Clinical Efficacy of Bone Reconstruction Surgery with Frozen Cortical Bone Allografts for Nonunion of Radial and Ulnar Fractures in Toy Breed Dogs

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Abstract

Objective To evaluate the effectiveness of frozen cortical bone allografts (FCBA) in the treatment of severe radial and ulnar atrophic nonunion fractures.

Animals Toy breed dogs with nonunion of radial and ulnar fractures ($n = 15$).

Methods Severe atrophic nonunion fractures were treated with FCBA (eight infected and seven non-infected fractures). Radiographs obtained immediately after surgery, and 1, 2, 3, 6 and 12 months later were evaluated and scored for the periosteal reaction at the bone regeneration sites, the healing process in the bone connection areas at both the proximal and distal sites, and the bone remodelling process within the allografts.

Results Improvements in the fracture-healing process and weight-bearing function were observed in all cases. Radiographic scores at the bone connection areas and within the allograft improved significantly over time ($p < 0.05$). There were not any significant differences in radiographic scores between the infected and non-infected groups.

Clinical Significance Bone reconstruction with FCBA is effective in the treatment of radial and ulnar nonunion fractures associated with large bone defects, regardless of the infection status of the surgical site.

Keywords
- frozen cortical bone allograft
- radius
- nonunion fracture
- radiographic analysis
- bone infection
- surgical treatment

Introduction

Nonunion is a serious complication in small animal orthopaedics,[1] particularly in the treatment of radial and ulnar fractures in Toy breed dogs. Several factors contribute to a nonunion, including inappropriate surgical treatment, instability of fracture sites, poor blood supply and infection. It has been reported that 60% of nonunions in dogs occur in the radius and the ulna, with 25% in the tibia and 15% in the femur.[1] The risk of nonunion is particularly high for distal radial and ulnar fractures in Toy breeds due to poor vasculature development at the distal radial metaphysis compared with large breed dogs.[2] Generally, nonunions are classified into two groups: biologically active nonunions resulting from insufficient mechanical stability at the fracture site and biologically inactive nonunions arising from a deficiency in blood supply, tissue necrosis and a lack of bone.[3] Biologically active nonunions from mechanical instability are classically called hypertrophic nonunions. Biologically inactive nonunions are also called avascular or avital non-unions with or without bone defects. Atrophic nonunions typically occur as avascularized non-unions. Due to a lack of force transmission, the bone ends undergo resorption with an active process requiring blood supply.[4]

Atrophic nonunions can result in bone defects of various sizes. The debridement of the atrophic bone ends is often

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necessary for the treatment of bone defects, which further enlarges the defect area and shortens the affected bone length. Such cases require reconstruction of the bone defects. To reconstruct such defects, various techniques have been used such as autologous or allogeneic cancellous bone graft, the use of artificial bone materials such as β-tricalcium phosphate and the use of growth factors such as bone morphogenetic protein.[5] Among them, frozen cortical bone allograft (FCBA) has been used for reconstruction procedures.[6] To date, the clinical outcome of FCBA application in atrophic non-unions in Toy breed dogs has not been evaluated. The goal of this study was to evaluate the effectiveness of FCBA application combined with autologous cancellous bone graft and fibroblast growth factor in the reconstruction of antebrachial bones of Toy breed dogs affected by nonunions after the failed initial treatment of radial and ulnar fractures. In addition, we clinically evaluated the effect of bacterial infections on the healing process of radial and ulnar nonunion fractures treated with FCBA.

Materials and Methods

Case Selection

Fifteen nonunion fractures in 14 dogs treated with FCBA were included (►**Table 1**). These cases were referred to Veterinary Medical Teaching Hospital, Nippon Veterinary and Life Science University, after a failed initial attempt to treat radial and ulnar fractures between 1994 and 2014. Nonunion fractures were classified as biologically inactive or biologically active based on the descriptions by Weber and Čech.[3] Nonunion fractures were further divided into infected and non-infected groups based on bacterial isolation from the bones debrided during surgery.

Preparation of Frozen Cortical Bone Allografts

Long radius bones were harvested aseptically from healthy Beagles that were used in unrelated continuing studies approved by the Experimental Animal Committee of Nippon Veterinary and Life Science University, Tokyo. The approval number of the unrelated recent studies is 27J-9. These studies had nothing to do with this present study. Sectioned bone grafts were wrapped in sterile gauze immersed in gentamicin (10 mg per 500 mL) and lactated Ringer's solution and sealed in plastic tubes (BD Falcon, Franklin Lakes, New Jersey, United States). The grafts were kept in a freezer (SANYO, Osaka, Japan) at a temperature of –70°C. The grafts for respective surgeries were selected based on the diameter and length of the recipients' bone defects. During the surgery, the frozen grafts of the appropriate sizes were thawed in 37°C sterile physiological saline, and soft tissues and bone marrow inside the medullary cavities were removed and the grafts were trimmed to accommodate the size of each recipient's bone loss (►**Fig. 1**).

Surgical Procedures and Postoperative Management

The patients underwent various examinations to confirm their health status, including physical examination, haematology, blood gas tests, electrocardiogram examinations and thoracoabdominal radiographs. Following the injection of droperidol (0.25 mg/kg intramuscular [IM]), propofol (7 mg/kg

Table 1 Breed, sex, age and body weight characteristics of each patient and the details of the operation

Case	Age (mo)	Sex	Breed	Body weight (kg)	Time from injury to nonunion treatment (d)	Postoperative follow-up period (d)
Infected group						
1	12	Male	Yorkshire Terrier	2.1	75	3,810
2	69	Spayed female	Toy poodle	2	87	2,688
3	12	Neutered male	Toy poodle	3.1	541	2,395
4	18	Female	Papillon	2.2	76	2,178
5	13	Male	Toy poodle	2.8	113	1,289
6	22	Male	Toy poodle	4.1	366	2,262
7	12	Spayed female	Toy poodle	2.35	209	4,209
8	48	Neutered male	Toy poodle	3.4	144	4,083
Uninfected group						
9	62	Male	Toy poodle	2.8	1,085	1,835
10	19	Male	Toy poodle	2.8	218	1,946
11	60	Female	Toy poodle	1.3	25	1,206
12	46	Female	Toy poodle	2.6	924	875
13	35	Female	Toy poodle	1.9	610	1,168
14	30	Neutered male	Chihuahua	1.4	214	975
15	30	Neutered male	Chihuahua	1.4	231	954

Note: The average age was 32 ± 20 months and the average weight was 2.5 ± 0.8 kg (mean ± standard deviation), d = days; mo = months.

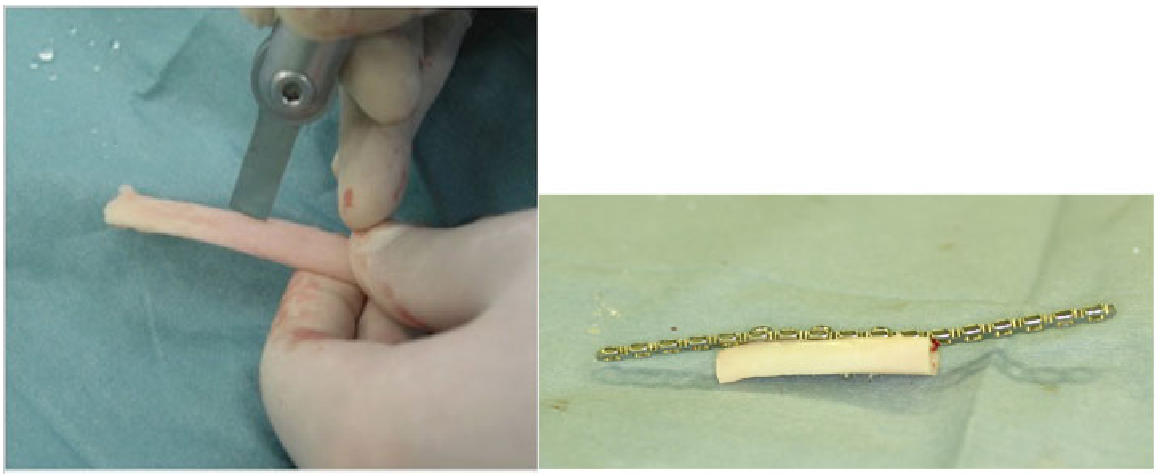

Fig. 1 Trimming of the grafts with the soft tissues completely removed to accommodate the size of each recipient's bone losses.

intravenous [IV]) was administered and anaesthesia was maintained with isoflurane. In addition, we performed nerve block anaesthesia, using lidocaine 1% and bupivacaine hydrochloride 0.5%, from the lateral side of the proximal region of the forelimb (dose of 0.1 mL/kg for each drug), and from the medial side of the forelimb (dose of 0.15 mL/kg for each drug). These anaesthetic agents blocked the radial, ulnar, musculocutaneous and median nerves.[7]

In all cases, the radial and ulnar shafts were exposed via a cranial approach of the forelimb. After exposing the nonunion sites, the previously placed implants were removed and bone at the fracture site was debrided and submitted for bacterial culture and antibiotic sensitivity tests. The edges of the fractured bones were debrided with sagittal saws until bleeding was evident from the cross sections. The areas were rinsed with physiological saline with povidone iodine 5% (Meiji Seika Pharma Co., Ltd., Tokyo, Japan) added. The length of the bone defect was measured after the debridement and the length of the bone graft was adjusted accordingly. After appropriately contouring the plates to the surface geometry of the cranial side of the contralateral radius, the grafts were fixed with the plates to the proximal and distal radius fracture fragments (►**Fig. 2**). The plates used included 1.5-mm plates (Ortho Medical GmbH, Duerbheim, Germany) and 2.0-mm reconstruction plates (MIZUHO, Tokyo, Japan) with 1.1-mm diameter screws (Veterinary Orthopedic Implants, St. Augustine, Florida, United States) or 1.5- to 2.0-mm-diameter cortical screws (Johnson & Johnson, Tokyo, Japan; ►**Table 2**). Approximately 1.5 mL of cancellous bone grafts were obtained from the proximal humerus on the same side of the reconstructive surgery. The cancellous bone grafts were impregnated with 200 µg of fibroblast growth factor 2 (FGF-2; Fiblast spray; Kaken Pharmaceutical Co., Ltd., Tokyo, Japan) before being placed near the interface between the FCBA and host bones. When the distal radial fracture fragments were sequestrated or non-vital, or the size of the fracture fragments was considered too small, we performed carpal panarthrodesis by fixing the distal end of the plate to the third metacarpal bone after resecting the distal fracture fragments (►**Fig. 3**). In cases with preoperative skin defects or excessive skin tension at the time of closure, a pocket flap graft procedure was performed.[8]

All the patients were treated with antibiotic medication after surgery with appropriate adjustment based on culture and sensitivity test result. For pain management after surgery, opioids such as a fentanyl hydrochloride salt were administered as necessary for 3 days, because it has been known that non-steroidal anti-inflammatory drugs have

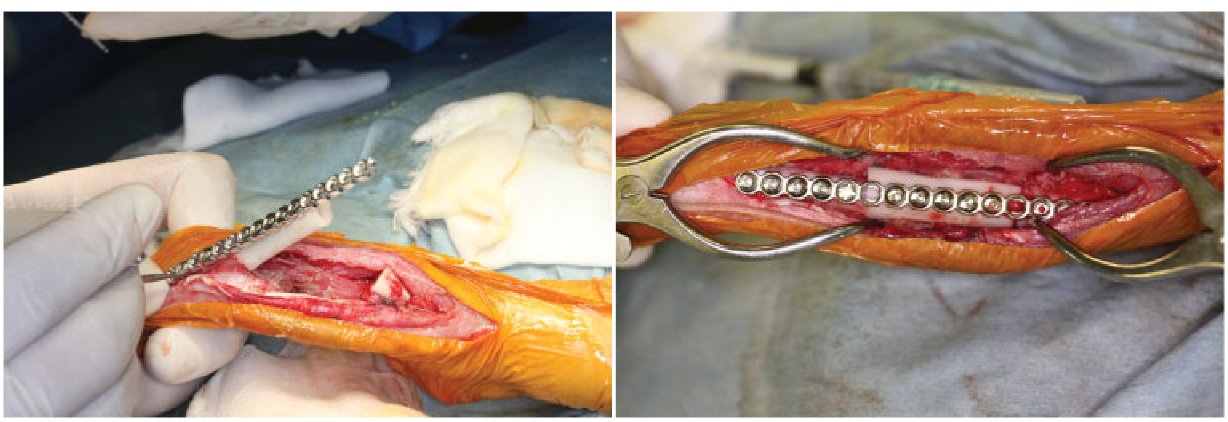

Fig. 2 Bone reconstruction surgery with frozen cortical bone allografts for non-union of radial and ulnar fractures.

Table 2 Details of operation

Case	Implants initially used in fracture treatment	Plates we used	Screws we used (mm)	Classification of nonunion
Infected group				
1	Intramedullary pin	Reconstruction plate	2.0 × 11.0	Non-activity, defect
2	Plate	Dynamic compression plate	2.0 × 8.0	Non-activity, dystrophic
3	Intramedullary pin	Reconstruction plate	2.0 × 11.0	Activity, oligotrophic
4	External fixator plate	Reconstruction plate	2.0 × 12.0	Non-activity, necrosis
5	Intramedullary pin, external fixator	Reconstruction plate	1.5 × 7.0; 2.0 × 8.0	Non-activity, necrosis
6	Plate	Reconstruction plate	2.0 × 14.0	Activity, moderately hypertrophic
7	External fixator	Reconstruction plate	2.0 × 10.0	Non-activity, atrophy
8	Intramedullary pin, wire	Reconstruction plate	2.0 × 11.0	Non-activity, dystrophic
Uninfected group				
9	Plate	Reconstruction plate	1.5 × 14.0	Non-activity, atrophy
10	External fixator	Reconstruction plate	1.5 × 4.0; 2.0 × 8.0	Non-activity, dystrophic
11	External fixator	1.5-mm miniplate × 2	1.5 × 9.0; 2.0 × 3.0	Non-activity, atrophy
12	Plate	Reconstruction plate	1.5 × 4.0; 2.0 × 8.0	Non-activity, atrophy
		1.5-mm miniplate	1.5 × 7.0	
13	Intramedullary pin, plate	Reconstruction plate	1.5 × 9.0; 2.0 × 2.0	Non-activity, atrophy
		1.5-mm miniplate	1.5 × 4.0	
14	Plate	1.5-mm miniplate	1.0 × 5.0; 1.5 × 6.0	Non-activity, atrophy
15	Plate	1.5-mm miniplate × 2	1.0 × 6.0; 1.5 × 5.0	Non-activity, dystrophic

Note: The non-union classification was performed according to Weber and Čech.[3]

suppressive effects on bone formation in experimentally fractured models in the dog.[9] A Robert Jones bandage was applied to the affected limbs in all cases for approximately 7 days after the operative treatments. The patients were confined to complete cage rest for approximately 2 weeks after surgery. After a postoperative period of 3 weeks, the patients were allowed to bear weight gradually and increase their walking distance and time in stages. Postoperative examinations were conducted in all cases at 1- to 3-month intervals. In some cases, staged implant removal was performed to promote bone healing.

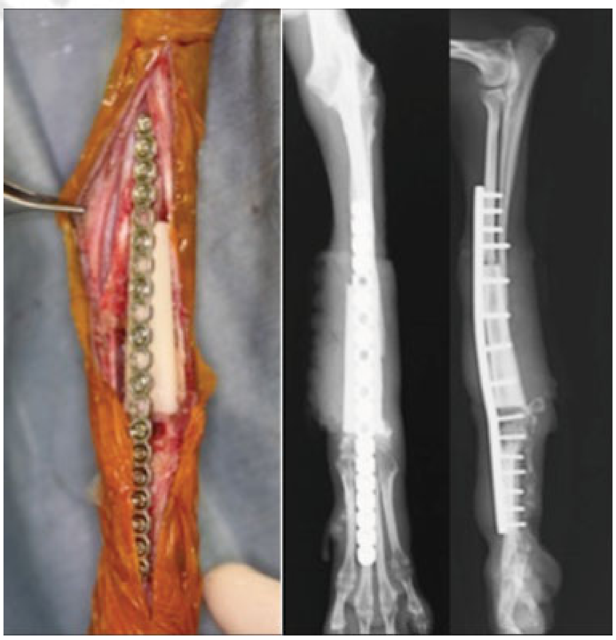

Fig. 3 Bone reconstruction surgery with carpal panarthrodesis by fixing the distal end of the plate to the third metacarpal bone.

Radiographic Parameter Scores

Bone remodelling process and bone healing following FCBA application were evaluated radiographically immediately postoperation and 1, 2, 3, 6 and 12 months postoperatively and were assigned a radiographic parameter score (RPS) based on a scoring system described previously.[10] Based on the radiographic presentation, the bone healing scores were determined by accumulating scores in the following four findings: (1) the presence or absence of periosteal reaction, (2) the degree of unions between the preserved bone allografts and autologous bones in the bone connection areas at both proximal and distal sites, (3) the change in the preserved bone allografts and (4) the presence or absence of complications. In this method, the highest score of 12 denoted bone remodelling. Conversely, the score would be zero regardless of the scores calculated in accordance with the four findings, if complications, such as refracture and reabsorption of the preserved bone allografts, were observed (►**Table 3**).

Table 3 Radiographic parameter scores (RPS)

	Points	
A. Periosteal reaction		
Absent	0	
Minimum	1	
Medium (<50%)	2	
Moderate (50–75%)	3	
Complete (>75%)	4	
B. Graft host bone union	Proximal limb	Distal limb
Radiotransparent line (total)	0	0
Radiotransparent line (partial)	2	2
Absent radiotransparent line	4	4
C. Graft changes		
Reaction absent	0	
Partial absorption	1	
Moderate remodelling	2	
Complete remodelling	3	
Total organization	4	
D. Complications (fracture or graft resorption)		
Total points	0	

Note: Radiographic scoring was based on the Fernandes and colleagues[10] report. In this analysis, the maximum score was 12.

Replacement Rate of Allograft

The allograft length was measured with lateral radiographs. The allograft length was the distance, in a straight line, from its proximal end to its distal end. The radial length of the affected side was a straight-line distance between the proximal end of the radius and the distal end of the radius. The replacement rate of allograft (RPA) was calculated using the following formula: allograft length (mm)/radial length of the affected side (mm) $\times$ 100.

Percentage of Radial Shortening Analysis

Plain lateral radiographs of the affected and healthy limbs before and after the operation were used to measure the percentage of radial shortening (PRS). The length of the healthy limbs was the straight-line distance from the proximal end of the radius to the distal end of the radius. The length of the affected limbs was the total distance of the direct lines passing through the diaphyseal axis of the radius in each fracture fragment. The PRS was calculated by the following formula: PRS $=$ length of the reconstructed radius/length of the radius of healthy limbs $\times$ 100.

Statistical Analysis

All results are reported as mean $\pm$ standard deviation (SD). For each time point, the value for the infected group was compared with that for the non-infected group. Differences between groups were statistically analysed by use of the Mann–Whitney *U* test or the two-sample *t*-test. We defined significance as a *p*-value less than 0.05.

Results

Reconstructive surgeries were conducted in 11 Toy Poodles, 1 Papillon, 1 Chihuahua and 1 Yorkshire Terrier (8 males and 6 females; median age 26 months [range: 12–69 months]; median weight 2.5 kg [range:1.4–3.4 kg]). The dogs were referred at an average of 328 days (range: 25 and 1,086 days) after the failed initial attempt of repair with intramedullary pins or plates. The postoperative follow-up period ranged from 875 to 4,209 days (average of 2,125 days; ►**Table 1**).

Details of the operations performed are shown in ►**Table 2**. Thirteen cases were biologically inactive nonunions and 2 cases were biologically active nonunions.

Improvement of radiographic signs for union was observed in all cases after the reconstruction surgeries with FCBA. Allograft rejection was not noted during the observation period. Following the second postoperative week, all patients were able to step and weight-bear with their affected limbs. By the third month after surgery, motor function recovered and general activities of daily living were not affected in all dogs.

A summary of the management for the infected and non-infected groups is shown in ►**Table 4**. The length of the allograft used for bone reconstruction (mean $\pm$ SD) was 27.6 ± 12.0 mm ($n = 8$) in the infected group and 21.8 ± 9.9 mm ($n = 7$) in the non-infected group. The RPA was $36.1 \pm 16.0\%$ ($n = 8$) in the infected group and $34.2 \pm 12.5\%$ ($n = 7$) in the non-infected group. The PRS was $87.3 \pm 8.4\%$ ($n = 8$) in the infected group and $86.21 \pm 7.8\%$ ($n = 7$) in the non-infected group. No significant differences were observed between the two groups regarding the allograft length, the RRA and the PRS. Arthrodesis was performed in three of the eight infected cases and in six of the seven non-infected cases. Pocket flap graft procedure was performed in two of the eight infected cases and in three of the seven uninfected cases. FGF-2 was used in two of the eight infected cases and in three of the seven non-infected cases. A staged implant removal was performed in seven of the eight infected cases. Of the seven cases, all the plates and screws were removed in five cases. No implants were removed after the first reconstructive surgery in one case. Conversely, the implants were removed in stages for six of the seven non-infected cases. Of the six cases, all the plates and screws were removed in two cases. No implants were removed after the first reconstructive surgery in one case.

Time-dependent changes of the radiographs from cases 6 and 7 are shown in ►**Figs. 4** to **7**. The variations in the RPS are shown in ►**Figs. 8** to **11**. However, the time-series comparison showed no significant differences between the two groups.

The bacterial culture tests from the excised bone or implants were positive in all eight fractures in the infected group, with a high prevalence of the *Staphylococcus* species (►**Table 5**). We used antibiotic therapy for 3 to 11 weeks.

Table 4 A summary of the treatments for the infected and uninfected groups

Case	Allograft length (mm)	Replacement rate of allograft (%)	Percentage of radial shortening analysis (%)	FGF2	Arthrodesis	Pocket flap grafts	Destabilization
Infected group							
1	31.3	51.9	91.8	(+)	(+)	(–)	2
2	8.9	11.1	95.8	(+)	(–)	(–)	3
3	35.0	38.6	87.2	(+)	(–)	(–)	2
4	35.8	59.8	84.9	(–)	(+)	(+)	3
5	43.1	44.0	96.9	(–)	(+)	(+)	1
6	31.3	33.0	88.3	(+)	(–)	(–)	3
7	11.8	21.0	70.4	(+)	(–)	(–)	3
8	23.8	29.5	83.3	(+)	(–)	(–)	3
Uninfected group							
9	31.7	38.4	89.4	(–)	(+)	(–)	3
10	15.1	18.6	88.5	(–)	(–)	(–)	3
11	23.9	39.1	94.8	(–)	(+)	(–)	2
12	36.0	44.1	88.8	(–)	(+)	(+)	1
13	20.4	38.1	70.6	(–)	(+)	(+)	2
14	19.1	46.6	82.1	(+)	(+)	(+)	2
15	6.6	14.7	89.3	(+)	(+)	(–)	2

Note: Depending on the stage of the removal, the cases were divided into the following three groups: group 1, where no implants were removed after the first reconstructive surgery; group 2, where some of the screws were removed; and group 3, where all the plates and screws were removed in several stages.

Discussion

The present study assessed the clinical efficacy of FCBA with autologous cancellous bone graft and FGF-2 in 15 nonunion fractures. In all cases, anatomical reduction of fracture was achieved with radiographic evidence of bone healing between the host bones and grafts. Bone remodelling of the graft was observed in all cases in the two groups. The weight-bearing function of the affected limbs was clinically recovered, and there were not any cases involving refracture or implant

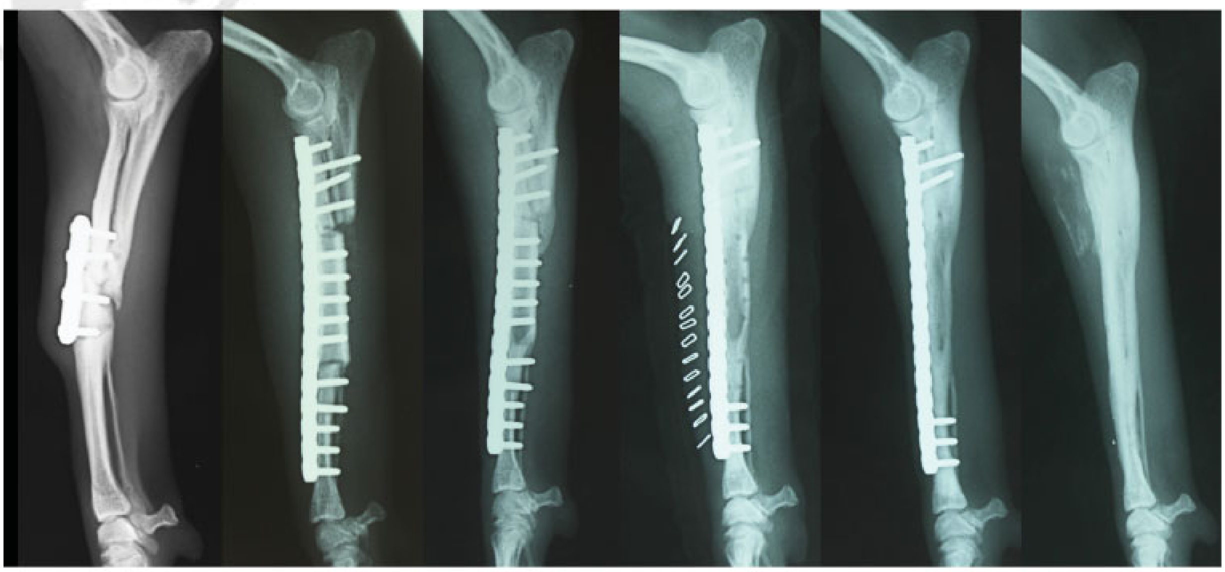

Fig. 4 Time-dependent changes in the radiographs from case 6 (lateral view). Pre-op, preoperative radiograph; Post-op, postoperative radiograph.

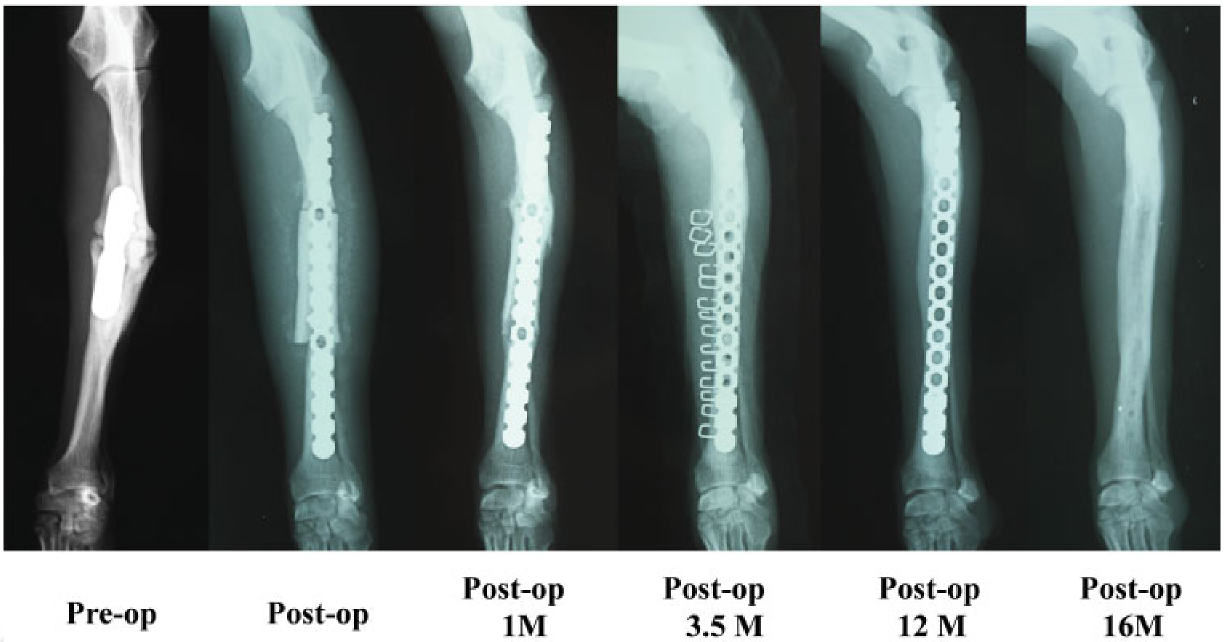

Fig. 5 Time-dependent changes in the radiographs from case 6 (anteroposterior view). Pre-op, preoperative radiograph; Post-op, postoperative radiograph.

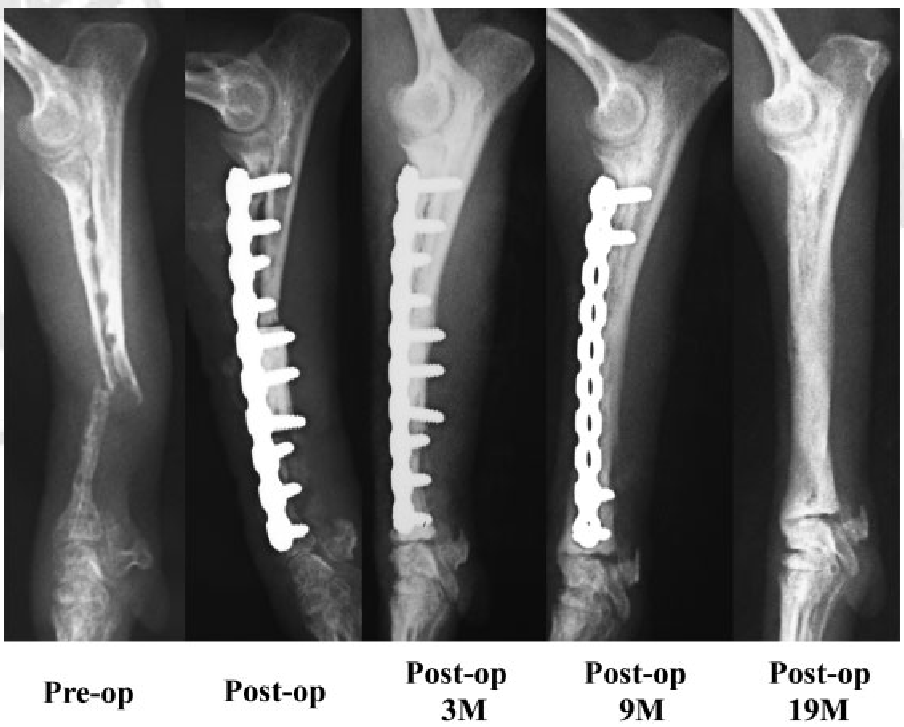

Fig. 6 Time-dependent changes of the radiographs from case 7 (lateral view). Pre-op: preoperative radiograph; Post-op: postoperative radiograph.

damage where repeated surgery was necessary. Regarding the minor complications in the present study, recovery of forelimb functions including weight-bearing tended to be delayed about a few weeks after surgery compared with the healing process after the fixation for fresh fractures. In addition, in cases where arthrodesis was not performed, a reduction in the range of motion in the carpal joint was found. This finding might be related to fibrosis of the extensor tendons around the radius, because of multiple surgical interventions. However, even in cases of infection during surgery, clinical findings suggesting post-surgical infection were not observed.

The RPS for bone healing and remodelling process significantly increased at 1 month after surgery and successively over time in the two groups. The radiographic bone healing was observed at 3 months after surgery in both proximal and distal bone connection areas. Bone remodelling within the bone

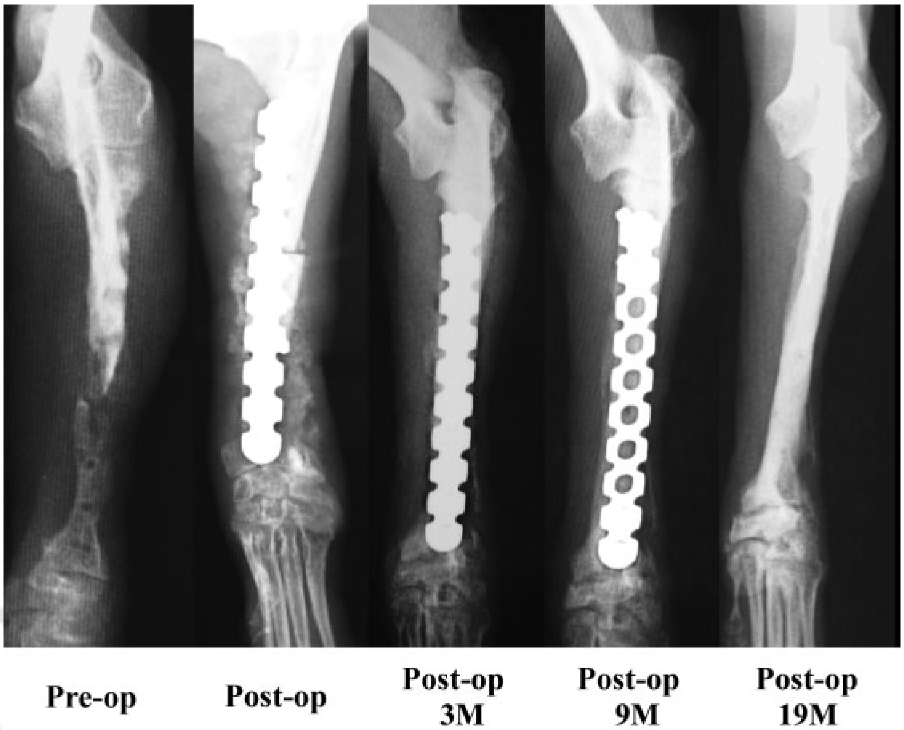

Fig. 7 Time-dependent changes in the radiographs from case 7 (anteroposterior view). Pre-op, preoperative radiograph; Post-op, postoperative radiograph.

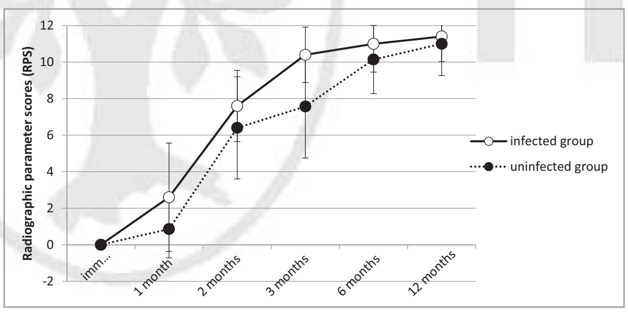

Fig. 8 The variation in the radiographic parameter scores (RPS). The results are presented as the mean ± SD. In both the infected and uninfected groups, RPS increased at 1 month after surgery and successive increases in the scores were observed over time. SD, standard deviation.

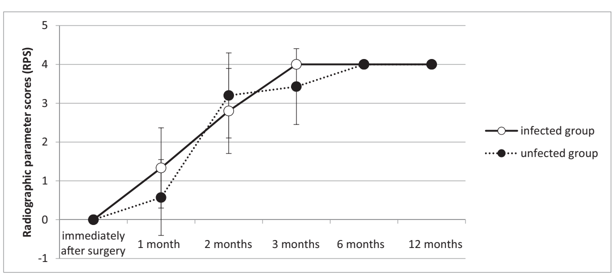

Fig. 9 The variation in the radiographic parameter scores (RPS) at the proximal bone connection areas. The results are presented as the mean ± SD. Bone unions were observed at 3 months after surgery at the proximal bone connection areas in both the infected and uninfected groups. SD, standard deviation.

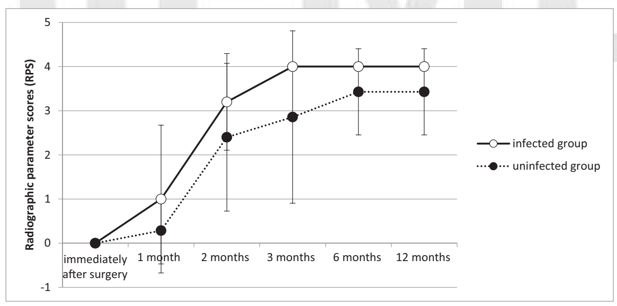

Fig. 10 The variation in the radiographic parameter scores (RPS) at the distal bone connection areas. The results are presented as the mean ± SD. Bone unions were observed at 3 months after surgery at the distal bone connection areas in both the infected and uninfected groups. SD, standard deviation.

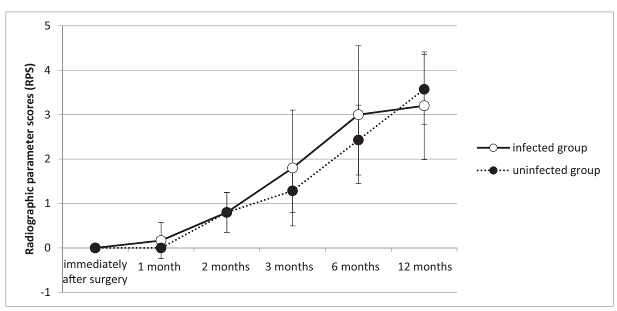

Fig. 11 The variation in the radiographic parameter scores (RPS) in the preserved bone allografts. The results are presented as the mean ± SD. The scores in the preserved bone allografts increased over time during the 12-month observation period in both the infected and uninfected groups. SD, standard deviation.

Table 5 The incidence of bacteria detected in the infected group

Case	
1	*Enterococcus* sp.
2	*Coagulase-negative Staphylococci*, *Corynebacterium* sp.
	Staphylococcus aureus (MSSA)
3	*Staphylococcus aureus* (MRSA)
4	*Staphylococcus aureus* (MSSA)
5	*Escherichia coli, Enterococcus faecalis, Acinetobacter lwoffii*
6	*Staphylococcus aureus* (MRSA)
7	*Pseudomonas aeruginosa*
8	*Enterococcus* sp.

Abbreviation: MSSA, methicillin-resistant Staphylococcus aureus.

allografts was also noted during the 12-month observation period. There were not any significant differences in RPS over time between the two groups.

Generally, the application of preserved bone allograft is not recommended for infected fractures.[11] Moreover, for distal radius osteosarcoma, even in cases where sparing surgery is performed after the tumour resection, there may be infection or construct failures, such as a fracture of the radius or a loosening or breakage of the bone screws or bone plates.[12] However, it was reported that the condition was managed without allograft removal in 75% of human cases that used preserved bone allografts at the infected sites.[13] A study by Sinibaldi[6] suggested that strong fixation devices, the contact between grafted and host bones, and cancellous bone grafts were important in the bone healing when applying preserved bone allografts. In this study, bone healing was achieved in infected nonunion fractures of the radius and ulna in Toy breed dogs, through the following specific procedures. These procedures included debridement of the infected tissues, lavage of the affected areas, the internal fixation of the preserved bone allografts with plates and screws, the implementation of autologous cancellous bone grafts after securing sufficient contact areas in the boundary between the allografts and graft beds and the administration of appropriate antibiotic medications based on the results of drug susceptibility testing. The etiological agent identified from the bacterial culture tests was predominately the *Staphylococcus* species (►**Table 5**). A careful selection of antibiotic medication was required as *methicillin-resistant Staphylococcus aureus* was detected in cases 3 and 6 (►**Table 5**). No case showed signs of infection after discontinuation of the antibiotic medication. Infection can cause nonunion. Thus, it was very important to perform bacterial culture tests of the sequestra and implants removed from the surgical wounds, as well as performing antibiotic susceptibility testing for postoperative antibiotic therapy. In addition, there is a risk that the bone graft may function as a biological film and become a breeding ground for bacteria in bone reconstruction surgeries with FCBA. In cases of osteomyelitis, it is known that necrotic tissues, such as the sequestrum in the affected sites, or the areas surrounding the implants that restrict vascularity, become breeding grounds for bacteria.[1] Therefore, it is necessary to perform complete removal of all the existing implants, adequate debridement of the tissues of suspected infection and lavage of the areas.

The PRS was above 80% in all cases; substantial shortening of the radius and ulna was not observed in this study. It was reported that lameness occurred when the percentage of femoral shortening was $\leq$80%.[14] When substantial shortening of the radius and ulna occurs, the preservation of limb length is also considered necessary owing to the risk of carpal hyperextension.

In the cases with a carpal panarthrodesis, the weight-bearing function of the affected limbs recovered after surgery. Radial and ulnar fractures in Toy breeds occur commonly at the distal one-third to one-fourth, and the size of the distal fracture fragments tends to be small.[15,16] The size is further reduced with the development of nonunion after fractures due to the progression of osteoporosis and bone resorption. This occasionally makes it difficult to provide fixation with more than two screws. Under such circumstances, an effective approach is to perform carpal panarthrodesis between the grafts and the radial carpal bones after resecting the distal radius fracture fragments, as shown in this study.

A Pocket flap graft procedure was performed with good results in all cases. In the cases of bone and adjacent soft-tissue atrophy from continued disuse of the chronically affected limbs, the transverse diameter of the carpal joints becomes thicker after the reconstruction of the antebrachial bones (►**Figs. 2** and **3**). This study suggested that pocket flap graft procedure for the treatment of skin defects is effective in dealing with cases in which surgical wound closures are difficult due to skin loss.

Excellent results were obtained in all the cases that underwent the staged removal of the implants. Gradual destabilization of the fixation strength in the fractured sites with the staged implant removal was considered as an effective method of increasing biomechanical stress on the grafted bones and promoting bone union as well as remodelling. However, these procedures should be performed based on radiographic observations, and early implant removal is not recommended. Implant-induced osteoporosis may occur when radial and ulnar fractures are treated via internal fixation of plates and screws. The occurrence of implant-induced osteoporosis is associated with circulatory deficits in the cortical bones immediately deep to the plates and with stress shielding effects of the implants.[17]

In the use of fresh bone allografts that were not treated by freezing, there was a risk that the allografts may trigger a graft rejection response due to the disparities in the major histocompatibility antigens between the donor and recipient.[18] In contrast, the immunogenicity of allografts frozen at –20 to –80°C decreases as the treatment destroys cells present in the allografts.[19] It has been reported that the freezing of allografts is helpful in promoting bone regeneration, even in cases of dog leukocyte antigen mismatched donor–recipient pairs.[20] This study did not evaluate the dog erythrocyte antigen and the dog leukocyte antigen in either of the graft donors or the nonunion cases. Nonetheless, all cases achieved bone healing and remodelling with the bone grafts, which may indirectly indicate that the antigenicity was reduced by freezing the grafts. The following features of FCBA are considered advantageous: high strength in structural support, possession of osteoinductive factors in association with the release of growth factors from the matrix and minimal foreign body reaction. A disadvantage of FCBA includes the lack of an ability to form new bone.

In the field of regenerative medicine, it is well known that three elements (bone-forming cells, cell matrices at the treatment site and growth factors) are all required to enhance bone regeneration.[21] In an attempt to reconstruct bones after nonunion, we used autologous cancellous bone grafts as the source of bone-forming cells, FCBA as the scaffold and structural support, and growth factors inherent to the bone grafts. We also used FGF-2 in five cases (two of the eight infected cases and three of the seven uninfected cases). In a previous study, we had obtained promising results when using a combination of these treatments for nonunion of diaphyseal femoral fractures in dogs.[22] Fibroblast growth factor 2 enhances bone formation by inducing undifferentiated mesenchymal cells to differentiate into preosteoblasts and stimulating preosteoblast proliferation.[23] Fibroblast growth factor 2 also plays a stimulatory role in osteoclast differentiation and bone resorption. Thus, FGF-2 has been recognized as a factor that promotes bone turnover.[23–26] In addition, FGF-2 can promote blood vessel formation by increasing the expression of vascular endothelial growth factors in mesenchymal cells.[27,28] However, FGF-2 has a short duration of action and a half-life of 17 hours when administered locally. Nonetheless, FGF-2 enhances bone formation by stimulating the initial responses when administered in a single dose. Using an in vivo application of FGF-2, a remarkable improvement in callus and osteoclast formation was reported, indicating that the capacity of FGF-2 to promote bone turnover made early bone healing possible.[29,30] Preserved cortical bone allografts have an osteoinductive capacity.[31] Preserved cortical bone allografts release growth factors such as bone morphogenetic protein and insulinlike growth factor 1 following integration into the graft beds.[32] Therefore, the combination of FGF-2 and preserved cortical bone allografts should be beneficial. However, it remains unclear how much of an effect FGF-2 had on the bone healing between the host bones and the grafts, and on bone remodelling within the grafts. This lack of understanding was likely fostered by the extremely short reaction time of FGF-2 and the limited single liquid administration.

In the cases of infected nonunion of the radius and ulna, the results also suggested that bone reconstruction surgery with FCBA is effective when performed with careful attention to infection. Given the high risk of nonunion in Toy breeds, bone reconstructive surgeries with FCBA can be considered effective for these complications. The results of this study indicated that FCBA was an effective treatment in severe atrophic nonunion with bone defect, regardless of the infection status of the surgical site.

Conflict of Interest
The authors cite no conflict of interest.

Author contributions
Shuntaro Munakata, Yasushi Hara and Nobuo Kanno contributed to conception of study, study design, acquisition of data and data analysis and interpretation. Daichi Katori, Yasuji Harada and Norihiro Muroi contributed to acquisition of data and data analysis and interpretation. Shinya Yamaguchi and Hiroyuki Akagi contributed to study design, acquisition of data and data analysis and interpretation. Kei Hayashi contributed to data analysis and interpretation. Yukari Nagahiro contributed to conception of study, study design and acquisition of data. All authors drafted and revised and approved the submitted manuscript.

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