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Short Communication

CLINICAL STUDY OF COLLAGEN MEMBRANE GUIDED BONE REGENERATION IN LONG BONE FRACTURES IN DOGS

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SUMMARY

Bone healing is a complex mechanism and of great concern in large bone defects. Treatment remains challenging for bone defects as bone regeneration is required in large quantity and may be beyond the potential for self-healing. Guided bone regeneration (GBR) technique could be an effective approach for management of such complications. The aim of this study was to evaluate the use of bioresorbable collagen membrane as guided bone regeneration in long bone fractures with bone loss in dogs. Six dogs with long bone fractures accompanied with bone loss in radius-ulna, femur and tibia were surgically treated with bone plating and collagen membrane was placed at the fracture site. Out of six dogs, five dogs were surgically treated with locking compression plate system and one dog was surgically treated with Veterinary cuttable plate system depending on the body weight of the patient. Following the fracture fixation with suitable bone plating, the fracture site was surrounded by a sterile collagen membrane and secured to the bone using polyglactin 910 no 2.0. The fracture healing was evaluated based on clinical, radiographical and biochemical parameters.The lameness grading based on weight bearing showed grade V lameness before surgical stabilization of the fracture. Post operatively five dogs progressed to grade I lameness and one dog progressed to grade II by the end of 90th post-operative day. Follow up radiographs taken on 7th, 15th, 30th, 45th and 60th postoperative days evinced good bone healing in five dogs and one dog with femur fracture showed plate bending by 15th post-operative day. It was concluded that the collagen membrane used for guided bone regeneration provided a good biocompatibility, biodegradability, and acted as barrier for epithelial infiltration and promoted good bone healing.

Keywords: Biocompatibility, Biodegradability, Collagen membrane, Guided bone regeneration

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Treatment of bone defects represents a great challenge, as bone regeneration is required in large quantity and may be beyond the potential for self-healing (Dimitriou *et al.*, 2012). The major obstacle in bone healing and new bone formation is the rapid formation of connective tissue which prevents osteogenesis. The concept of Guided Bone Regeneration (GBR) involves using a bioabsorbable or non-resorbable membrane that acts as a barrier to prevent soft-tissue invasion into the defect, create and maintain a space over the bone defect and under the periosteum-forming a 'chamber' to 'guide' the bone regeneration process (Meinig, 2010 and Retzepi and Donos, 2010). Ideally, osteo-progenitor cells should colonize the space over the defect; however, these cells grow relatively at slower rate. The membranes used in GBR prevent the ingrowth of rapidly proliferating epithelial and connective tissue cells into the defect (Wessing *et al.*, 2018). This barrier membrane guides has been widely investigated and employed for the treatment of bone defects in maxillofacial surgery, especially to improve the alveolar bone quality before implant placement (Von Arx *et al.*, 2005). Collagen membranes are preferred owing to their bioresorbability, hemostatic function, which leads to early wound stabilization, chemotactic properties toward fibroblasts, and permeability, which facilitates nutrient transport (Schwarz *et al.*, 2008). The present study was conducted on six dogs irrespective of age, breed and sex with long bone fractures. These fractures were surgically treated with bone plates *viz*., locking compression plating (n=5) and veterinary cuttable plate (n=1) with application of collagen membrane. The collagen membrane (Healiguide) used in this study is a bioresorbable high purity type-I cross linked membrane with porosity lesser than the penetrable size of an epithelial cell. The different sizes of collagen membrane used in the study were 15×20 mm or 20×30 mm or 30x40 mm in pre-sterilized pack (Fig. 1). All the animals were thoroughly examined for loss of function, abnormal motility, deformity or change in angulation of affected limb, pain and crepitation at the fracture site. Clinical signs and neurological status of the dogs were recorded preoperatively on the day of presentation. Following initial clinical assessment, the dogs were subjected to pre-operative radiographic examination in two orthogonal views. The owners were advised to withhold food for about 12 hours and water for about 6 hours to dogs prior to surgery. Atropine sulphate @ 0.04 mg/kg body weight was administered subcutaneously as pre-anaesthetic followed by xylazine hydrochloride @ 1 mg/kg body weight

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Fig. 1. Guided bone regeneration (Healiguide) used in the study

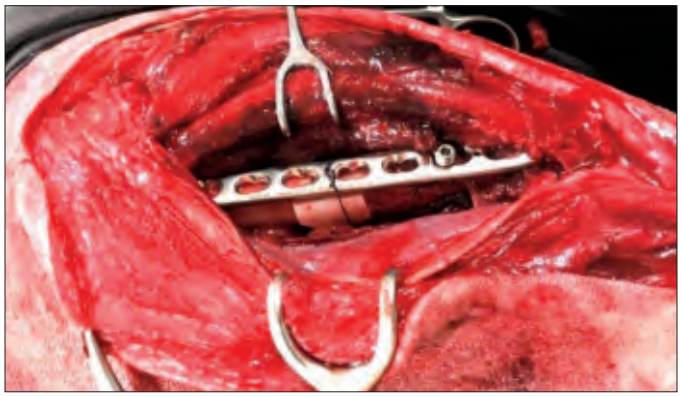

Fig. 2. Fracture site surrounded by a sterile collagen membrane and secured to the bone using polyglactin 910 no 2.0

intramuscularly. General anaesthesia was achieved with intramuscular administration of ketamine hydrochloride @ 10 mg/kg body weight and maintained with intravenous infusion of propofol @ 4 mg/kg body weight. Standard surgical approaches were made for radius-ulna, femur and tibia fractures 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 with screws. Following the fracture fixation with suitable bone plating, the fracture site was surrounded snugly by the collagen membrane. The membrane was secured at the fracture site with polyglactin 910 no 2.0 (Fig. 2). Soft tissue closure was done immediately after the membrane placement. Post-operatively, Ceftriaxone sodium was administered @ 25 mg/kg body weight intramuscularly twice a day for 7 days and Injection Meloxicam@ 0.3 mg/kg body weight administered intramuscularly once daily for 3 days. Owners were advised to restrict the movement of the animal for the first 2 weeks of surgery and then to allow leash walking for the next few weeks. Evaluation of fracture healing was carried based on clinical evaluation,

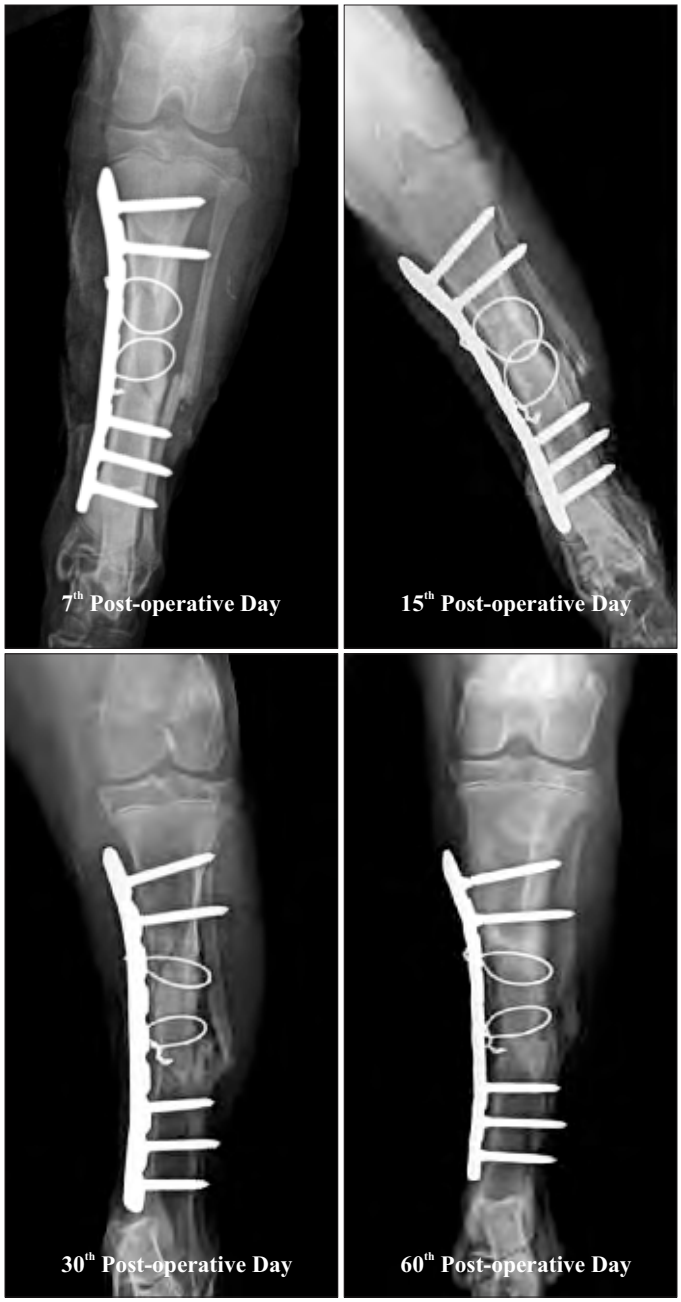

Fig. 3. Tibial fracture immobilized by Locking compression plating with Collagen membrane as GBR

lameness grading, radiographic evaluation, haemato-biochemical evaluation on 15th, 30th, 45th and 60th post-operative days and whenever needed, on later dates to assess the progress of bone healing.

Out of six dogs, three were Non-descript and three German Shepherd. The mean age of the dogs was 15.83 ±6.73 months ranging from 3 to 48 Months. The body weights of the dogs ranged from 6-20 kg with a mean of 16.33±2.15kg. The cause of fractures was automobile accident in three dogs, fall from height in two dogs and in one case it was physical trauma. The fractures occurred in three male dogs and three female dogs. The mean time gap

between the time of fracture and treatment was 4.33 ±0.88 days with a range of 1-7 days. The preoperative radiographs revealed comminuted middiaphyseal closed fracture of radius-ulna in two dogs, comminuted mid diaphyseal closed fracture of femur in two dogs, and comminuted mid diaphyseal closed fracture of tibia in two dogs. The collagen membrane used in this study was found to be biocompatible as no anaphylactic reactions were noticed clinically. As the study was conducted in clinical cases, no owner allowed for second surgery to confirm inflammatory reaction to membrane and its bioresorbability. Lee *et al.* (2003), Amoian *et al.* (2016) and Li *et al.* (2018) expatiated that resorbable collagen membranes did not release any toxic byproducts during resorption and observed no evidence of inflammatory reaction to membrane or necrosis of cells in histologic findings and asserted that lack of antigenicity is one of the most important advantages of collagen membrane. The collagen membrane used in the present study was a bilayered membrane. It has outer smooth compact layer which contacts with soft tissue acting as barrier against epithelial cell infiltration. The inner side has rough porous surface that contacts with the bone giving space for osteoblasts attachment. Kim *et al.* (2009) and Kozlovsky *et al.* (2009) stated that on the smooth surface, although there were penetrations by individual cells, there was no destruction of the structure of membrane or formation of tissue structure. While on the rough surface, osteoblasts attached themselves to the collagen fibers of the membrane by penetrating and adhering to the loose, porous structure which aided in bone regeneration. The collagen membrane used was type-I cross-linked collagen membrane with porosity lesser than the penetrable size of an epithelial cell and found to be sufficiently rigid to snug closely around the defect site. Liu and Kerns (2014) quoted that cross-linking of collagen membrane is associated with prolonged biodegradation as well as reduced epithelial migration, decreased tissue integration, and decreased vascularization. The higher the degree of cross-linking, the longer the resorption rate. This was also opined by Rothamel *et al.* (2005), Verissimo *et al.* (2010) and Lee and Kim (2014) who stated that type I highly cross-linked collagen membrane was found to be associated with a nearly complete continuous layer of lamellar bone with osteoblastic activity after 30 days in a rabbit model compared to only fibrous connective tissue in the non- membrane group. All the six dogs in the present study showed partial weight bearing from the 1st post-operative day. All six dogs showed normal weight bearing at rest, favors affected limb while walking from 7th day. Three dogs achieved complete weight bearing by the 15th post-operative day, one dog by 30th post-operative day, one dog by 60th postoperative day and other dog by 90th post-operative day. Lameness grading (Vasseur *et al.*, 1995) based on weight bearing was recorded pre-operatively showed grade V lameness before surgical stabilization of the fracture. In five dogs progressed to grade I lameness and one dog progressed to grade II by the end of 90th post-operative day. Evaluation of immediate post-operative radiographs revealed proper placement of the plate and screws, good alignment and apposition of the fracture fragments. Immobilization was considered satisfactory with locking compression plating and veterinary cuttable plating in five dogs. One dog with femur fracture immobilized with locking compression plate showed plate bending on 15th post operative day. Plate removal was done in that dog and the fracture fragments were immobilized with intramedullary pining. Radiographs obtained on the 15th post-operative day depicted proper position and good alignment of the fracture fragments with good callus formation, bridging the fracture site. However, the radiolucent fracture line was still discernible in all the dogs. By 30th post-operative day, evidence of callus formation with adequate radio-density was noticed and the fracture line between fracture fragments became faint. One dog with tibia facture immobilized with locking compression plate showed periosteal callus at the posterior aspect of the tibia bridging the fracture site. By 60th post-operative day, bridging bony callus was observed and the fracture line disappeared showing clear restitution of cortico-medullary continuity. The sequential post-operative radiographs revealed progressive bone healing (Fig. 3). The haemoglobin level and packed cell volume on 15th post-operative day decreased initially and then the values gradually increased within normal physiological limits by 60th post-operative day. The total erythrocyte count elevated non-significantly which were within normal physiological limits. This might be due to the physical stress at the time of fracture, loss of blood during surgery, as well as haemodilution and anesthesia during internal fixation procedure. The total leukocyte count was higher on the day before surgery when compared to the postoperative period. Physiological leucocytopenia seen was suggestive of gradual decrease in inflammatory reaction. The differential leucocyte count like neutrophil count decreased on 15th and 60th post-operative day when compared to the day before surgery. Contrary to this, the lymphocyte count increased on 15th and 60th post-operative days within normal physiological limits. This indicated gradual decrease of inflammatory reaction. The monocytic and eosinophilic count showed nonsignificant variations at different time interval within normal physiological limits. The serum calcium values showed a gradual decrease by

15th post-operative day followed by increase in the value and reaching normal at 60th post-operative day. The serum phosphorous values showed a gradual increase without any significant variation and the values were within the normal range. The serum alkaline phosphatase values showed a gradual increase by 15th post-operative day followed by increase in the value and reaching normal at 60th post-operative day indicating increased chondroblastic proliferation to cause bone formation during bone repair.

CONCLUSION

Based on clinical, radiographic and haemo-biochemical parameters it was concluded that the use of collagen membrane aided in improved bone healing as shown by restitution of cortico-medullary continuity by 60th post-operative day which reduced the fracture healing time for comminuted fractures. The collagen membrane used for guided bone regeneration provided a good biocompatibility, biodegradability, and acted as barrier for epithelial infiltration and promoted good bone healing. The results obtained do serve as basic clinical research outcome, however future controlled studies with larger sample size need to be undertaken in a clinical setting to validate the bioresorbability and biocompatibility of collagen membrane as guided bone regeneration and to define their therapeutic use in augmenting or accentuating fracture healing in long bone fractures with bone defects in dogs.

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