

THE EFFECT OF AUTOGENOUS, NON-VASCULARIZED PERIOSTEUM TRANSPLANTATION ON ALLOGRAFT BONE GRAFTING FOR LARGE CORTICAL LOAD-BEARING BONE DEFECT – A RABBIT MODEL

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ABSTRACT

Segmental bone allografts are widely used in managing large cortical bone defects. To improve host-graft union, the effect of allograft on a large cortical tibial bone defect augmented with a non-vascularized periosteal flap was studied. Twelve mature Australian white rabbits (*Oryctolagus cuniculus*) were divided into intervention groups and a control group. Bone defects in the intervention groups were treated with segmental allografts wrapped with a non-vascularized periosteal flap. The control group was treated with allograft transplantation alone. Healing was evaluated at the end of the 2nd, 4th, and 6th weeks with plain radiographs, CT scan, and histology. In the intervention groups, the bony union was achieved at both ends of the allografts at the 4th to 6th weeks. Solid callus encasing the whole allograft segments at the end of the 6th week. In the control group, the union did not occur at both ends of the allograft segments even up to the end of the 6th week. No callus formation surrounding the allograft segments. Fragmentation and telescoping of the allograft segment into the medullary cavity of the host were observed. The use of autogenous, non-vascularized periosteal flap modified the healing process of allograft and maintained the integrity of the allograft.

Key words: Allograft, bone grafting, large cortical defect, periosteum, union

INTRODUCTION

The periosteum is a specialized connective tissue that forms a fibrovascular membrane covering the entire surface of bone except for the articular cartilage, ligament at the site of tendon insertions, and the surface of sesamoid bones (Neel, 2003; Allen *et al.*, 2004; Dwek, 2010). It consists of two histological layers; an outer fibrous layer containing blood vessels and nerves supplying the bone, and the inner cambium layer adjacent to the bone, in

which the cambium (osteogenic) cells reside (Allen *et al.*, 2004; Colnot, 2009; Wang *et al.*, 2017). The cambium cells are osteogenic progenitor cells of mesenchymal origin (Driscoll, 1999; Simon *et al.*, 2003; Colnot, *et al.*, 2012). In children, the cambium layer contributes to the increase of the diameter of the bone during growth (Bisseret *et al.*, 2015). In adults, the cambium cells may be activated after the mechanical stimulus (trauma), infection, and tumorous growth. Under these circumstances, this layer is capable of inducing callus tissue formation and osteogenesis (Simon *et al.*, 2003; Zhang *et al.*,

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2005; Giannoudis *et al.*, 2007; Colnot, 2009; Colnot *et al.*, 2012; Mercurio *et al.*, 2012; Wang *et al.*, 2017). This ability for the cambium cells to differentiate into chondrogenic or osteogenic cells has stimulated interest in their use (Nather & Goh, 2000; Neel, 2003; Vögelin *et al.*, 2005; Chang & Knothe-Tate, 2012; Ferretti *et al.*, 2012; van Gastel *et al.* 2012).

A large segmental bone defect is a common problem faced in orthopedic practice and various surgical options are available for bridging such defect (El-Mowafi *et al.*, 2005). The use of a large segmental bone allograft has become an accepted method of replacing bone defects (McAuliffe, 2003; Vidal *et al.*, 2020). But there are many possible complications associated with this approach. Non-union, delayed incorporation of graft and failure of an allograft is well observed and documented (Finkemeier, 2001; Betz, 2002; Karaoglu *et al.*, 2003; Zhao & Zeng, 2003; El-Mowafi *et al.*, 2005; Giannoudis *et al.*, 2007; Vidal *et al.*, 2020). To improve the host-graft union, autogenous vascularized periosteum overlying allografts were used to augment the incorporation of the graft in reconstructive surgery (Karaoglu *et al.*, 2003; Neel, 2003; Barckman *et al.*, 2013).

It is interesting to explore whether the beneficial effects of periosteal grafts transplanted for augmentation of the healing of large segmental bone grafts are the same if the vascularity of the periosteum is not surgically restored during transplantation. The current study was designed to determine whether free autogenous non-vascularized periosteum can survive transplantation *in-vivo* and to observe its effects on the incorporation of the allograft.

MATERIALS AND METHODS

A total of 12 mature male New Zealand white rabbits (*Oryctolagus cuniculus*) aged between 3-5 months old and weight of 2-3 kg were used in this study. The animals were randomly divided into two groups to form an intervention group (9 rabbits) and a control group (3 rabbits). Two assessment periods were chosen to show the differences in the graft incorporation and healing changes as previous studies indicated histological evidence of fusion-healing changes at around 4-8 weeks (Bax *et al.*, 1999; Nather & Goh, 2000; Karaoglu *et al.*, 2003; Vögelin *et al.*, 2005). As such, the intervention group was further subdivided into 3 groups of 3 rabbits according to the time of observations (at second, fourth, and sixth weeks, respectively).

Two orthopedic surgeons with previous experience in animal studies and handling performed the administration of the anesthesia and the surgery. All the rabbits were anesthetized using a cocktail

of intravenous Ketamine 35 mg/kg and Xylazine 5 mg/kg. A prophylactic single dose of antibiotic (Ampicillin 25 mg/Kg) was also administered intramuscularly. Under full aseptic technique, a surgical incision was made longitudinally over the anteromedial aspect of the right leg proximal to the ankle joint to expose the tibial bone. A 3 cm segment of the tibia was exposed and osteotomized at the distal third using a saw with saline irrigation. This segment of bone was kept as fresh frozen allografts in a freezer at -40°C. They were stripped bare of their surrounding soft tissues before freezing and to be used for the subsequent rabbits. The defect in the right tibia was replaced with a previously prepared fresh-frozen allograft and stabilized with a Kirschner wire with a diameter of 2 mm passed retrogradely through the intramedullary canal of the proximal tibia. The wire was cut flushed the bone and buried it subcutaneously.

The bone defects in the rabbits from the control group were treated with allograft transplantation alone. In the intervention group, another similar incision was made on the left leg. The wound was made to expose the periosteum, which was incised and stripped circumferentially away from the bone. The free periosteum of the left tibia was then transferred to the right side and wrapped around the allograft segment and anchored with several sutures. The wounds were irrigated with normal saline and then closed in layers. The leg was immobilized in a Plaster of Paris (POP) cast, extending from above the knee joint to the metatarsal of the foot with the knee kept in 90 degrees flexion and ankle in plantigrade position.

Radiological and histological evaluations were performed at the end of the second, fourth, and sixth weeks. Three rabbits from the intervention group and a rabbit from the control group were selected at each time of observations. They were subjected to plain radiographs and CT scans of the right tibia with an infusion of intravenous radio-opaque dye. These were performed under general anesthesia. After the radiological evaluations, the rabbits were euthanized by giving an overdose intravascular injection of pentobarbitone 60 mg/kg. The right tibia was harvested by disarticulating through the knee and the ankle joints. The specimen with its surrounding soft tissue was kept submerged in 10% formalin for hematoxylin and eosin (H&E) staining and examinations.

RESULTS AND DISCUSSION

The ideal model of a bone graft should have all the qualities of osteoconduction, osteoinduction, osteogenesis, and structural integrity (Betz, 2002; Finkemeier, 2002; McAuliffe, 2003; Giannoudis *et*

al., 2007; Eskandar *et al.*, 2008). An autogenous tricortical bone graft possesses all these, hence, remains as the gold standard option. However, harvesting leads to donor site morbidity and the source is limited (Betz, 2002; Finkemeier, 2002; Giannoudis *et al.*, 2007). Researchers turned to innovating and developing composite grafts that can combine these qualities in a graft model (Giannoudis *et al.*, 2007). The current study was designed in line with this philosophy with the periosteal tissue providing the osteoinductive and osteogenetic properties, whereas the allograft acted as a scaffold (osteoconduction) and structural support. Rabbits were used for this study considering the short duration of fracture healing in them. Only mature male rabbits were used to standardize the gender and to avoid the effects of hormonal fluctuation in female rabbits on bone healing (Arnsdorf *et al.*, 2008; Eskandar *et al.*, 2008).

Two weeks post-transplantation: early healing changes

All the samples from both groups presented with very mobile osteotomy sites. The plain radiographs showed clear gaps at the osteotomy sites (Figure 1a). In some of the specimens from the intervention group, indistinct callus was seen partially surrounding the proximity of the allograft (Figure 2a). Histological evaluation revealed attempted healing at the osteotomized sites in specimens from both groups (Figure 3a). The transplanted periosteum in the intervention group remained adhered to the allografts with no spaces in between them. The periosteum had lost their cellular components and became thicker, but they maintained their architectural pattern (Figure 3b). These were evidence that the periosteum was undergoing repair and surviving in the new environment. The allografts in both groups showed remnants of cells in the lacuna spaces and there were no surviving cells seen.

Four weeks post-transplantation: restoration of periosteal functions and neovascularization

Plain radiographs of the rabbits from the intervention group showed the presence of callus at the host-graft junctions indicating union at both proximal and distal ends. Callus formations were also seen encasing the allografts segments. However, the fracture lines were still visible (Figure 2b). CT images with contrast media enhancement confirmed the presence of these encasing callus (Figure 2c). In the control group, the osteotomy sites remained mobile. Some callus was present over the osteotomy sites but not on the allograft segment as seen in the intervention group. The allograft segment appeared smooth without any callus or other soft tissue cover (Figure 1b). Histologically, an excessive healing process was present at both host-graft junctions

(Figure 1d). Callus was seen produced by the survived periosteum in the intervention group as well (Figure 3c). In some of the specimens, the presence of intraosseous callus containing normal osteocytes and new blood vessels was seen in the allograft segments (Figure 3d).

Six weeks post-transplantation: retainment of the integrity of allograft

All specimens from the intervention group achieved the union of the host-graft junctions with abundant callus formation (Figure 2d). Exuberant callus was also observed encasing the whole allograft segments and present in the intramedullary space, protecting the graft and maintaining its integrity (Figure 3e & 3f). On the other hand, the allograft segment in the control group was seen fractured and fragmented at the distal host-graft junction leading to telescoping of the graft into the medullary cavity of the host bone despite having abundant surrounding callus (Figure 1e).

Vascularized versus non-vascularized transplantation of periosteum

The use of vascularized periosteum to augment the healing and incorporation of the bone grafts was proven and reported in previous studies (Driscoll, 1999; Neel, 2003; Vögelin *et al.*, 2005; Karaoglu *et al.*, 2013; Barckman *et al.*, 2013). Zhao and Zheng (2003) concluded that large bone defects can be repaired with vascularized tubulate periosteum with or without combination with bone filling materials. The current study was in favor of the results reported by these studies. On the other hand, Askar and Sabuncuoglu (2003) and Eskandar *et al.* (2008) did not believe that a transplanted periosteum could provide the osteogenetic properties and healing relied directly on the host's recipient bed.

But vascularized transplantation requires microsurgical expertise and takes a longer operational time. Not all surgical institutions can provide the services. In the current study, non-vascularized periosteum was used to augment the healing of allografts. It is technically less demanding than restoring vascularity. The periosteum graft has been proved to be able to initiate osteogenesis in the new environment (Eskandar *et al.*, 2008). But one of the major concerns was to determine whether the free non-vascularized periosteum can survive the transplantation and retained its biological and physiological functions. This was observed in the results obtained from the intervention group of the current study.

The surgical technique of harvesting the periosteum is critical for the success of the transplantation. The cambium layer containing the osteogenic cells delicately adhere to the fibrous layer and can be damaged or left behind on the bone when

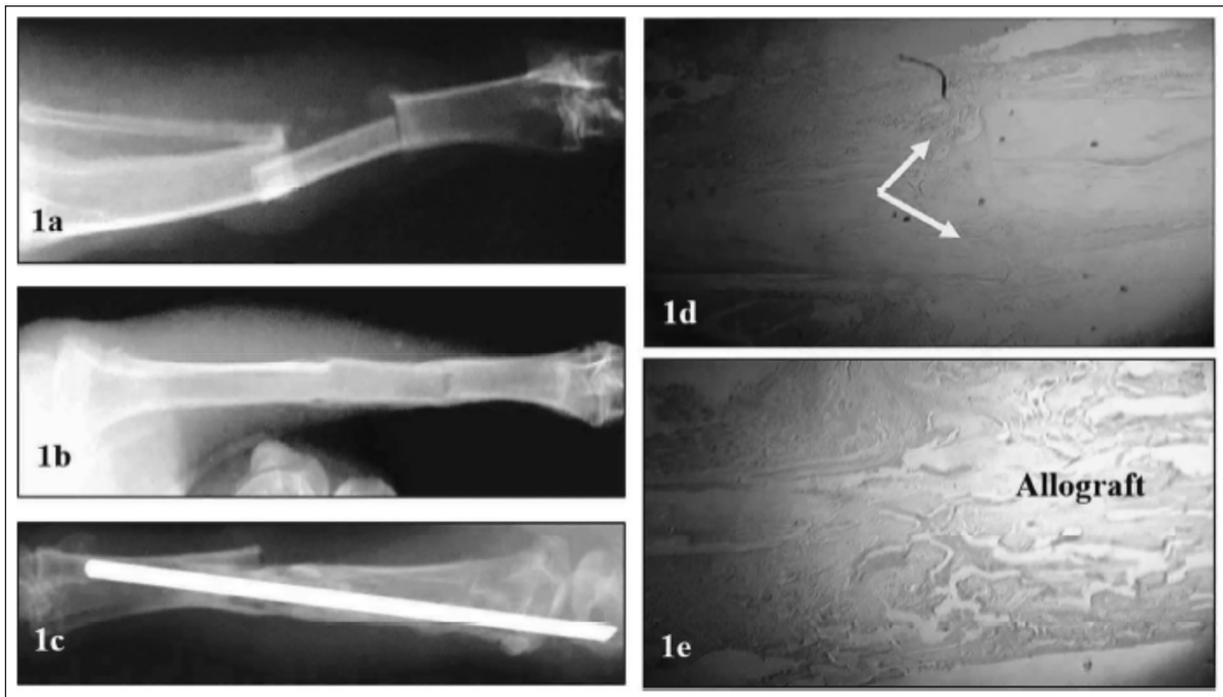


Fig. 1. Evaluation of the right tibiae from the control group. Plain radiographs were taken at the end of (a) 2 weeks, (b) 4 weeks, and (c) 6 weeks. (d) Evidence of the healing process at the host-graft junction started to appear at the end of the fourth week (as shown by the white arrow). (e) At the end of the sixth week, the allograft was seen fractured, fragmented, and telescoped into the medullary cavity of the host bone, despite having abundant surrounding callus.

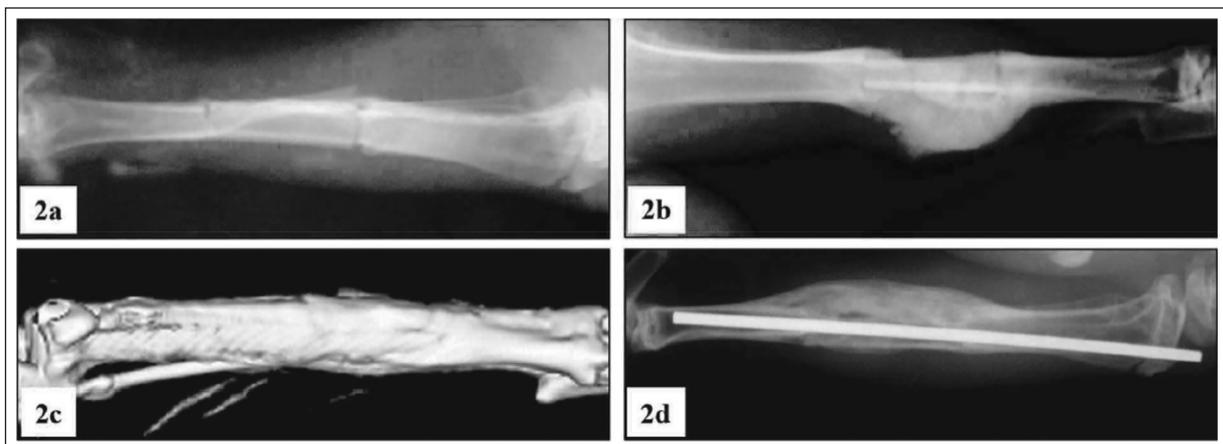


Fig. 2. Radiological evaluation of the right tibiae from the intervention group. (a) At the end of the second week, the osteotomy sites were still visible with minimal callus surrounding the allograft segment. (b) An abundance of callus was seen at both ends and around the allograft at the end of the fourth week on (b) plain radiograph and (c) 3D reconstructed CT image. (d) Bony union was evident at the host-graft junction with exuberant callus was seen encasing the whole of the allograft segment at the end of the sixth week.

the periosteum is harvested (Brownlow *et al.*, 2000; Driscoll & Fitzsimmons, 2000; Simon *et al.*, 2003; Zhang *et al.*, 2005; Colnot, 2009; Dwek, 2010; Colnot *et al.*, 2012; Mercurio *et al.*, 2012; Bisseret *et al.*, 2015). Technical precautions should be taken during periosteal harvesting and transplantation.

CONCLUSION

The free non-vascularized periosteum survived the *in-vivo* transplantation and retained its beneficial biological and physiological functions. The allograft healing and host-junction incorporation was

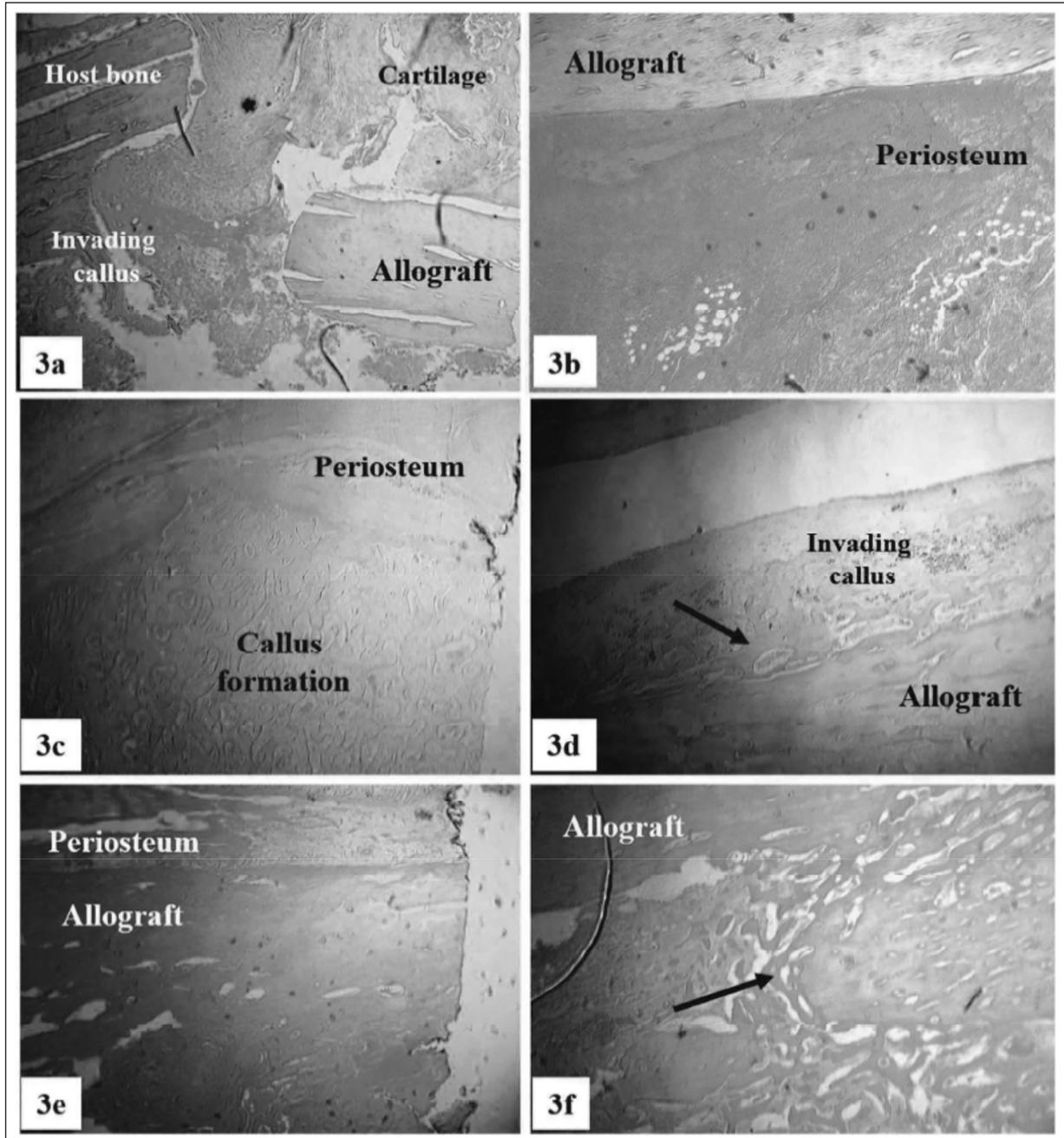


Fig. 3. Histological evaluation of the right tibiae from the intervention group. At the end of the second week, (a) attempted healing was visualized at the host-graft evident by the presence of callus, and (b) the periosteum remained to adhere to the allograft and became thickened, indicating that it was undergoing repair and surviving in the environment. At the end of the fourth week, (c) the survived periosteum started to produce callus, and (d) evidence of intraosseous callus formations with neovascularization (as shown by the black arrow). (e and f) The integrity of the allograft was maintained with parts of the allograft being replaced by new bone (as shown by the black arrows).

modified by the transplanted periosteum by hastening the callus formation at the host-graft junctions. The integrity of the allograft was protected by the formation of callus encasing the graft as well as the presence of intramedullary callus.

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