Allogeneic Platelet Gel with Autologous Cancellous Bone Graft for the Treatment of a Large Bone Defect

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Introduction

Large bone defects that are caused by extensive trauma or osteomyelitis can be treated by various surgical methods. Reconstruction by vascularized bone transfer, the Ilizarov intercalary bone transport method and cancellous bone grafting are the most widely used [1]. The repair of bone defects with these methods is generally successful; however, delayed fracture healing associated with repeated surgical procedures, prolonged ambulatory impairment and high costs is still common [2, 3], so additional surgical techniques have been developed, such as the use of external fixation devices [4, 5]. It is also known that the healing of bone fractures is to some extent impaired in diabetes, which has been extensively studied in animals [6–11] and less well in humans [12–16]. Recently, autologous platelet-rich plasma (PRP) in the form of activated platelet gel and recombinant morphogenetic proteins have been used with various results for healing wounds, ulcers, fractures and in maxillofacial settings [17–38].

These healing effects were attributed to the numerous growth factors (GFs) released by the platelets after activation [35, 39–41]. Some that have been identified so far include: platelet-derived growth factor, transforming growth factor-α & -β (TGF-α & -β), epidermal growth factor, fibroblast growth factor, insulin growth factor, platelet-derived epidermal growth factor, platelet-derived...
angiogenesis factor, interleukin-8, tumor necrosis factor-α, connective tissue growth factor, granulocyte macrophage colony-stimulating factor, keratinocyte growth factor, and angiopoietin, as reviewed by several authors [41–44].

Several recent studies on humans have provided promising clinical results when using platelet releasates as a source of GFs for the regeneration of long bone as well as foot and ankle defects [45–47]. It has been demonstrated that better and stronger bone was yielded with the use of platelet gel as compared to reconstruction with conventional methods [48, 49], and that bone density and growth were enhanced [50, 51].

The majority of clinical studies with platelet gel have been performed by using autologous platelets obtained preoperatively. However, this may not be the best solution in the cases of diabetes, since it has been shown that the expression of platelet GFs is decreased in diabetic animals [52] and that the local delivery of insulin and PRP as a source of additional GFs can considerably improve diabetic fracture healing in experimental animals [53–55].

These data led us to use a graft composed of allogeneic platelet gel mixed with autologous cancellous bone in order to improve the healing of a long bone defect in a diabetic patient who had been previously treated unsuccessfully. In this way, the healing potential of GFs obtained from a high number of allogeneic platelets was combined with the potential of autologous osteogenic and other stem cells from the cancellous bone, whereas the plasticity of the resulting mixture was used for the remodeling.

**Materials and Methods**

**Patient**

H.P., a 50-year-old male, was diagnosed as type 2 diabetic in 1998. He was treated for 4 years by peroral antidiabetic therapy (glimepirid 1 × 3 mg/day combined with metformin 2 × 850 mg/day). On June 9, 2004, he suffered a comminuted fracture of his left distal tibia and was initially treated with primary osteosynthesis. One and a half months later, he was admitted to our hospital with a necrosis that exposed osteosynthetic material on the tibia (fig. 1). We removed the osteosynthetic material and the necrotic bone from the tibial defect and installed a tubular monolayer AO/ASIF external fixator from the tibia to the foot. The infection of the fracture with *Enterobacter cloacae* was susceptible to trimethoprim/sulfamethoxazole and was treated accordingly; a vacuum seal on the skin defect was applied. The resolution of the infection was clinically and microbiologically assessed, whereas the skin defect was surgically covered with a free flap transfer. Six months after his injury (December 11, 2004), the fracture still showed no signs of healing, so it was diagnosed as a defected pseudoarthrosis. Since the tibial surface of the talocrural joint was damaged, debridement, cancellous bone implantation and arthrodesis of the ankle appeared to be the best treatment options.

**Surgical Procedure**

We approached the fracture from the anterior aspect, debrided the necrotic tissue, prepared the talocrural joint for arthrodesis, and filled the resulting bone defect of 45 ml, as measured with CT-assisted imaging, with a graft under perioperative antibiotic coverage with cephalosine (fig. 2). At the same time, a graft consisting of autologous cancellous bone harvested from the patient’s left iliac crest and suspended in the activated allogeneic platelet gel was prepared.

**Preparation of Platelet Gel Mixture**

The platelet gel originated from a standard allogeneic random single donor platelet concentrate that was ABO- and RhD-matched, serologically HIV, HBV, HCV and Lues-negative, HCV RNA-negative, leukocyte-depleted and irradiated. A standard single donor platelet concentrate was prepared from 450 ml of whole blood, containing $7 \times 10^9$ platelets in 50 ml of citrated plasma and stored in a plastic bag designed for platelet storage at 20–24°C on an automatic agitator for up to 5 days. Leukocyte depletion was performed by using a commercial filter (BioP05 Plus, Fresenius HemoCare, Bad Homburg, Germany) with 10–15% platelet loss postfiltration. The platelet concentrate was then irradiated with a cobalt irradiator. All platelet-related procedures, including the bacteriology controls, were performed according to the recommendations for blood banking procedures [56].

Finally, 50 ml of the graft mixture consisted of 25 ml of lightly compressed autologous cancellous bone from the anterior iliac crest and 25 ml of allogeneic platelet concentrate with approximately $1.4 \times 10^9$/platelets per 1 ml (which is around 5 times the
physiologic number of platelets in the blood). The final graft volume of 50 ml contained approximately $30 \times 10^9$ of platelets. The cancellous bone was ground in a manual grinder. The ingredients were mixed and 1.50 ml of human thrombin (100 IU/ml) in 40 mM CaCl$_2$ (Tissucol, Beriplast P, ZLB Behring, Marburg, Germany) were added for the activation of platelets. This resulted in the formation of a mixture with the appropriate plasticity in 2 min. The resulting gellous graft was modeled according to the defect, and the final graft volume of 45 ml was implanted. This was followed by closure of the wound, and negative pressure suction was placed subcutaneously, outside of the graft, in order to minimize the removal of GFs. All procedures were carried out within a sterile operation field.

Postoperative Care and Follow-Up
The suction tube was removed on the second day following the operation, after draining a total of 600 ml of partially serous and bloody fluid. The other postoperative care was uneventful. The patient was discharged from hospital 6 days after the operation and was regularly examined in the outpatient clinic; he was x-rayed at 2, 6, and 12 months. At 6 and 12 months, the bone structure was assessed by CT scan. The potential immune reactions relating to the use of allogeneic platelets were monitored by screening the patient for the presence of anti-HLA/class I antibodies and anti-HPA (human platelet antigens) antibodies at week 14 after the procedure. The standard in-house platelet immunofluorescence test and antigen capture ELISA test (PAK-12, GTI, Brookfield, Wisc., USA) were used for screening antibodies.

Results
Three months after the operation, the external fixator was removed and the patient began partial weight bearing. The monitoring of healing with x-ray and CT scans after 6 and 12 months showed that his bone defect had been solidly bridged by the implant and that it had fused with both the proximal and distal part of the fracture, and showed the consistency of normal bone (fig. 3). By that time, the patient could fully load his leg, and both legs were of the same length. No side effects were observed and no platelet or HLA class I antibodies were detected.

Discussion
Bone formation in long bone defects still represents a clinical challenge, especially in patients with diabetes. Recently, various platelet-derived GFs have been used as an adjunct in various clinical settings. In the majority of these, autologous platelet gel was used as a source of GFs [42, 57]. However, autologous platelets might have some disadvantages, especially in diabetic patients. The expression of GFs in platelets of diabetic animals and the potential of autologous PRP for healing fractures are decreased [52, 55]. Autologous platelet gel preparation requires preoperative apheresis or blood draws from the patient. This procedure adds to the risk and cost of surgery [33].
Being a standard blood bank product, single donor platelets are very easy to acquire; they are available in large quantities, and are safe and affordable. They are highly standardized in terms of platelet content, residual leukocyte and red blood cell content, the centrifugal forces used for their isolation, the temperature of the centrifugation, techniques of separation and processing and the composition of preservative solution. In addition, they are constantly resuspended on agitators in a standard manner. On the other hand, autologous platelet preparations are subject to enormous variability [42, 58], and this hinders serious studies of their clinical efficacy. Although there is some evidence that the GFs are released to some extent from the stored platelet concentrates, the majority of GFs remain intact in the platelet granules if they are appropriately stored for 5 days [59]. Based on these facts, we were of the opinion that allogeneic platelets constitute a viable alternative to autologous preparations obtained by preoperative apheresis. Therefore, a standard platelet concentrate from the blood bank was used as a source of activated platelet gel.

For the prevention of immune and bacteriological side effects, among which alloimmunization to HLA class I and HPA antigens is the most common [60, 61], the platelets were leuko-reduced and irradiated. In fact, there was no evidence of immune reactions or transfusion-transmitted infection following the procedure.

The clinical results in the present case were confirmed by radiographic evidence. The combined autologous/allogeneic graft showed successful incorporation into the defective pseudoarthrosis, and the patient achieved a satisfactory clinical improvement with no side effects. To our knowledge, this is the first report to use allogeneic platelets mixed with autologous cancellous bone for the treatment of a fracture with bone loss. A wider prospective clinical study is required to demonstrate the effectiveness and reproducibility of this therapeutic approach.

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References


