Indian J. Vet. Surg. 46(1): 17-19 June 2025

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Evaluation of demineralized bone matrix graft with tissue adhesive in healing of long bone fractures stabilized with interlocking nail in dogs

Ruchi Patwa[1], P. Bishnoi[2†], A.K. Bishnoi[3], Sakar Palecha[3] and Pratishtha Sharma[3]
Rajasthan University of Veterinary and Animal Sciences, Bikaner- 334001(Rajasthan)

[1]PG Scholar, [2]Professor and Head, [3]Assistant Professor, Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Science, Bikaner

DOI: 10.5958/0973-9726.2025.00004.7 *Received: July, 2022*

The present study was conducted on 6 clinical cases of dogs having closed long bone fractures stabilized with intramedullary interlocking nail (ILN). After fracture fixation with ILN, demineralized bone matrix (DBM) was applied at fracture site and tissue adhesive (TA) was applied at fracture ends. Preoperative signalment, clinical and radiographic evaluation was done in all cases. Fracture healing was evaluated by recording weight bearing/lameness score (1-6), and radiographic evaluation at regular intervals. The results showed that the tissue adhesive when used along with demineralized bone matrix at the fracture ends prevented callus formation and resulted in delayed or non-union of fracture.

Key words: *Bone fracture, Demineralised Bone Matrix, Fracture healing, Interlocking nailing, Tissue Adhesive*

Demineralized bone matrix (DBM) is an osteoconductive and osteoinductive material depending on the processing of bone and donor characteristics with no limitation of quantity (Drosos *et al.*, 2015). Bone morphogenic proteins (BMPs) present in the DBM stimulate transformation of local undifferentiated mesenchymal cells into osteoblasts (osteoinduction), and the collagenous framework allows migration of the newly regenerated tissue (osteoconduction) into the site (Reddi, 1995).

Biological adhesives have been used for the repair of soft tissues, and chondral and osteochondral fractures (Shah and Meislin, 2013). Adhesives primarily promote bone graft fixation at recipient site for their maintenance (Esteves *et al.*, 2014). The adhesives are compatible when they are in contact with bone structures, and are effective in fixing the grafts (Hochuli-vieira *et al.*, 2017). Butyl-2-cyanoacrylate is non-histotoxic with strong tissue binding properties even in non-dry environment has been used in the fixation of fractures, osteotomies and in treatment of craniofacial and mandibular injuries (Yilmaz and Kuyurtar, 2005). Cyanoacrylate has low viscosity, which provides rapid fixation of fracture fragments and it does not cause inflammation, histotoxicity and cortical bone necrosis (Akcal *et al.*, 2014). The present study was conducted with the objective to evaluate the effect of tissue adhesive along with demineralized bone matrix in healing of long bone fractures stabilized with interlocking nail in dogs.

Six clinical cases of client owned dogs reported for treatment of long bone fractures were included in the study. Clinical examination was done to evaluate the general health status, limb and bone involved, swelling at fracture site and the extent of movement of fracture fragments. Age, sex, breed and body weight of the animal, etiology and type of fracture and limb and bone involved were recorded in all the cases. Lameness was graded pre- and post-operatively (Carr and Dycus, 2016). Preoperatively radiographic assessment was done (orthogonal views- medio-lateral and cranio-caudal) to determine the fracture type, location, medullary cavity diameter to select ILN of appropriate size and cortex to cortex measurement to select screws of suitable length by measuring the silhouette of the contra-lateral bone. The fracture was stabilized by open reduction and internal fixation using ILN as per the standard surgical technique (Johnson, 2013). The ILN (5-10 mm diameter and 14-22 cm length) of surgical grade stainless steel (Siora Surgicals Pvt. Ltd., India) with 4 proximal and 4 distal holes with a distance of 10 mm between the holes were used. Commercially available xenogeneic demineralized bone matrix graft (DBM Osseograft, Advanced Biotech Products Pvt. Ltd., India) was used as an osteoinductive and osteoconductive material along with N-butyl-2 cyanoacrylate tissue adhesive (Truseal, Suture India Ltd.). Once the fracture fragments were stabilized with ILN, the DBM graft (0.5 g) mixed in normal saline to form a paste like consistency was applied at the fracture gap and around the fracture site (Fig. 1). The tissue adhesive was applied on the fracture ends for fixation of the graft (Fig. 2).

After surgery, the limb was protected with modified Robert Jones bandage for 2-3 weeks. Postoperatively, broad-spectrum antibiotic ceftrixone (20 mg/kg body wt., for 7-10 days, NSAID, meloxicam (0.02 mg/kg body wt. for 3 days) and calcium-phosphorus oral supplement (for 30 days) were advised. The animal owners were instructed to

†Correspondence; E-mail: drpbishnoi29@gmail.com

***Fig. 1**: DBM graft paste application at fracture site.*

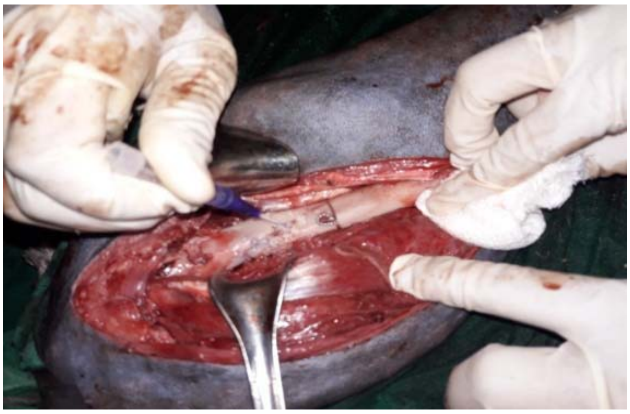

***Fig. 2**: Tissue adhesive application at fracture site*

***Fig. 3**: Callus formation away from fracture site*

restrict the movement of the dogs especially during the early phase of fracture healing. Skin sutures were removed after 10-15 days of surgery.

Postoperatively all the animals were clinically evaluated for weight bearing on the operated limb, lameness, flexion-extension of limb joints, limb muscular strength, surgical wound healing and any complications related to implant, fracture fixation and wound healing. The number of days when the dog first started weight bearing was noted. Similarly, the extent of weight bearing while standing, walking and running was also noted. Lameness grading was done with slight modification in the numerical rating scale described by Carr and Dycus (2016). Postoperative radiographic assessment was done at regular intervals to evaluate the implant position, fragment alignment and callus formation. The radiographic fracture healing was recorded (Table 1) as per scale (1-3) given by Leow *et al*. (2016).

Table 1: Grading of radiographic fracture healing at different time intervals.

Case No.	2nd week	4th week	6th week
C1	4	4	NRF
C2	8	4	NRF
C3	4	8	4
C4	NRF	NRF	NRF
C5	10	8	8
C6	4	6	8

NRF- Not reported for follow up evaluation

The average time of first weight bearing was 5.66±0.71 days. Similarly Raghunath *et al*. (2012) have reported earliest weight bearing by 3rd to 5th day in dogs treated for segmental fractures of tibia and femur by static intramedullary (IM) nailing. However, Priyanka *et al.* (2019) have observed first weight bearing by 1st to 10th postoperative day in all cases of tibial fracture stabilized with IM pinning, except 4 cases where it was observed after 20 days.

The lameness grade improved up to grade 3 in C2 and C4 at 8th postoperative week, while in C1 at 4th postoperative week. In C3, lameness grade improved up to grade 1 by 4th week but further again decreased to grade 6 by 8th week. In C5, lameness grade improved up to grade 4 by 8th week, whereas in C6, lameness grade improved up to grade 5 by 8th week (Table 3). In the case (C6) with femur head dislocation along with fracture of femur; after 2 weeks of femoral fracture fixation, femur head and neck ostectomy (FHO) was performed. Priyanka *et al.* (2019) have reported mean weight bearing score of 0.24±0.09, 2±0.32, 3.28±0.39 and 4.86±0.28 at 0, 15, 30-45 and >60 days, respectively, in dogs.

Postoperatively radiographs were taken at regular intervals for the evaluation of implant position and fracture reduction, callus formation and visibility of fracture line, and fracture healing (2nd, 4th and 6th weeks) (Table 1). The grade of fracture healing in C1 was 4 at 2nd and 4th week, in C2 it was 8 and 4 at 2nd and 4th week, in C3 grades of fracture healing were 4, 8 and 4 at 2nd, 4th and 6th week, respectively. Asif *et al*. (2011) in their study observed minimum callus formation and slight periosteal reaction on 30th postoperative day and complete bridging callus at 60th postoperative day.

N-butyl cyanoacrylate has been widely used as holding material to fix the fracture (Akcal *et al*., 2014), as an easy and reliable method for treatment of segmental fracture in rat tibia fracture model. In their study of Xavier and Leite (2012) also found that the adhesive stabilized the bone graft within the first few weeks and did not interfere with the consolidation of the osteotomies, or the integration of the bone graft in rabbit. However, in the present study, fracture healing was delayed in all cases. In the initial 2-3 weeks of fracture fixation, callus was formed away from fracture site in 2 cases. After 4 weeks, the gap at fracture site increased due to bone demineralization. In one case, callus was not formed at fracture site but away from the fracture site callus was observed after 5th week (Fig. 3). In one case (C1) no callus was formed up to 4 weeks. Bas *et al.* (2012) reported the use of N-2-butyl cyanoacrylate (NBCA) glue in onlay cortical graft fixation to enhance bone formation, but it interfered in trabecular bone formation between the graft and recipient site. NBCA resisted the healing process and graft incorporation at recipient site due to high amount of unabsorbed adhesive at the interface of graft and recipient bone (De Santis *et al*., 2017). In the present study, discharge from the suture site was observed in cases C3, C5 and C6. Other postoperative complications such as deviation of limb (at stifle joint), nail dislodgement from distal end of femur bone, muscle atrophy, shortening of limb, fracture of another bone (tibia at proximal end), loosening and bending of screw, and demineralization of bone with increased gap at fracture site were recorded. ILN was removed in case 2 and in case C5 cerclage wire was removed.

From the results of this study, it was concluded that cyanoaerykate tissue adhesive when used with demineralized bone matrix at the fracture ends prevented callus formation and resulted in delayed or non-union of fracture. Hence further studies are needed involving more number of cases before tissue adhesives can be used in routine clinical settings in dogs.

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