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A Clinical Study on the Healing of Comminuted Long Bone Fractures in Dogs Repaired with Locking Compression Bone Plate along with Hydroxyapatite Bone Graft with Collagen Membrane as Guided Bone Regeneration

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ABSTRACT

Background: Treatment of comminuted long bone fractures in dogs is burdened by the risk of bone healing complications. The immediate complications are due to soft tissue damage while the late complications of fracture healing include delayed union, non-union and malunion. Bone grafting with locking compression bone plate and barrier membranes as guided bone regeneration aids in enhanced fracture healing with minimal complications.The aim of this study was to evaluate the use of hydroxyapatite bone graft with collagen membrane as guided bone regeneration in long bone comminuted fractures in dogs.

Methods: Six dogs irrespective of age, breed and sex and suffering from comminuted long bone fracture in Radius, Femur and Tibia were surgically repaired with suitable bone plate as internal fixation technique. The hydroxyapatite bone graft with collagen membrane was used as an adjunt to the fracture site.

Result: Out of six dogs, one dog showed early bone healing, four dogs depicted slow healing on radiographic evaluation and one dog with femur fracture immobilized with LCP showed screw pull out by 15th post operative day.

Key words: Bone healing, Bone regeneration, Collagen membrane, Hydroxyapatite bone graft, Osteoconduction, Resorption.

INTRODUCTION

The goal of fracture treatment are to; encourage healing, restore function of the affected bone and the soft tissue and to obtain a cosmetically acceptable appearance. The long bone fractures with bone defects presents a significant clinical problem which needs to be addressed prudently. (Oh *et al.*, 2006). Bone grafts and bone substitutes have specific characteristics applicable to fracture repair and bone healing and should be selected based on those properties. Synthetic bone graft substitutes can be used to fill bone voided spaces which have led to extensive research in tissue engineering based on the development of osteoconductive scaffolds with osteoinductive growth factors either co-delivered with or to aid in in-situ recruitment of osteogenic cell sources (Giannoudis *et al.,* 2005 and Guda *et al.*, 2013). Osteoconductive scaffolds are designed to provide asuitable substrate for the in-growth of bone tissue,supporting vasculature and intendedto function as space maintainers for bony in-growth (Carloreis *et al.*, 2011 and Guda *et al.*, 2013). To support the space maintenance function, barrier membranes are used as guided bone regeneration to prevent in-growth of faster growing fibrous tissues in bone defect spaces (Queiroz *et al.,* 2006 and Guda *et al.*, 2013). Hydroxyapatite bone graft is the most common synthetic bone graft substitute used with collagen membrane in guided bone regeneration due to their osteoconductivity and ability to form a direct bond to host bone. Therefore, this study was designed with the objective of evaluating the osteoconductive property of hydrooxyapatetite bone graft along with collagen membrane as guided bone regeneration in comminuted fractures.

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MATERIALS AND METHODS

The study included 6 dogs presented with traumatic single long bone comminuted fractures of femur (n=2), tibia (n=3)

and radius and ulna (n=1). All the dogs were clinically healthy and had no other concurrent illness. The ethical approval for conducting the research was taken under IAEC No.43/Cvsc/Rnagar/2020. Clinical signs and neurological status of the dogs were recorded. Following initial clinical assessment, the dogs were subjected to pre-operative radiographic examination in two orthogonal views. Preoperative radiographs of six dogs were shown in Fig 1. Surgical intervention was advised to the owners and the dogs were called off-feed and off water for 12 and 6 hours, respectively, prior to surgery.

Inj. Atropine sulphate @ 0.04 mg/kg body weight was administered subcutaneously as pre-anaesthetic medication followed 10 minutes later by inj. xylazine hydrochloride @ 1 mg/kg body weight intramuscularly. General anesthesia was induced with intramuscular injection of ketamine hydrochloride @ 10 mg/kg body weight, after 10 minutes and was maintained with intravenous infusion of inj. propofol @ 4 mg/kg body weight.

Standard surgical approaches were made for radius-ulna through cranio lateral approach, femur through lateral approach and tibia through medial approach as recommended by Johnson (2013). Following the surgical exposure of the fracture site, the fracture fragments were aligned, reduced and held with bone holding forceps to restore the length and correct rotational orientation before securing the plate. Locking compression plate was then placed over the bone and the plate was held in position with plate holding forceps and secured to the bone with locking screws placed on either end with proximal most and distal most screws placed initially and bone plating was accomplished by insertion of additional screws in both proximal and distal fragments leaving the fracture line. Following the fracture fixation with suitable bone plating, 0.5 gm of sterile hydroxyapatite bone graft (Sterile synthetic Nano crystalline Hydroxyapatite bone graft by Eucare Pharmaceuticals (P) ltd.) was placed in a sterile petridish and mixed with 1 ml of fresh blood collected from cephalic vein (Franch *et al.,* 2006). The graft was placed with a small bone curette to fill the bone defect at the fracture site (Fig 2). The bone defect filled with the graft was surrounded snugly by the collagen membrane (Sterile reconstituted Type-I Collagen membrane by Advanced Biotech products (P) ltd.). The membrane with bone graft was secured at the fracture site with polyglactin 910 no 2.0 (Fig 3). Soft tissue closure was done immediately after graft placement.

Post-operative care included;Inj.Ceftriaxone sodium @ 25 mg/kg body weight,intramuscular twice a day for 7 days as an antibiotic and inj. meloxicam@ 0.3 mg/kg body weight, one a day, intramuscular for 3 days as analgesic. Owners were advised to restrict the movement of the dogfor the first 2 weeks of surgery followed by leash walk for the next few weeks.

RESULTS AND DISCUSSION

The study included six dogs of Non-descript (n=3) breed, Labrador retriever (n=2) and Terrier (n=1) dog. The mean age of the dogs was 11.33±2.41 months (range 5 -18 months). The body weights of the dogs ranged from 8-32 kg with a mean of 15.83±3.67 kg. The cause of fractures was; automobile accident (n=4), fall from height (n=1) and physical trauma (n=1). The mean time gap between the time of fracture and treatment was 5.66±0.71 days (ranging from 3-8 days). The dogs were equally distributed based on gender.

The Collagen membrane used for guided bone regeneration was found to be satisfactory as it covered the bone defects and aided in proper placement of bone graft at bone loss site. Evaluation of immediate post-operative radiographs revealed proper placement of the plate and screws, good alignment and apposition of the fracture fragments (Fig 4). Immobilization was considered satisfactory with locking compression plating in five dogs. In one dog suture dehiscence with screw pullout from plate seen by 15th postoperative day due to unrestricted activity of the dog and improper post operative care by the owner . The hydroxyapatite bone graft applied at the fracture site appeared as radio opaque granular structure at the fracture site in immediate post-operative radiographs which can be appreciated in Fig 4. Appearance of callus with adequate radio-density and radiolucent fracture line at the fracture site was observed on 45th post-operative day. By 60th post-operative day evidence of callus formation



Fig 1: Pre operative view of radiographs.

with reduced fracture gap was discernible and the graft had integrated with bridging callus and increased radio opacity. By 90th post-operative day, fracture line disappeared and good radio dense callus was evident at fracture site and cortical continuity was established. The sequential postoperative radiographs revealed progressive bone healing (Fig 5).



Fig 2: Sterile Hydroxyapatite bone graft incorporated in the collagen membrane surrounding the fracture site with a smallbone curette to fill the bone defect.

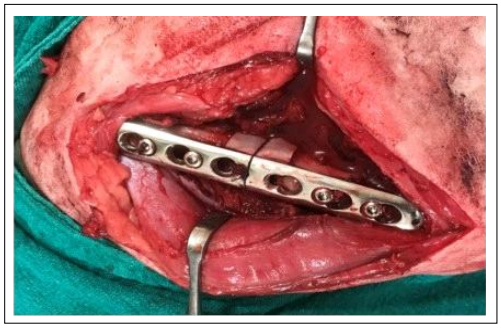


Fig 3: The Collagen membrane with Hydroxyapatite bone graft was secured at the fracture site with polyglactin 910 no 2.0.

Out of six dogs, one dog showed early bone healing by 60thpost operative day with cortico medullary continuity where as four dogs depicted slow healing as cortico medullary continuity was noticed by 90th post operative day.The healing time of each bone was given in Table 1. In these four dogs the fracture gap along with distinct graft integrated with bridging callus was seen suggesting the slow resorption of the graft with slow bone healing by 90th post-operative day. Slow bone healing was adduced to slow resorption of hydroxyapatite. Even though hydroxyapatite showed minimal resorbability it acted as a scaffold for bone ingrowth by providing a fixed structure for calcification to occur proving its osteoconductivity. In the present study a sterile hydroxyapatite bone graft (Sybograft) nano crystalline granules of particle size 200-300 microns was used. It provided a good surface for osteoconduction and biocompatibility. Similar findings were noticed by Blokhuis *et al.* (2000) who stated that the biocompatibility of hydroxyapatite is attributed to its hexagonal crystal structure and similarity to the mineral phase of bone tissue. The porosity of the hydroxyapatite influences its osteoconductivity by serving as a framework for the migration of blood vessels and deposition of new bone. The similar views were expressed by Wahl and Czernuszka (2006); Corinaldesi *et al.* (2009); Anderud *et al.* (2014); Bansal *et al.* (2009); Basile *et al.* (2015) and Santos *et al.* (2015).

One dog with femur fracture immobilized with LCP showed screw pull out by 15th post-operative day. Plate removal was done in that dog and the fracture fragments were immobilized with intramedullary pining with adequate weight bearing noticed by 60th post operative day.



Fig 4: Immediate post operative radiographs.

Table 1: Details of healing time taken for each bone.

Age of the dog	Type of the bone	Establishment of corticomedullary continuity time
1.4 year old male labrador	Mid diaphyseal comminuted tibial fracture	By 90 post operative days
1.8 year old dog female terrier	Proximal diaphyseal comminuted tibial fracture	By 100 post operative days
7 month old dog female non descriptive	Mid diaphyseal comminuted radius ulna fracture	By 80 post operative days
1.4 year old dog male non descriptive	Mid diaphyseal comminuted femur fracture	By 90 post operative days
6 month old dog female labrador	Mid diaphyseal comminuted tibial fracture	By 60 post operative days

The haematological and serum biochemical values fluctuated non significantly within the physiological limits through different post-operative days in all the dogs.

The Collagen membrane used for guided bone regeneration was satisfactory in all the six dogs. It is a bio-resorbable, bilayered, high purity type-I cross linked membrane with porosity lesser than the penetrable size of anepithelial cell. The porous and compact layers of collagen membrane not only enable osteogenic cell migration but also prevent the invasion of fibroblasts which helps in bone ingrowth. This was in correlation with Jegoux *et al.* (2011) and He *et al.* (2015). The study had a major limitation of controlled studies (with similar bone, almost similar age group, similar type of fractures) and larger sample size in clinical setting to validate the osteoconductive properties of hydroxyapatite bone graft along with bio-resorbable collagen membrane.



Fig 5: Progressive radiographs in a dog with tibial fracture immobilized with Locking compression plate and circlage wire along with hydroxyapatite bone graft and collagen membrane.

CONCLUSION

The clinical study concluded that collagen membrane used as guided bone regeneration and hydroxyapatite bone graft employed in the treatment of long bone fractures with bone defects proved to be good in promoting bone healing. Despitethe minimal resorb-ability of hydroxyapatite, it was an effective osteoconductive scaffold in promoting bone healing.

Conflict of interest

All authors declared that there is no conflict of interest.

REFERENCES

Anderud, J., Jimbo, R., Abrahamsson, P., Isaksson, S.G., Adolfsson, E., Malmstrom, J., Kozai, Y., Hallmer, F and Wennerberg, A. (2014). Guided bone augmentation using a ceramic space maintaining device. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology*. **118:** 532-538.

Bansal, S., Chauhan, V., Sharma, S., Maheshwari, R., Juyal, A. and Raghuvanshi, S. (2009). Evaluation of hydroxyapatite and beta-tricalcium phosphate mixed with bone marrow aspirate as a bone graft substitute for posterolateral spinal fusion. *Indian Journal of Orthopaedics.* **43(3):** 234.

Basile, M.A., Dayala, G.G., Malinconico, M., Laurienzo, P., Coudane, J., Nottelet, B., Ragione, F.D. and Oliva, A. (2015). Functionalized PCL/HA nanocomposites as microporous membranes for bone regeneration. *Materials Science and Engineering* C. **48:** 457-468.

Blokhuis, T.J., Termaat, M.F. and Den Boer, F.C. (2000). Properties of calcium phosphate Ceramics in relation to their *in vivo* behavior. *Journal of Trauma.* **48:** 179-86.

Carloreis, E.C., Borges, P.B., Araujo, A., Mendes, M.V.F., Guan, L. and Davies, J.E. (2011). Periodontal regeneration using a bilayered PLGA/calcium phosphate construct. *Biomaterials*. **32:** 244.

Corinaldesi, G., Pieri, F., Sapigni, L. and Marchetti, C. (2009). Evaluation of survival and success rates of dental implants placed at the time of or after alveolar ridge augmentation with an autogenous mandibular bone graft and titanium mesh: A 3- to 8-year retrospective study.International *Journal of Oral and Maxillofacial Implants.* **24:** 1119-1128.

Giannoudis, P.V., Dinopoulos, H. and T siridis, E. (2005). Bone substitutes: An update. Injury. 36.

Guda, T., Walker, J.A., Singleton, B.M., Hernandez, J.W., Son, J.S., Kim, S.G. and Wenke, J.C. (2013). Guided bone regeneration in long-bone defects with a structural hydroxyapatite graft and collagen membrane. *Tissue Engineering Part A*. **19(17-18):** 1879-1888.

He, M., Xue, J.,Geng, H., Gu, H., Chen, D., Shi, R. and Zhang, L. (2015). Fibrous guided tissue regeneration membrane loaded with anti-inflammatory agent prepared by coaxial electrospinning for the purpose of controlled release. *Applied Surface Science*. **335:** 121-129.

Jegoux, F., Goyenvalle, E., Cognet, R., Malard, O., Moreau, F. and Daculsi, G. (2011). Mandibular segmental defect regenerated with macroporous biphasic calcium phosphate, Collagen membrane and bone marrow graft in dogs. *Archives of Otolaryngology- Head and Neck Surgery*. **136:** 971.

Johnson, A.L. (2013). Fundamentals of Orthopedic Surgery and Fracture Management. In Textbook of Small Animal Surgery. [Fossum, T.W. (eds)], 4th Ed Elsevier Health Sciences.

Oh, T., Rahman, M.M., Lim, J.H., Park, M.S., Kim, D.Y., Yoon, J. H., Kim, W.H., Kikuchi, M., Tanaka, J.,Koyama, Y. and Kweon, O.K. (2006). Guided bone regeneration with beta tricalciumphosphate and poly L-lactide-co-glycolide-co-epsilon-caprolactone membrane in partial defects of canine humerus. *Journal of Veterinary Science.* **7(1):** 73-77.

Queiroz, T.P., Hochuli-Vieira, E., Gabrielli, M.A. and Cancian, D.C. (2006). Use of bovine bone graftand bone membrane in defects surgically created in the cranial vault of rabbits. Histologic comparative analysis. *International Journal of Oral and Maxillofacial Implants*. **21:** 29.

Santos, C., Piedade, C., Uggowitzer, P.J., Montemor, M.F. and Carmezim, M.J. (2015). Parallel nanoassembling of a multifunctional GO/HapNP coating on ultrahigh-purity magnesium for biodegradable implants. *Applied Surface Science*. **345:** 387-393.

Wahl, D.A. and Czernuszka, J.T. (2006). Collagen-hydroxyapatite composites for hard tissue repair. *European Cells and Materials*. **11:** 43-56.