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Femoral fracture repair by demineralized bone matrix combinations in canines

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ABSTRACT

Autogenous cancellous bone and demineralized bone matrix combinations used in treatment of experimentally created femoral fractures with bone loss in 48 mongrel dogs of either sex divided equally into 4 groups. Group 1 was kept as control without any graft or implant, while group 2,3,4 animals received autogenous cancellous bone graft, demineralized bone matrix with tricalcium phosphate and demineralized bone matrix with hydroxyapatite implants respectively. Intramedullary pinning was used in all the groups for immobilization of fractured segments.

Early functional limb usage was noticed in demineralized bone matrix with tricalcium phosphate implanted group followed by demineralized bone matrix with hydroxy apatite and autogenous cancellous bone grafted groups.

Radiologically the segmental defect was visible clearly even up to twelfth week in control animals. Bridging of the gap and union of graft to host bone was observed at ninth week in cancellous bone grafted animals. The union was visible at sixth week in demineralized bone matrix implanted groups. Periosteal reaction was prominent in control and cancellous bone grafted animals. The present investigation proved the efficacy of DBM + TCP; DBM + HA implants as good substitutes for autogenous cancellous bone grafts in treating fractures with bone loss in animals.

Key words: Bone, Demineralized bone matrix,Femoral fracture repair,Fracture repair

Autogenous cancellous bone graft is preferred due to its superior osteogenic property and lack of immunological reactions (Reneger 1983). However, it involves risk to the patient like additional surgical intervention, high postoperative morbidity and weakening of bone at the donor site. Moreover, the available transplantable tissue may not be sufficient to fill up a large fracture gap. To encompass the complications and challenges scientists developed biomaterials. Among these demineralized bone chips, powder and matrix were used to induce bone formation in extra skeletal tissue (Urist and Dowell 1968). In this study the demineralized bone matrix with tricalcium and hydroxy apatite combinations were evaluated, and the same were compared with autogenous cancellous bone grafts and untreated group in canines.

MATERIALS AND METHODS

Apparently healthy dogs (48) of either sex weighing 15-20 kg divided into 4 groups of 12 animals each. In all the animals, a complete midshaft transverse fracture and segmental defect of 2cm × 2 cm size were created using wire saw, in the distal fractured segment under general anaesthesia (Fig. 1). Intramedullary pinning was done in all the animals to stabilize the fractured fragments. The defect was left unfilled in group 1 (control) animals. In the group 2, autogenous cancellous bone harvested from iliac crest of the same animal was used to fillup the segmental defect. In groups 3 and 4 combination of demineralized bone matrix with tricalcium phosphate implants were used, respectively, as implants. These implants were interested aseptically into segmental defects, and retained in position by suturing soft tissues over the graft or implant. Post operatively all the animals received antibiotic treatment with daily dressing of wound. The limb was immobilised by applying aluminium splits moulded to the contour and normal angulation of the limb. Following fracture repair changes in clinical symptoms like rectal temperature, pulse respiratory rates, skin wound healing, gait, deformity, extent of edema, swelling of thigh region and discharges from the wound for were recorded 15 days. The performance of each animal was graded according to clinical evaluation of functional limb usage (Braden and Brinker 1973).

Grade	Description of limb usage
I	No functional limb usage. Animal carried limb most of the times.
II	Slight functional limb usage. Animal able to keep limb touching ground at rest/walk without any weight bearing.

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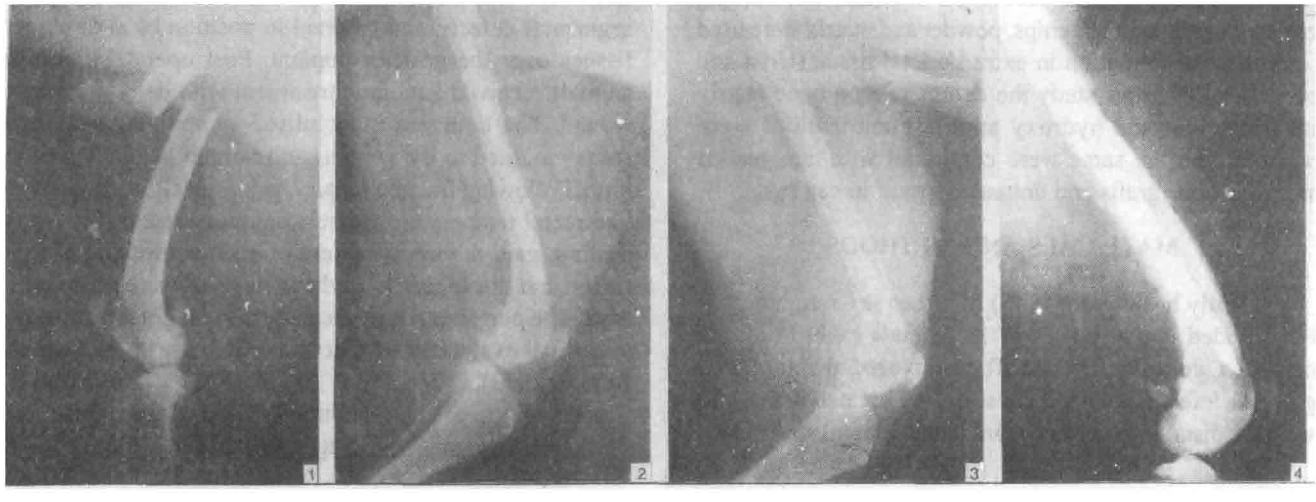
III Limping on limb and partial weight bearing.
IV Full weight bearing on limb. Full functions at standing walking and running.

Medio-lateral skiagrams were taken immediately following the creation of fracture with segmental defect and after immobilization with intramedullary pin skiagram were also taken at 3, 6, 9 and 12 weeks postoperatively. Fracture healing was assessed based on visualization of callus, extent of ossification, con solidation of callus and gap filling.

RESULTS AND DISCUSSION

All the animals evinced pain, however, it was more in group 2 which might be due to additional surgical trauma caused by harvesting of cancellous bone from the iliac crest. Angulation at the fracture site was observed in few animals, which were very active and removed the external splints. Hence it is imperative that in fractures with bone defect external immobilization is essential, Anderson (1965) also stressed this. Mild degree of lameness during progression for the first 10 days might have been due to pain of surgical trauma. Control group animals reached grade I degree of limb usage at the end of second week and grade II in 5 weeks. Postoperatively 4 animals of this group carried the limb throughout the period of observation. There was a gradual improvement in the functional limb usage up to 9 weeks at which time it was close to grade III. Group 3 animals with DBM+TCP regained functional limb usage earlier followed by DBM+HA and cancellous bone grafted animals. Grade-IV limb usage was recorded at 6 weeks in group-3, whereas the same was observed at eighth week in group-4 and group-2. The control animals showed slight gait abnormality even up to 12th week. Early weight bearing capacity and functional limb usage in implanted/grafted group could be attributable to perfect reduction, alignment, and segmental defect filling and fracture fixation. Einborn *et al.* (1984). In treated groups slight edema and swelling might have been due to local reaction of tissues to the grafts or implant or also due to the operative stress (Bommaiah *et al.* 1976). Externally the periosteal callus at the fracture site was observed as a palpable swelling in control and cancellous bone grafted animals and not in other groups. The amount of external callus formed is directly related to the amount of movement present at the fracture site (Anderson 1965). The bigger size of external callus in group 2 is suggestive that cancellous bone graft failed to provide rigid immobilization and the same was also observed in control group.

Skiagram taken immediately after fracture repair showed identical midshaft transverse fractures with rectangular segmental defects of distal segment. Radiographically the alignment of fracture ends was maintained throughout the period of observation. Skiagram taken at the end of third week showed moderate periosteal reaction. The fracture line with defect was visible clearly in control group, whereas group-2 animals showed good periosteal reaction on both the segments group-3 animals showed low density gap with fracture line. Low density implant and fracture gap was visible in group-4 by the end of third week. At sixth week both control and cancellous bone grafted group animals showed extensive and diffuse periosteal reaction along the lateral aspect of bone. Good bridging of fracture gap and blurring of the defect was noticed in group-3 (Fig. 2). Though cortices continuity was established, the fracture gap was still visible as a thin line in group 4. Dense ossification with excess periosteal cuffs was noticed in group-2 animals. Perfect fixation of fractured ends, defect filling and implant union were the features in DBM+TCP group, with uniform density of the implant and host bone (Fig.3). The radiographic union of implant with host bone was a feature in group-4 also. At the end of the



Figs 1-4. **1.** Skiagram showing complete fracture of femur with 2×2 defect in distal end. **2.** Skiagram showing good fracture repair with blurring of defect in group III in 6 weeks. **3.** Skiagram showing uniform density of implant and host bone (9 week). **4.** Skiagram showing union of graft host bone with defect filling in 2 weeks in group 2.

ninth week defect filling was seen in control animals and even at 12 weeks also the defect was clearly observed. The animals group-2 showed union of the graft with host bone with defect filling (Fig.4). Group-3 animals showed uniform density of the fractured area with smooth merging of cortices and defect filling. Group-4 also evidenced fully incorporated implants with uniform radiographic density. The radiographic observations corresponded to the clinical findings that DBM+TCP and DBM+HA implanted groups of animals showed early radiographic union and functional limb usage. There was no implant rejection or host reaction. However, DBM alone or bone induction protein i.e., osteogenic alone was less potent inducer of bone formation. Hence, DBM was coupled with TCP and HA to provide good results. Tuli and Singh (1978) observed new bone formation at 9 weeks and fully mature bone by 12 weeks with defect filling using DBM implants in treating segmental defects of ulna in rabbits. Glowacki *et al.* (1981) reported on clinical and radiographic assessment of craniofacial reconstruction using DBM. Sastry (1989) also confirmed the observation on DBM implants. The new bone is induced by DBM was strong as the bone formed after grafting with autogenous cancellous bone. It can thus be conducted that DBM preparations with TCP or HA mechanical strength, defect filling and early functional limb usage in animals in experimentally created fracture. The same can be used safely as substitutes for autograft, without any reactions.

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