ABSTRACT

Purpose. To evaluate the effect of autogenous platelet-rich plasma (PRP) for fresh-frozen allografts in tibial defect reconstruction in rabbits.

Methods. 40 adult New Zealand white rabbits underwent tibial defect reconstruction with autografts (n=12), allografts without PRP (n=12), or allografts with PRP (n=12) and were observed for 12, 16, and 24 weeks (4 for each period). Tibias of the remaining 4 rabbits were used as donor allografts, and the remaining allografts were procured from recipient rabbits. A 1.5-cm cortical segment of the tibia was osteotomised, and then fixed with a 9-hole mini-compression plate and 2 cerclage wires. Allografts were stripped off the periosteum and soft tissues and medullary contents, and then stored in a freezer at -80°C. All allografts were deep frozen for at least 4 weeks before transplantation. 7 ml of whole blood was drawn to prepare 1 ml of PRP. The PRP was then mixed with 1.0 ml of human thrombin to form a platelet gel. The PRP gel was then packed into the medullary canal of the allograft and applied on the cortical surface before tibial defect reconstruction. Rabbits were sacrificed at 12, 16, and 24 weeks. The specimens were assessed for bone union at host-graft junctions and for bone resorption, new bone formation, callus encasement, and viable osteocyte counts.

Results. There were 4 specimens in each group at each observation period. Osteoid bridging the gap at host-graft junctions was noted in all specimens in the autograft and allograft-with-PRP groups at week 12 and in the allograft-without-PRP group at week 24. Bone union in allografts without PRP was delayed. All indices for biological incorporation (resorption index, new bone formation index, callus encasement index, and viable osteocyte count) were significantly greater in the autograft than allograft-without-PRP groups, except for the resorption index at week 24, whereas the differences were not significant between the autograft and allograft-with-PRP groups. The differences between the 2 allograft groups were usually not significant, except for the resorption index.

Conclusion. PRP-augmented allografts behaved similarly to autografts for tibial defect reconstruction in rabbits. PRP increased bone union and bone resorption.

Key words: growth factors; platelet-rich plasma; tibia; transplantation, homologous
INTRODUCTION

Options for bridging large bone defects include vascularised autografts, non-vascularised autografts, allografts, custom-made prostheses, and bioceramics. Use of allografts has advantages of less surgical morbidity and reduced operative time and hospital costs, but involves the risks of disease transmission, non-union, and lack of biological incorporation. In a cat study, biological incorporation of non-vascularised cortical autografts was good, with a bone resorption rate of 13% at 12 weeks, a cortical new bone formation rate of 4.3% at 16 weeks, and a callus encasement rate of 8% at 16 weeks. In contrast, reparative activity was minimal in cortical allografts, with a bone resorption rate of 1.8% at 9 months, and a cortical new bone formation rate of 0.74% at 9 months. Moreover, allografts may lose much of their strength over time.

To improve remodelling and incorporation of the allografts, recombinant growth factors such as bone morphogenic proteins (BMPs), platelet-rich plasma (PRP), bone-marrow–derived mesenchymal stem cells (MSC), and a combination of these have been used. PRP can be obtained from the patient’s own blood and is much cheaper than BMPs. PRP enhances bone regeneration and density, as it contains a variety of growth factors including platelet-derived growth factor, transforming growth factors-beta, insulin-like growth factor, epidermal growth factor, and epithelial cell growth factor. These factors are initiators of bone healing at the fracture site and host-graft junction. They are known to stimulate osteoblasts in vivo and in vitro and influence angiogenesis given the presence of some angiogenic factors.

The effect of PRP on bone grafts in non-weight-bearing bones has been reported. PRP has a profound effect on wound healing enhancement and bone regeneration on cancellous cellular marrow grafts. Grafts with PRP achieve greater bone density and a radiographic maturation rate 1.62 to 2.16 times that of grafts without PRP. In a goat study where the defect of the mandibular angle was filled with an autogenous particulate bone grafts with and without PRP, histological and histomorphometric evaluation showed PRP significantly enhanced bone healing. We evaluated the effect of PRP on deep-frozen tibial allografts in adult rabbits.

MATERIALS AND METHODS

Between January 2008 and July 2008, 40 adult, male, New Zealand white rabbits with a mean weight of 3.22±0.52 kg underwent tibial defect reconstruction with autografts (n=12), allografts without PRP (n=12), or allografts with PRP (n=12) and were observed for 12, 16, and 24 weeks (4 for each period). Tibias of the remaining 4 rabbits were used as donor allografts, and the remaining allografts were procured from recipient rabbits.

General anaesthesia was used with ketamine (35 mg/kg) and xylazine (5 mg/kg) for induction, and with isoflurane and oxygen for intubation. An incision was made on the right shin of the rabbit’s leg to expose the mid-shaft of the tibia (Fig. 1). A 1.5-cm cortical segment of the tibia was osteotomised using an oscillating saw (Fig. 1). This defect size is critical because the defect does not heal by fracture callus alone. It must be filled with a bone graft to achieve bony continuity. In the autograft group, the segment was flushed with normal saline to remove all blood and debris, and then re-placed into its original bed and fixed with a 9-hole mini-compression plate with 3 screws each proximally and distally and 2 cerclage wires (Fig. 1). The wound was flushed with 50 ml of normal saline containing 250 mg of cloxacillin and 250 mg of ampicillin. The soft tissues and skin were closed with absorbable vicryl sutures, and a below-knee plaster-of-Paris cast was applied. Postoperatively, cephalixin (10 mg/kg 6 hourly) was administered for one week. Activities inside the cage were allowed. The cast was removed after 6 weeks.

Figure 1 (a) Mid-shaft segment of right tibia is exposed. (b) A 1.5-cm mid-shaft segment is excised. (c) Excised segment is replaced with a graft. (d) The graft is fixed with a 9-hole mini plate and 2 cerclage wires.
For allograft procurement, the periosteum and soft tissues and the medullary contents were stripped off. The allografts were flushed with normal saline to remove all blood and debris, and then soaked in 20 ml of normal saline solution containing 250 mg of ampicillin and 250 mg of cloxacillin, and then stored using the sterile double jar technique\textsuperscript{31} in a freezer at -80ºC. All allografts were deep frozen for at least 4 weeks before transplantation. For allograft transplantation, the deep-frozen allograft was thawed in 50 ml of normal saline containing 250 mg of ampicillin and 250 mg of cloxacillin for about 30 minutes.

For preparation of PRP, 7 ml of whole blood was drawn from the marginal auricular vein using a 10-ml syringe containing 1 ml of anticoagulant citrate–dextrose solution during the operation. It was purified using a platelet concentrate collection system\textsuperscript{21} and centrifuged twice, first at 3000 rpm for 225 s to obtain the ‘buffy button’ fraction, and then at 3000 rpm for 780 s to further separate the buffy coat, which was the PRP. About 1 ml of PRP was obtained from 7 ml of blood. The PRP was then mixed with 1.0 ml of human thrombin\textsuperscript{32} to form a platelet gel before infiltrating into allografts.\textsuperscript{21} The thrombin concentration was 500 IU/ml. The final ratio between thrombin and plasma was 1:7. The PRP gel was then packed into the medullary canal of the allograft and applied on the cortical surface before tibial defect reconstruction.

Bone union was evaluated clinically (mobility at the host-graft junction), radiographically (callus at fracture sites), and histologically (bridging of fracture gap by osteoid). At the end of each observation period, the rabbit was sacrificed under general anaesthesia with intramuscular pentobarbitone (>200 mg/kg). The whole right tibia was procured by disarticulation at the knee and ankle. Soft tissues were dissected to expose the tibia, and photographs and radiographs were taken. The plate and wires were removed, and mobility at both host-graft junctions was evaluated. The specimen was then fixed in 10% buffered formalin. The distal and proximal ends of the host tibia were excised to leave a central 3.5 cm portion containing the graft (1.5 cm) and the proximal (1 cm) and distal (1 cm) host-graft bone junctions. The specimen was sectioned longitudinally into 2 halves in the plane perpendicular to the plate. Undecalcified sections of the specimen (5-µm thick) were cut and then stained with Toluidine blue. Histological examination and histomorphometry was performed in the middle 50% of the graft (middle 7.5 mm).

![Figure 2](image-url)

**Figure 2** Host-graft junctions of specimen at 12 weeks (Toludine blue, x40): (a) osseous union and small resorption cavities (RC) in the cortex are seen in autografts; (b) fibrous tissue (with no bone union) and few RC in the cortex are seen in allografts without platelet-rich plasma (PRP); and (c) osseous union and numerous RC in the cortex are seen in allografts with PRP.
Bone union was defined as bridging of the host-graft junction with osteoid tissue seen in histological sections stained with Toluidine blue at a magnification of 40x (Fig. 2). The following indices were assessed using an image analyser system interfaced with a CCD camera to quantify biological healing of the graft. The resorption index was defined as the total area of all resorption cavities seen in both cortices, and was expressed as a percentage of the total area of both cortices quantitated on an undecalcified section stained with Von Kossa at a magnification of 40x. The cortical new bone formation index was defined as the total area of new bone formed in the cortices expressed as a percentage of the total area of both cortices measured on an undecalcified section stained with Toluidine blue at a magnification of 200x using an average of 3 microscope fields. The callus encasement index was defined as the total area of callus around the central 7.5 mm of the graft expressed as a percentage of the total area of both cortices measured on an undecalcified section stained with Toluidine blue at a magnification of 20x. The osteocyte index was defined as the total number of lacunae occupied with osteocytes expressed as a percentage of the total number of lacunae in each microscope field using an undecalcified section stained with Toluidine blue at a magnification of 200x and taking the average of 3 microscope fields. All these indices were quantitated in the central 50% of the graft segment to reflect biological incorporation of the graft itself.

The differences in histomorphometric indices between groups at each observation period were compared using the Student’s t test. A single-factor analysis of variance, followed by a Tukey post hoc test was used to compare all indices between the 3 observation periods for each group. A p value of <0.05 was considered statistically significant.

## RESULTS

There were 4 specimens in each group at each observation period. Osteoid bridging the gap at host-graft junctions was noted in all specimens in the autograft and allograft-with-PRP groups at week 12 and in the allograft-without-PRP group at week 24 (Table 1). Bone union in allografts without PRP was delayed.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of specimens with bone union</th>
<th>12 weeks</th>
<th>16 weeks</th>
<th>24 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autograft (n=4)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Allograft without platelet-rich plasma (n=4)</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Allograft with platelet-rich plasma (n=4)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

### Table 1

**Histological bone union at host-graft junctions**

### Table 2

**Indices for biological incorporation of bone**

<table>
<thead>
<tr>
<th>Index</th>
<th>Group</th>
<th>1 (autografts)</th>
<th>2 (allografts without platelet-rich plasma)</th>
<th>3 (allograft with platelet-rich plasma)</th>
<th>Groups 1 vs. 2</th>
<th>Groups 2 vs. 3</th>
<th>Groups 1 vs. 3</th>
</tr>
</thead>
</table>
All indices for biological incorporation (resorption index, new bone formation index, callus encasement index, and viable osteocyte count) were significantly greater in the autograft than allograft-without-PRP groups, except for the resorption index at week 24. The differences were not significant between the autograft and allograft-with-PRP groups (Table 2). The differences between the 2 allograft groups were usually not significant, except for the resorption index (Table 2). The indices were usually greater (but not significantly) in the allograft-with-PRP group.

There were more viable cells in the centre of the transplant in the autograft than allograft-with-PRP groups (cellular index, 10 to 15% vs. 4 to 7%).

DISCUSSION

PRP contains various growth factors and is an effective promoter of bone healing. It can be easily obtained from the patients’ own blood on the day of surgery and thus carries no risk of any transmissible disease. PRP is more economical than rh-BMP2 or BMP7. Autografts combined with PRP achieve quicker maturation of bone transplants and higher bone density. PRP also has the potential to improve soft-tissue healing.

Allografts are useful for orthopaedic and maxillofacial reconstructive surgeries, but they carry risks of disease transmission and non-union. Although autografts are the gold standard for bone defect reconstruction, there are problems related to donor-site morbidity and limited supply. Allografts combined with PRP increase the healing rate of bone defects. Other growth factors such as BMP2 and BMP7 also increase bone healing. A combination of MSCs and PRP with allografts accelerates bone healing and remodelling processes, as does a combination of allogenic PRP and high surface ceramic scaffolds (calcium-deficient hydroxyapatite), but a combination of MSC and PRP did not improve bone healing further.

Allografts are relatively inert. In our study, when PRP was added into allografts, the resorption activity increased significantly and accelerated bone union. This finding is consistent with other studies. Nonetheless, the increases in the other indices of bone healing were not significant.

ACKNOWLEDGEMENTS

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REFERENCES