after surgery the panoramic radiograph reveals a proper integration of the bone graft and a good consolidation of the fracture.

cells from the surrounding vital bone migrate to the bone graft where they settle, proliferate and differentiate into osteoblasts and form new bone tissue. The process can be accelerated by a higher number of osteoblasts. Adding bone marrow derived progenitor cells can enhance the local concentration of osteoprogenitor cells which are capable of differentiating into osteoblasts (*Caplan and Bruder, 2001*).

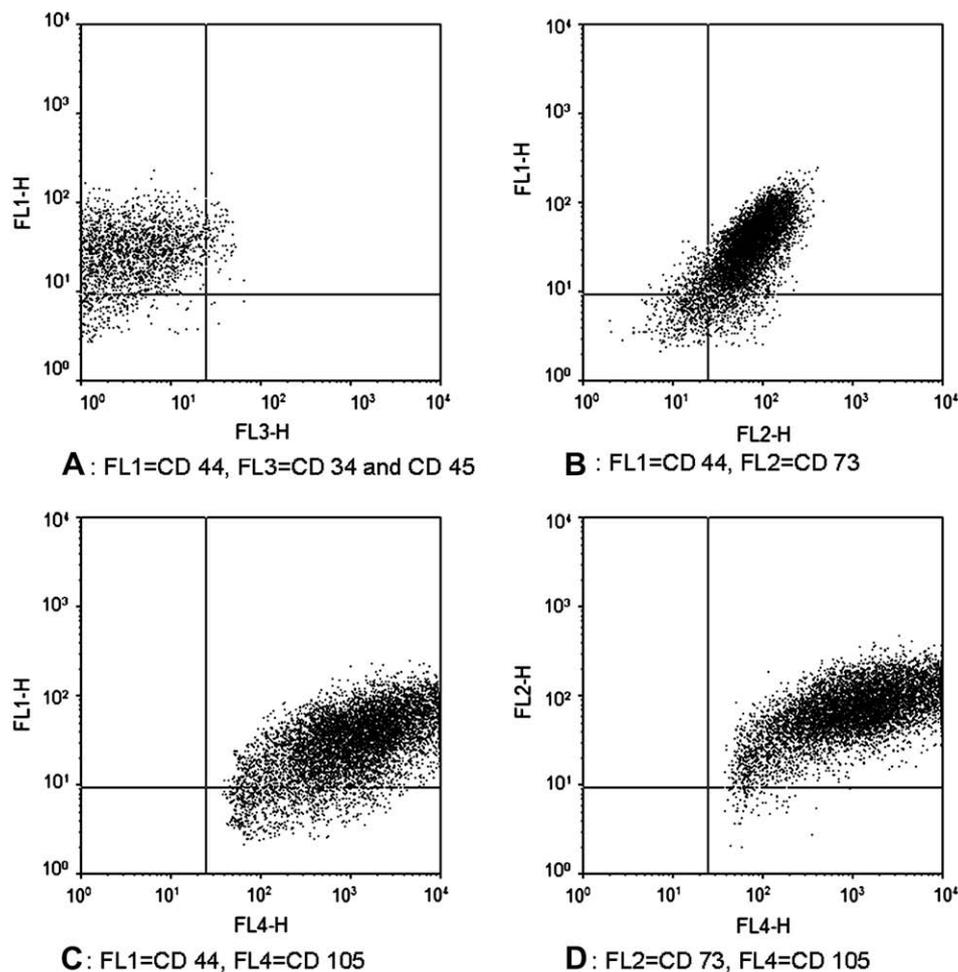


**Fig. 7** — Morphology of cultured MSCs stained with methylene blue (A). Adipocyte differentiated from MSC. The adipocyte is rounded and filled with lipid droplets which might fuse to form vacuoles that can be stained by Oil Red O, a lipophilic red dye (B). Chondrocyte nodule cultivated by differentiation of MSCs. The extracellular protein aggrecan is specific for chondrocytes. The immunohistochemical staining indicates the presence of aggrecan by its red fluorescence (C). Osteoblasts differentiated from MSCs. Alkaline phosphatase is stained blue (D). Collagen Type I is immunohistochemically indicated by the brown staining (E). Calcification shows a black colour in the van Kossa staining (F).

*Yuan et al. (2007)* showed in the dog model that critical-sized segmental defects of the canine mandible can be repaired by osteogenically induced BMSCs with a biodegradable  $\beta$ -TCP scaffold.

*Connolly et al. (1991)* found bone marrow injection to be successful in the treatment of eighteen of twenty patients with tibial nonunions. He also showed that concentration

of bone marrow cells by the use of a centrifuge could increase osteogenesis further (*Connolly et al., 1991*). *Hernigou and Beaujean (2002)* found a positive correlation between the volume of mineralized callus 4 months after injection of bone marrow progenitor cells, and the number of cells injected. They consider this method as appropriate for the treatment of nonunions (*Hernigou et al., 2006*).



**Fig. 8** – Flow cytometrical analysis of stem cells. In the upper left quadrant of A, cells which are positive for CD 44 and negative for CD 34 and negative for CD 45 can be seen. B displays the same cells as in A. Cells which are positive for CD 44 and CD 73 are in the upper right quadrant. Only cells of interest are shown and can be examined for other surface molecules. C shows that these cells are positive for CD 44 and CD 105. D displays cells which are positive for CD 73 and CD 105.

While it is impossible to determine whether the addition of MSCs helped this case of nonunion of a mandibular fracture to obtain osseous union, concentrated bone marrow aspirate is an interesting and promising approach to revitalizing sites with impaired healing. It was shown that multipotent MSCs were actually transplanted in the presented case and in contrast to the classic laboratory procedure, the separation via density gradient, that this new method allows processing of stem cells “chair-side”. Future research must address the characterization of the cells and the quantification of the new bone formation in controlled clinical trials.

## CONCLUSION

There is evidence in the literature supported by the results of the presented case, that bone formation can be enhanced with the application of bone marrow derived progenitor cells. Transplantation of connective tissue progenitors was designed to compensate for a deficiency in the number or function of local connective tissue progenitors as may occur in regions of previous trauma,

infection, previous radiation, tissue defects, scars or compromised vascularity. This technique is convenient to apply and may improve the outcome of grafts and bone substitute materials or even replace them (*Muschler and Midura, 2002; Muschler et al., 2004*), but further clinical trials are needed.

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