

## Biomechanical assessment of freeze-dried allograft cortical bone plate graft in canine bone defect model

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**ABSTRACT:** Freeze-dried cortical bone can be used as a biological plate, either alone or in combination with other internal fixation devices, to stabilize fractures. In addition to it conferring mechanical stability, freeze-dried cortical bone may enhance fracture-healing and increase the bone stock. This study examined the effect of a freeze-dried allograft cortical bone plate (FACBP) on the biomechanical properties of an implant site in a canine bone defect model. Twelve adult mongrel dogs (around 4.8 kg) were used. A segmental critical-size defect (5 mm in length) at ulna diaphysis was created using an oscillating saw. The experimental animals were divided into two groups: eight dogs treated with an absorbable bone plate (FACBP) fixed by metal bone screws (Group A) and four dogs treated with a commercial stainless steel bone plate and metal bone screws (Group B). Bone healing was assessed by radiography, Dual-energy x-ray absorptiometry and a three-point bending test. The FACBP incorporated in the host bone produced complete remodeling of the cortical bone. There was no significant difference in the bone mineral density and biomechanical tests between the FACBP application site and normal ulna or a stainless steel bone plate of the ulna. These results suggest that FACBP facilitates recovery from a bone fracture by assisting in the induction of new bone formation in a defected fracture.

**Keywords:** bone defect model; dual-energy x-ray absorptiometry; absorbable bone plate

There has been increasing interest on bone transplants in orthopedic surgery. Bone transplants can be used to treat fractures, nonunion and osteomyelitis. Other orthopedic indications include limb lengthening, reconstructive procedures after a tumor resection, arthrodeses and osteointegration of joint replacement prostheses. The clinical use of these techniques extends beyond the field of orthopedics to include periodontal, maxillofacial and neurosurgery (Sinibaldi, 1989; Wilson et al., 2005). Although autogenous bone is the gold standard that all alternatives must either meet or exceed, autografts have significant limitations, such as an additional surgical incision, donor site morbidity, weakened donor bone sites, an inadequate amount and an inappropriate form (Enneking et al., 1980; Burchardt, 1987; Finkemeier, 2002). These limitations have prompted increasing interest in alternative bone grafts.

The most common allograft is freeze-dried and deep-frozen. Preservation is obtained by either freezing or freeze-drying. Both allow extended storage but reduce the immunogenicity of the graft and may alter its mechanical strength. Allografts are processed and preserved in a variety of ways in order to maintain the osteoinductive and osteoconductive capacity of the material as well as to reduce its immunogenicity (Burchardt, 1987; Vajaradul, 2000).

Bone density changes secondary to plate application have been documented using biomechanical testing, photon absorptiometry, direct and morphometric caliper measurements, ashing procedures, histological and fluorescence microscopy and microradiography (Faulkner et al., 1991; Freeman et al., 1996; Grier et al., 1996; Millis et al., 1998; Ebbesen et al., 1999; Schneider et al., 2004).

Dual-energy x-ray absorptiometry is the method most commonly used for a quantitative *in vivo*

assessment of the bone mineral status in humans (Theodorou and Theodorou, 2002), and is also the method most commonly used in dogs (Freeman et al., 1996; Grier et al., 1996; Lauten et al., 2001). The technique can be performed quickly and has a low radiation dose but relatively high precision and accuracy (Grampp et al., 1993).

Freeze-dried allograft cortical bone (FDACB) can act as a biological plate, either alone or in combination with other internal fixation devices, to stabilize fractures (Chandler and Tigges, 1998; Brady et al., 1999). As well as conferring mechanical stability, FDACB may enhance fracture-healing and increase the bone stock (Malinin et al., 1984; Wilson et al., 2005). Previous reports suggested that cortical allografts unite, remodel and mature with the host bone (Haddad and Duncan, 2003). However, the long-term effect of a FDACB graft on the implant site is not known.

This study examined the effect of long term FDACB plate application on the implant site density and strength in dogs. The change in cortical bone density was determined by comparing the strength of the implant site of the ulna with that of a normal contralateral ulna or a stainless steel bone plate of the ulna.

## MATERIAL AND METHODS

### Animals and experimental design

Twelve healthy adult mongrel dogs, weighing 4–6 kg ( $4.8 \pm 1.1$  kg), and aged from 2–6 years ( $4.1 \pm 1.7$  years) were used in this study. The basic experimental model consisted of an ulna diaphysis transverse defect (5 mm in length), which was created using an oscillating saw under continuous saline irrigation. The experimental animals were divided into two groups. Eight dogs with a transverse defect ulna were given an absorbable bone plate treated with FDACB and a metal bone screw (Group A). Four dogs with a transverse defect ulna were treated with a stainless steel bone plate and screws (Group B).

Preparation of freeze-dried allograft cortical bone plate cortical bone was obtained from healthy two year old mongrel dogs weighing 25 kg. The ends of the long bones were cut away using a bone band saw. The midshafts were mechanically scraped clean of soft tissues, including the cartilage, periosteum and bone marrow, and washed in cold Ringer's lactated solu-

tion. The cortical bone was trimmed to the required size using a metal bone plate as a guide. The bones were dried and washed with deionized water for 1 h at 4°C with stirring performed several times. The bones were defatted in a 1 : 1 ratio of chloroform/methanol at 24°C for seven days and kept in flowing air for 1 h at room temperature to remove the residual chloroform and methanol by evaporation. The defatted bones were washed with deionized water, with stirring, for 2 h at 4°C, and frozen at –80°C. The cortical bone plate was placed in a freeze-drying chamber that had been pre-cooled to –80°C and a vacuum of  $10^{-2}$  mtorr was applied. Complete freeze drying took approximately six days. At that time, the moisture of the bone was approximately 5%. The freeze-dried bone was then double-wrapped in sterilized packs of which one side was paper and the side was plastic film. The materials were sterilized by exposure to EO gas and stored at room temperature until needed.

### Surgical procedure

Prior to surgery, the patients received 25 mg/kg cephalexin (Methilexin<sup>®</sup>, Union Korea Pharm, Korea) intramuscularly for prophylaxis. The experimental animals were premedicated with 0.02 mg/kg subcutaneous atropine sulfate (Atropine Sulfate Daewon<sup>®</sup>, Dae Won Pharm, Korea), induced with 5 mg/kg intravenous propofol (Anepol IN<sup>®</sup>, Ha Na Pharm, Korea) and maintained with enflurane and oxygen. The animals were placed in the lateral recumbency position and all surgical procedures were carried out under sterile conditions. A 5-cm longitudinal skin incision was made at the cranio-lateral aspect of the left ulna. The treatment group was given an absorbable bone plate treated with freeze-dried allograft cortical bone with a metal bone screw. (Figure 1) The control group was stabilized with a metal plate and screws according to the guidelines of the association for a study of internal fixation (ASLF). The muscle attachment was repaired and the skin was close in layers. The animals were allowed to freely bear weight immediately after surgery, and were allowed to eat.

### Gross inspection

Postmortem (135 weeks), all the specimens were visually inspected to determine the presence and

distribution of bone formation at the bone defect. In addition, the alignment of the defect ulna was compared with the contralateral ulna.

### Plain radiography

The fate of the graft was examined by x-ray imaging at 2–4 weeks intervals for 135 weeks. The parameters examined were new bone formation, union of a defected gap, and absorption of a bone plate.

### Dual-energy x-ray absorptiometry

After 135 weeks, the dogs were euthanatized by an intravenous administration of sodium pentobarbital (Entobar Inj<sup>®</sup>, Han Lim Pharm, Korea). Each ulna was dissected from the surrounding soft tissues, with care taken to preserve the bone. The bone mineral density (BMD) and bone mineral content (BMC) were determined by peripheral dual-energy x-ray absorptiometry (GE lunar PIXImus2, GE Healthcare, USA). Quality control and calibration were carried out within 24 h of each scanning period. The ulna was divided into three regions of interest, general ulna shaft, implant region and defect region. (ROI; Figure 2) The analogous ROIs were scanned in the intact contralateral



Figure 1. A 5 mm bone defect was created in the middle of the ulna and was stabilized with freeze-dried allograft cortical bone plate and metal bone screw

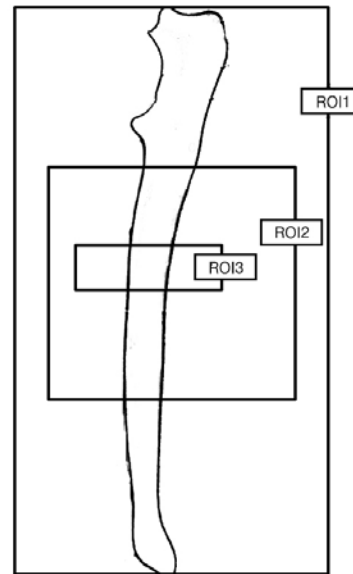


Figure 2. Illustration showing lateral view of the ulna and ROI using dual-energy x-ray absorptiometry. ROI1 = general ulna shaft, ROI2 = implant region, ROI3 = defect region

ulnas. Commercially available software (LUNAR Pixi Mus 1.43, GE Healthcare, USA) was used to determine the bone mineral density ( $\text{g}/\text{cm}^2$ ) and bone mineral content (g).

### Biomechanical testing

After x-ray absorptiometry, a Universal testing machine (WL2100C; WITH LAB CO., LTD Korea) was used for the biomechanical tests. The flexure stress of the ulna was determined by breaking the bones by 3-point bending (gap size, 30 mm) with the load being applied at a constant displacement of 15 mm/min to failure. The ulna broke in the defect region (Or midshafts ulna of the intact contralateral limb). The maximum load (N) and stiffness (N/min) were interpreted and calculated from the load-deflection curve, which was displayed continuously on a computer monitor.

### Statistical analysis

The results are presented as the mean  $\pm$  SEM. Statistical analysis of the data was carried out by one-way analysis of variance (ANOVA). A comparison of the BMD, BMC, ultimate load at failure and stiffness were made using a Student's *t*-test. A *P* value  $< 0.05$  was considered significant.

Table 1. Bone mineral density and bone mineral content of the general ulna shaft, implant region, defect ROI for Group A, Group B, and a normal ulna (mean  $\pm$  SEM)

	Group A	Group B	Normal ulna
ROI1(general ulna shaft)			
Bone mineral density(g/cm <sup>2</sup> )	0.37 $\pm$ 0.02	0.40 $\pm$ 0.006	0.35 $\pm$ 0.02
Bone mineralcontent (g)	1.76 $\pm$ 0.2	1.64 $\pm$ 0.13	1.51 $\pm$ 0.09
ROI2(implant region)			
Bone mineral density(g/cm <sup>2</sup> )	0.42 $\pm$ 0.02	0.39 $\pm$ 0.04	0.39 $\pm$ 0.02
Bone mineralcontent (g)	1.08 $\pm$ 0.08	1.09 $\pm$ 0.03	0.91 $\pm$ 0.07
ROI3(defect region)			
Bone mineral density(g/cm <sup>2</sup> )	0.42 $\pm$ 0.03	0.40 $\pm$ 0.03	0.39 $\pm$ 0.01
Bone mineralcontent (g)	0.23 $\pm$ 0.09	0.12 $\pm$ 0.03	0.09 $\pm$ 0.009

ROI = region of interest, Group A = freeze-dried allograft cortical bone plate, Group B = stainless steel bone plate

## RESULTS

### Surgical follow-up

All the dogs tolerated the surgical procedures well, and recovered fully without infection, fractures about the fixation or the need for additional surgical procedures. All dogs were able to fully bear weight on the limb.

### Gross inspection at 135 weeks

In Group A, complete healing of the defect site was observed in all eight dogs. The defect

site showed complete remodeling as a result of the marked adherence to the recipient bone. In Group B, complete healing of the defect site was observed in all four dogs. The defect site showed complete remodeling and fracture healing.

### Plain radiography

In both groups, the defect site was stable in all ulnas without evidence of screw loosening. In Group A, the radiographs demonstrated a healed fracture, the FDAB plate incorporated in the host bone and complete remodeling of the cortical bone (Figure 3). In Group B, the radiographs demon-

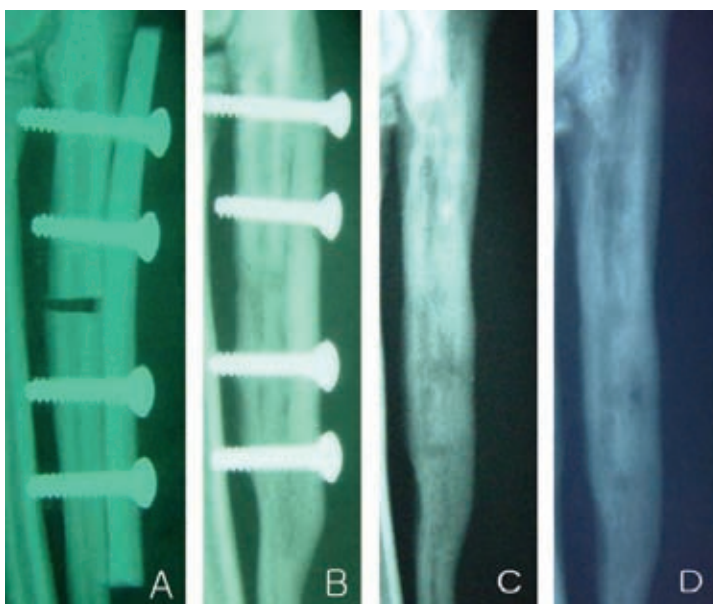


Figure 3. Radiographs demonstrating the healed defect site in Group A, with the freeze-dried allograft cortical bone plate uniting the host bone, new bone formation, and remodeling. A, B, C, D show 0, 18, 74, 135 weeks after surgery, respectively



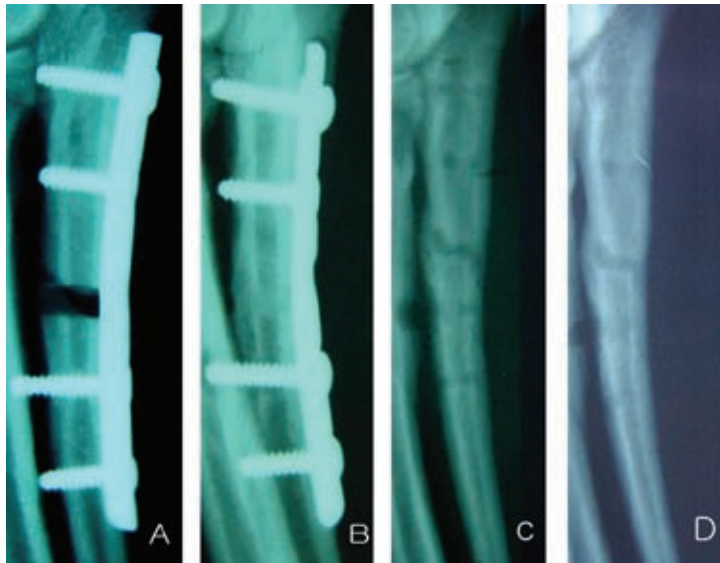


Figure 4. Radiographs demonstrating a healed defect site in Group B, with partial new bone formation in the implant site. A, B, C, D show 0, 18, 74, 135 weeks after surgery, respectively

strated a fracture union and complete remodeling of the cortical bone (Figure 4).

**Dual-energy x-ray absorptiometry**

Table 1 shows the BMD and BMC. BMD and BMC of the FDAB plate applied to the ulna (Group A) were similar to those of the stainless steel bone plate applied the ulna (Group B). There were no significant differences between the two groups ( $P > 0.05$ ).

**Biomechanical testing**

Eight ulnas from Group A and four ulnas from Group B (at 135 weeks) were used for the tree-point bending test. Ten normal ulnas were used

as the control. The maximum load (N) to fracture in Group A, Group B, and the control was  $6\,420 \pm 1\,166$ ,  $4\,210 \pm 1\,232$ , and  $5\,478 \pm 727$  N, respectively (Figure 5.). The stiffness (N/min) for fracture in Groups A, Group B, and the control was  $1\,572 \pm 158$ ,  $1\,019 \pm 173$ , and  $1\,438 \pm 110$  N/min, respectively (Figure 6.). The maximum load (N) and stiffness (N/min) in Group A was slightly higher than that of the normal ulna but the difference was not statistically significant.

**DISCUSSION**

The use of various forms of bone grafts to replace or supplement a diseased portion of the skeleton is common in clinical practice. The successful use of these tissues is dependent on various biological,

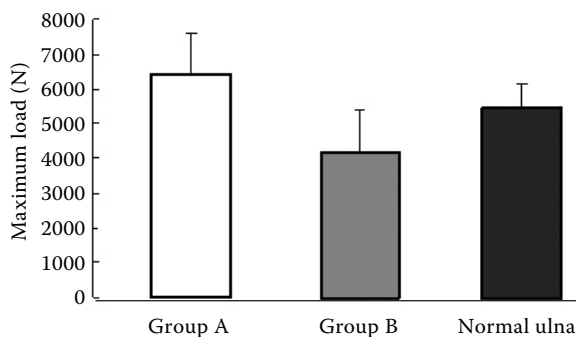


Figure 5. Biomechanical strength of the bone defect site at 135 weeks. The maximum load of the ulna in Group A was slightly higher than of the normal ulna and Group B. However, the difference was not statistically significant

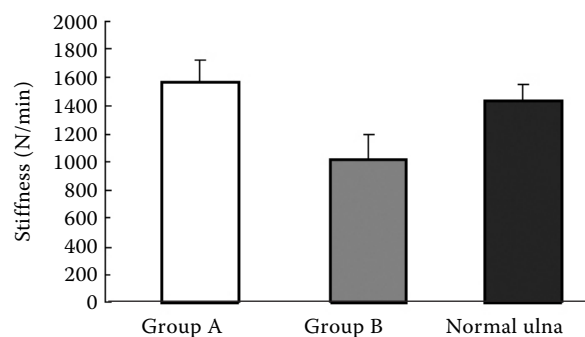


Figure 6. Biomechanical strength of the bone defect site at 135 weeks. The stiffness of the ulna in Group A was slightly higher than of the normal ulna and Group B. However, the difference was not statistically significant

physiological, and biomechanical factors, each of which affects the result of the transplant (Burchardt, 1987; Horowitz and Friedlaender, 1987).

This study showed that the effect of long term FDACB plate application on the host bone was similar to that of the control group. A significant loss of bone minerals (up to 50%) develops in a fractured bone during the first six months after an injury. After fracture healing, some recovery of the lost bone occurs (Finsen and Haave, 1987). It was previously reported that the bone mineral decreased rapidly after fracture, which continued for approximately five months. Towards the end of the first year after injury, there was slow restoration of the mineral content but no return to the initial values in most instances. Although the average maximum loss was approximately 45%, only 25% of the initial bone mineral was missing after one year (Andersson and Nilsson, 1979).

Fracture treatment by internal fixation with metal plates is a common practice. However, the application of a metal plate to the bone has an adverse effect on the bone remodeling process (Akeson et al., 1976; Woo et al., 1976; Slatys et al., 1978). In addition, it does not promote osteogenesis (Anderson, 1965). There are significant biological and biomechanical differences in fracture healing between internal and external fixation (Uthoff and Finnegan, 1983).

The three regions of interest of the ulna where the FDACB plate was applied canine limb were similar to the normal ulna. The long term FDACB plate site showed complete fracture healing and recovery of the bone defect. This suggests that the bone may, under some circumstances, show a local increase in strength after remodeling.

The main finding of this study was that the stainless steel bone plate groups showed a mean 35% lower maximum load and a 35% lower stiffness than the FDACB groups. The recorded differences indicate that the defect site gained strength once stabilized internally with the FDACB plate. Malinin et al. (1984) reported that refracturing of the radius fixed with an allograft cortical bone plate required 72.9% of the failure load needed to break a normal paired radius. In contrast, a stainless steel-plated radius required only 44.2% of the control load to refracture at eight weeks. These results correspond to those of an earlier study, which reported that an allograft cortical bone plate site was stronger than a stainless steel bone plate site. However, the allograft cortical bone plate site was weaker than the normal ulna. It is believed that in the previous

study, the fracture site had not healed, remodeled and united completely with the host bone. In addition, there was a difference in the observation period compared with the present study. In this study, there was complete healing of the defect site and incorporation in the host bone.

A stainless steel bone plate site creates compression at the interface between the bone cortex and the plate (Moyen et al., 1978; Jain et al., 1997). Local ischemia leads to cortical necrosis with increased haversian remodeling as well as early bone porosis to reestablish the cortical circulation. The production of newly formed lamellar bone normally occurs within 20 weeks after plate application (Perren et al., 1988).

There is a dynamic change in the allograft biomechanics during the incorporation and remodeling process. The biomechanical response to an allograft onlay strut has been well documented in a canine model (Kreuz et al., 1951; Emerson et al., 1992). The bone-plate allograft augmented the strength in the host bone and promoted healing of the fracture site (Malinin et al., 1984; Zhou et al., 2005a,b). After a 135 week follow-up, there was complete maturation and remodeling in the implant site. This study showed host bone fracture healing, anatomical alignment, union and incorporation in the host bone.

The freeze-dried allograft cortical bone plate united with the fractured bone by incorporating with the host bone, showing complete remodeling without complications. This material can be used clinically as an absorbable internal fixation material, and can assist in healing by inducing new bone formation in a large defect fracture.

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