

# Use of autologous bone marrow cells concentrate enriched with platelet-rich fibrin on corticocancellous bone allograft for posterolateral multilevel cervical fusion

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## Abstract

The outcomes of posterolateral multilevel spine fusion in difficult clinical settings, such as in an aged multi-diseased osteoporotic patient, remain unpredictable. The osteoprogenitor cells in bone marrow decrease with ageing without losing their osteogenic potential. Autologous bone marrow cells (BMCs) from iliac crest aspirate can be concentrated in the operating room and platelet-rich fibrin (PRF) can be obtained from a peripheral blood as a source of autologous osteoprogenitor cells and growth factors, respectively. We present the case of an 88 year-old multi-diseased osteoporotic patient affected by cervical stenosis and subjected to C3–C7 posterior decompression, instrumentation and posterolateral fusion, using an intraoperative 'tissue-engineered' composite made of corticocancellous bone allograft augmented with autologous BMCs concentrate from iliac crest aspirate enriched with PRF from peripheral blood. Lateral dynamic X-rays and CT scan showed consolidation signs at 3 months follow-up, with solid C3–C7 fusion at 6 months follow-up. This paper describes a simple and effective method for potentially improving the fusion rate in aged osteoporotic patients by using corticocancellous bone allograft augmented with autologous BMCs concentrate from the iliac crest, enriched with PRF from peripheral blood, rapidly obtained before the surgical procedure. Copyright © 2008 John Wiley & Sons, Ltd.

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## 1. Introduction

The time required for spinal fusion increases with advancing age and the fusion rate remains unpredictable in the ageing population (Denaro, 1991; Weller *et al.*, 1997; Malik *et al.*, 2008). Moreover, smoking, osteoporosis and systemic illnesses have an adverse impact on fusion rates in spinal surgery (Boden and Sumner, 1995; Di Martino

*et al.*, 2006). Bone formation is regulated by systemic and local growth factors and osteogenic cells, such as bone marrow (BM) stem cells. Previous studies have suggested that BM stem cells are reduced with ageing and that this could be responsible, in part, for age-associated bone deficits and reduced bone repair potential (Quarto *et al.*, 1995). However, it has been reported that the osteogenic potential of BM skeletal stem cells, commonly named mesenchymal stem cells (MSCs; Friedenstein *et al.*, 1974; Caplan, 1991; Bianco and Robey, 2001), does not decrease by at late adulthood (Leskela *et al.*, 2003). Therefore, bone formation may be improved by augmenting the ossification site with both osteoprogenitor cells,

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such as bone marrow cells (BMCs), and growth factors. BMCs from iliac crest aspirates can be concentrated in the operating room as a source of osteoprogenitors and platelet-rich fibrin (PRF) can be obtained from peripheral blood cells as a source of growth factors. Corticocancellous bone allograft acts as an osteoconductive and osteoinductive structural support, which can be combined with cells and growth factors, forming a 'tissue-engineered' construct ready to be used in the operating room.

In this report, the use of autologous BMCs concentrate mixed with PRF enriching corticocancellous bone allograft is described to improve the outcomes of posterolateral multilevel cervical fusion in an 88 year-old multi-diseased osteoporotic patient. The novelty of this report is the description of the use of a tissue-engineered construct in a one-step procedure for cervical spine fusion in a difficult clinical setting.

## 2. Case Report

An 88 year-old white man presented at our outpatient clinic for the occurrence of progressive motor and sensory deficits affecting the four limbs. The patient was a heavy smoker and also presented with chronic renal failure as a consequence of long-lasting hypertension, osteoporosis, hypothyroidism and diabetes.

X-rays and preoperative CT scan showed a retrolisthesis of C3 on C4 and C3–C6 cervical stenosis (Figure 1a, b). MRI could not be performed, as the patient had a pacemaker. ESP and EMG analysis confirmed cervical myelopathy in central cord syndrome. With the diagnosis of cervical spondylosis, the patient was a candidate for decompressive laminectomy and fusion with lateral mass screw fixation.

In order to improve bone formation, a construct composed of osteoprogenitor cells and growth factors upon an osteoconductive scaffold was combined in a one-step procedure to achieve a long and stable cervical fusion. The Institutional Ethical Review Board of the author's institution approved the human protocol for this study and informed consent was obtained.

### 2.1. Autologous BMCs concentrate preparation

After general anaesthesia, 60 ml BM aspirate were drawn from the posterior iliac crest (Muschler *et al.*, 1997) with multiple punctures. BM was transferred to a centrifugation chamber containing a specific membrane for buffy coat separation and centrifuged using the Smart PReP2™ system according to manufacturer's instructions. Using a two-step centrifugation process, mononuclear cells were separated from red cells and resuspended in plasma with a final volume of 10 ml.

An aliquot of these BMCs was used to evaluate cell viability by Trypan blue staining and to determine the percentage of CD34<sup>+</sup>/CD45<sup>+</sup> and CD34<sup>-</sup>/CD90<sup>+</sup> cells, representative of haemopoietic stem cells and MSCs, respectively, by flow cytometry (Becton Dickinson).

### 2.2. PRF preparation

120 ml of the patient's own blood were drawn 60 min before surgery. PRF was obtained using a fully automated system consisting of a processor and a disposable applicator. The biochemical process of the Vivostat™ system has been described elsewhere (Kjaergard *et al.*, 1997). Briefly, the process is initiated by biotin–batroxobin, which acts upon the fibrinogen in platelet-rich plasma; the completion of the process depends entirely on autologous thrombin producing a sealant that overcomes the potential infective and antigenic risk. The entire process was fully automated and microprocessor-controlled and 6 ml PRF were obtained in approximately 30 min.

### 2.3. Surgical technique

An extensive C3–C6 decompressive laminectomy was performed. Instrumentation consisted of two titanium bars with lateral mass polyaxial screw fixation. A freshly frozen iliac crest bone allograft was cut into long bars of corticocancellous bone and placed alongside the instrumentation. The BMCs concentrate was then combined with the PRF to form a gel-like tissue that

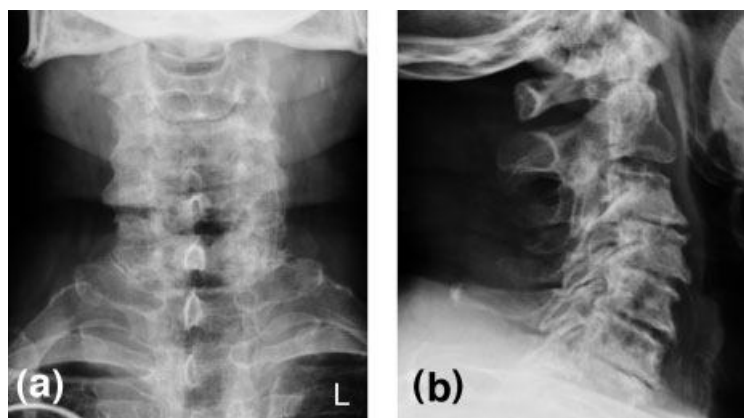


Figure 1. Preoperative cervical X-rays, showing a retrolisthesis of C3 on C4 and C3–C6 cervical stenosis (a–b)

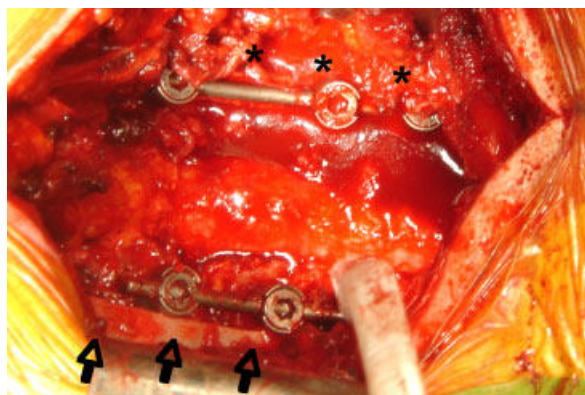


Figure 2. Intra-operative view of the posterior cervical decompression, instrumentation and apposition of the posterolateral bone graft. On the bottom side a long bar of corticocancellous bone (arrows) is shown alongside the instrumentation. On the top side autologous bone marrow cells concentrate enriched with platelet-rich fibrin (stars) has been added to cover the graft and obtain the composite

was used to cover the graft and obtain the composite (Figure 2).

### 3. Results and Discussion

The Trypan blue staining of the concentrated BMCs showed that all the cells were viable after centrifugation. Flow cytometry analysis of the BMCs concentrate showed that the  $CD34^+/CD45^+$  and  $CD34^-/CD90^+$  cells were 5.46% and 0.91%, respectively (Figure 3).

After surgery there were no neurological or wound-related problems and the patient showed improvements in hand movement control. A Miami-type collar was kept on for 45 days after surgery. Three months after surgery, standard and dynamic flexion/extension X-rays (Figure 4a, b) and CT scan showed signs of fusion without instability. At 6 months follow-up, the CT scan showed a solid C3–C7 fusion (Figure 4c–f).

BM is composed of a variety of cells, including osteoprogenitor cells (Friedenstein *et al.*, 1966). Two different types of stem cells co-reside within BM: haematopoietic stem cells and MSCs. Haematopoietic stem cells renew circulating blood elements. MSCs contribute to the regeneration of mesenchymal tissues, such as bone, cartilage, muscle, ligament, adipose tissue and stroma (Caplan, 1991; Pittenger *et al.*, 1999; Bianco and Robey, 2001). MSCs represent <1% of all cells present in the marrow (Muschler and Midura, 2002); indeed, these data have been concordant to our flow cytometry analysis.

The concentration system used allows a buffy coat to be obtained from whole BM aspirate to produce a mononuclear cell concentrate in a one-step procedure. The BM buffy coat obtained contains the total components of all mononucleated cells as well as platelets and other soluble factors that can play a role in osteogenesis.

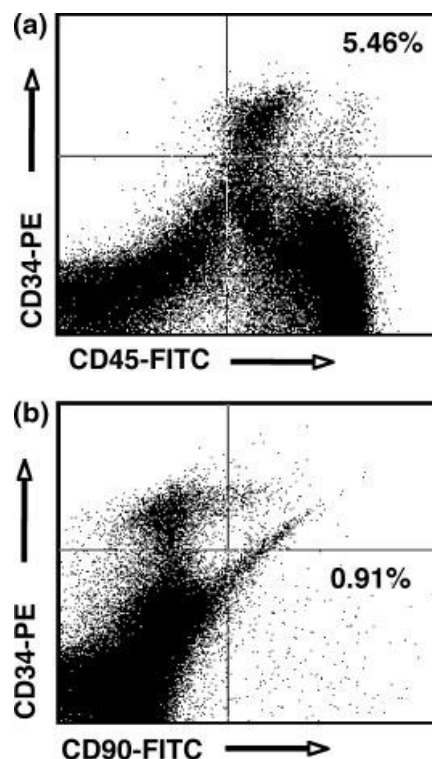
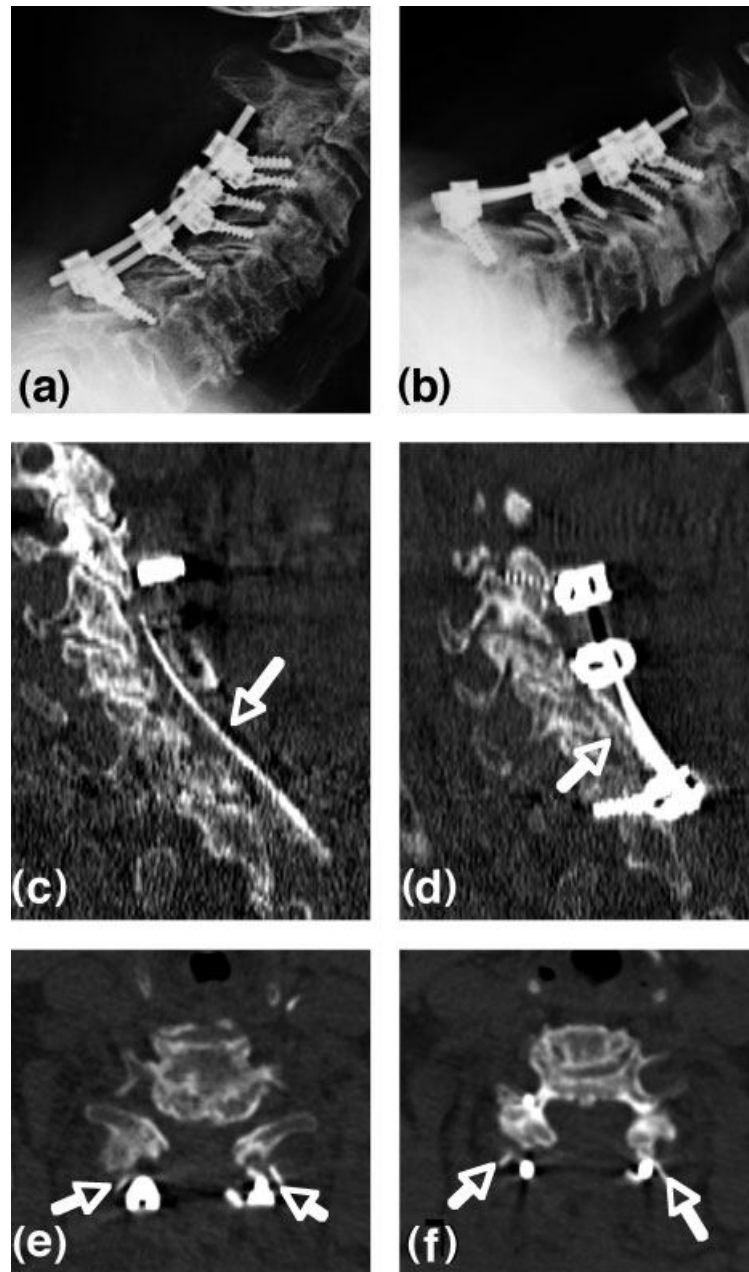


Figure 3. Expression of  $CD34^+/CD45^+$  (a) and  $CD34^-/CD90^+$  (b) by flow cytometry analysis from bone marrow mononuclear cells after the concentration procedure with SMART Prep2™ system

BM aspirated from the iliac crest has been used in orthopaedic (Connolly and Shindell, 1986; Tiedeman *et al.*, 1991; Garg and Gaur, 1995), craniofacial (Kitoh *et al.*, 2004) and spine surgery (Moro-Barrero *et al.*, 2007) as an adjuvant to bone grafting procedures, owing to their likely biological value and low risk. Further advantages of BM use in orthopaedic procedure can be achieved by the system used in our study, which allows BMCs from a marrow aspirate to rapidly concentrated into a small volume directly in the operating room, before starting surgery.

Moreover, we enriched the BMCs concentrate with autologous PRF, which is a source of growth factors such as platelet-derived growth factor, transforming growth factor, vascular endothelial growth factor and insulin-like growth factor. PRF has been shown to stimulate osteoblast-like cells *in vitro* and to enhance bone graft incorporation in maxillofacial applications *in vivo* (Slater *et al.*, 1995; Marx *et al.*, 1998). Platelet-rich plasma has also been shown to promote early maturation of bony fusion and has yielded good results in lumbar spine fusions clinically (Li *et al.*, 2004).

The process of bone regeneration requires three critical elements: osteogenic cells that have the ability to synthesize new bone; osteoinductive factors (i.e. growth factors and cytokines) that promote the osteoblastic differentiation of pluripotential stem cells; and an osteoconductive scaffold that facilitates neovascularization and supports bone ingrowth. Autologous bone is considered to be the graft material of choice for spinal fusion, since it has all the



**Figure 4.** Outcomes of the cervical fusion. Cervical dynamic flexion/extension (A, B) X-rays showed signs of fusion without instability 3 months after surgery. Representative cervical CT scan images showing bone fusion in the coronal (a–b) and sagittal (c–d) planes 6 months after surgery

above-mentioned properties essential for bone formation. However, it is difficult to obtain large autografts with the desired shape and mechanical strength. In our case, the old age and co-morbidities of the patient led us to choose bone allograft. Allograft is primarily osteoconductive with minimal osteoinductive potential but, because the donor cells are eradicated during tissue processing, this material is not considered to be osteogenic (Stevenson, 1999; Whang and Wang, 2003). Therefore, we used the principles of osteogenesis and tissue engineering to enrich our bone allograft (osteoconductive scaffold), seeding it with a cell–gel mixture, such as BMCs (osteogenic cells) concentrate with PRF (osteoinductive growth factors) in a one-step procedure.

Other studies have shown the effectiveness of the use of BMCs and platelet-rich plasma with bone allograft to induce bone formation (Dallari *et al.*, 2007; Filho Cerruti *et al.*, 2007). In this case we are reporting the effectiveness of this method in generating posterolateral cervical fusion in a difficult clinical setting where age and co-morbidities could compromise spinal fusion achievement.

It has been shown that BMCs are able to adhere and proliferate on bone allografts *in vitro* (Hofmann *et al.*, 2005). Moreover, PRF-derived growth factors also support the osteoprogenitor fraction of the BMCs to have a high potential for proliferation and bone differentiation (Slater *et al.*, 1995; Marx *et al.*, 1998). Therefore, it is plausible to conclude that the favourable process of spine fusion

observed in our patient was due to the presence of the concentrated BMCs in the bone graft.

*Ex vivo* expansion of bone marrow MSCs allows an increase in the number of osteoprogenitor cells, which can be loaded on an osteoconductive scaffold, giving further advances in improve bone formation. This approach has been successfully used for the reconstruction of long segmental defects in larger animals (Bruder *et al.*, 1998; Kon *et al.*, 2000) as well as in clinical studies (Quarto *et al.*, 2001). Bone tissue engineering using *ex vivo* expanded progenitor cells will probably be the approach of choice for difficult clinical cases in the future.

Another option in this patient could be the use of recombinant human bone morphogenic proteins (rhBMPs), such as BMP-2 and BMP-7. However, the high price of these drugs (in the range €4000–6000; Glassman *et al.*, 2007) led us to choose the described approach, which had a cost of €1800 (Vivostat™ system kit for the PRF plus Smart PReP2™ system kit for the BMCs concentrate).

The described one-step procedure allowed cervical fusion to be achieved in 6 months. It is generally well known that co-morbidities and old age decrease the fusion rate in spine surgery (Di Martino *et al.*, 2006). In the elderly population subjected to posterior cervical spine surgery fusion can be achieved, but the few reports available do not specifically consider patients above 85 years with several co-morbidities. In these studies the time required for spine fusion was not assessed; however, the evaluation of fusion lasted up to 15 months after surgery (Weller *et al.*, 1997; Malik *et al.*, 2008). The risks of the described procedure are correlated with the use of cadaveric bone grafts, with the risk of disease transmission (Tomford, 1995) as well as the risks related to blood and marrow manipulation.

In conclusion, this simple and effective method may improve the outcome of fusion rate in difficult clinical settings, such as aged multidiseased osteoporotic patients. However, our conclusions are limited by this single observation and randomized clinical trials are needed to confirm the effectiveness of this method.

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