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Research Paper

Radiographic evaluation of healing potential of stem cell-loaded scaffold in experimental bone defect

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ABSTRACT

Regeneration of critical size bone defects using tissue engineered constructs involving bone scaffolds and stem cells is being extensively evaluated. The main aim remains to develop an effective and affordable biomaterials in general and in veterinary medicine, in particular. The present study sought to explore the feasibility of using decellularized bovine (xenogenic) cancellous bone scaffold and allogenic mesenchymal stem cells (MSCs) for treating critical-size bone defects. The *in vivo* study was carried out in grey giant rabbits (n=48) equally divided in 4 groups as per the treatment protocol with left side critical size bone defect in radial diaphysis. The radiographic evaluation on day 30, revealed little radiopacity at defect site in Group I (self-repair without implant) and Group III (decellularized bovine bone scaffold, DBBG) while as in Group II (autologous implant) and Group IV (decellularized bone graft loaded with allogenic mesenchymal stem cells) larger area of radiopacity with external callus and bridging of defect was recorded. In Group I there was no apparent change at day 60th with unrepaired sections seen on radiograph but in Group II and Group IV complete union was seen with most extensive callus formation in Group IV and moderate amount of callus in Group III. Stem cell loaded bovine decellularized scaffold is an effective strategy to improve bone regeneration in critical size gap defects in rabbits.

Key words: Decellularized, grafts, mesenchymal stem cells, radio opaque and implant

Large bone gap defects are one of the most challenging problems for orthopedic surgeons. The defects commonly arise following musculoskeletal tumor resection, accidents, necrosis of bone, cysts etc (Chen *et al.*, 2005). Improvement and facilitation of the bone healing of large size bone defects is now-a-days commonly treated using bone tissue engineered construct which not only help in osteoconduction (like scaffolds) but osteo-induction (stem cells) as well (Giannoudis *et al*., 2000, Buchwalter & Mankin. 1998). To develop a cheap and readily available tissue engineered construct, the study evaluates healing potential of xenogeneic (bovine) decellularized bone with or without the stem cells in induced critical sized radial bone defect using plain radiograph.

MATERIALS AND METHODS

Preparation of decellularized bovine bone scaffold (DBBG): Bovine cancellous bone pieces of 1.5×1.5 cm^2 were prepared from cadaveric bones and then thoroughly washed with sterile phosphate-buffered saline (PBS), rinsed five times in hot water (4 min each time) for up to 20 min to remove lipids, and stored at −4°C before decellularization. For effective decellularization bone pieces were treated with Sodium Dodecyl Sulphate (SDS) solution@ 2.5%, 5%, 8% for 1hr, 3hr and 8hr respectively at 37℃ followed by washing with 75% ethanol and PBS to remove traces of SDS(Shahabipour *et al.*, 2013).

Cell seeding on Scaffold and culture method: Cryopreserved allogeneic bone marrow derived mesenchymal stem cells (BMSCs) in third passage (p3) @ 1×10^6 cells/ml. After sterilization, decellularized scaffolds were transferred in 24-well plates and seeded with 200 µl aliquots containing 2.5×10^6 cells and incubated at 37°C for 1 hr to allow cell attachment (Shahabipour *et al.,* 2013).

Experimental design: Clinically healthy grey giant rabbits (*Oryctolagus Cuniculus)* n=48 of either sex (n=48) with an average age of 6-10 months weighing 1.5-2.4 kg were randomly divided into 4 equal groups with n=12 animals per group. The animals were properly weighed and subjected to thorough physical and clinical examination before creation of critical size bone defect. The surgical site was aseptically prepared and anesthesia was induced using a combination of xylazine HCl (8mg/kg i/m) and ketamine HCl (80mg/kg i/m), the later was given five minutes after xylazine (Gugjoo *et al*., 2022). The critical size (15mm long) full thickness osteo-periosteal bone defect was created using oscillating saw under strict surgical asepsis in left radial diaphysis through medial approach (Akbar *et al*., 2017). Group I (Negative control) animals were given no treatment and no implant was used, Group II animals were kept as positive control with autografts used as implants, Group III animals were treated with decellularized bovine cancellous bone scaffold and Group IV animals were treated with decellularized cancellous bone scaffold loaded with allogeneic mesenchymal stem cells. Implants were snuggly held in place between the proximal and distal bone segments. Skin, fascia and muscles were closed using absorbable (vicryl 2-0) sutures. The test bone was supported by splints and sterile gauze for one week postoperatively (Maiti *et al.,* 2018). Regular dressings with antiseptic lotion was done for 5 days post operatively and administration of antibiotics and analgesics for 3 days post-surgery (Sadegh and Oryan, 2015).

Radiological assessment: Mediolateral radiographs were immediately taken after surgery using table top procedure at 14 mAs; 50 kVp and 90 cm FFD of each bone defect site to monitor the position of the implants (day 0). Subsequently radiographs were taken at 1month (n=48) and 2ndmonth (n=24) interval postoperatively to record the acceptance or rejection of scaffold with or without stem cells. The initiation and progression of bone formation and healing at critical size bone defect site was done at mentioned intervals. Overall radiographic scores for each group were calculated by adding mean scores for each group at different time intervals. Resorption of stem cell-loaded scaffold was qualitatively assessed (Maiti *et al.,* 2018).

RESULTS AND DISCUSSION

Qualitative assessment of radiographs demonstrated new bone tissue formation for various groups as shown in Fig. 1, 2, 3 and 4. In Group I (self-repair without implant) least radiopacity was seen at the defect site on day 30th with little bridging between two fracture ends and persistent unrepaired area seen on day 60th (Fig.1), Group III (decellularized bovine bone implant) show little radiopacity with some amount of callus formation and little implant resorption at defect site on day 30thpost-surgery while on day 60th obvious amount of callus between the defect site and moderate implant resorption was seen (Fig.3). In Group II (autologous implant) (Fig.2) and Group IV (decellularized bovine bone graft loaded with allogenic mesenchymal stem cells) (Fig.4) obvious amount of callus with large radiopaque area is seen between the fracture site on day 30th while as on day 60th complete union was seen in both the groups and complete implant resorption and remodeled callus was seen in Group IV on radiograph.

The present study focuses on the economical readily available bone scaffold loaded with the MSCs. This is done to treat dumb creatures and prevent them from sufferings. As there is good percentage of animals that are affected with the bone affections that fail to heal of its own. In veterinary or in human medicine, common modality for evaluating bone healing is the radiography (Eastaugh-Waring*et al.,* 2009). This is readily available economical diagnostic modality to evaluate bone healing. Considering all these factors, our study focuses on development of tissue engineered construct that can be used to promote bone healing and same can be confirmed through commonly available and economical diagnostic modality (Tawonsawatruk *et al.,* 2014). In our study, we were able to appreciate enhanced bone formation with tissue engineered construct than the autologous graft. Decellularized xenogeneic scaffold can be used in future to treat large bone defects without causing donor site morbidity (Chen *et al.,* 2016).DBB Gsare cost effective, easily available, easy to prepare and can even replace wide variety of scaffolds available in market due to their biomimietic nature, longer shelf life, cost effectiveness. These xenogeneic scaffolds can incite immune response but simultaneous use of the stem cells might have prevented such a reaction and thus, early bone healing than the scaffold group. Further, as the tissue engineered construct improves bone healing even than the autologous, it may be inferred that combined use of scaffold and MSCs offers a better assembly for early and improved bone healing (Maggi *et al.,* 2017). Autografts are considered to be the gold standards but they cause donor site morbidity, more pain, additional

stress, longer hospital stays with limited availability and thus, can be replaced with the tissue engineered construct.

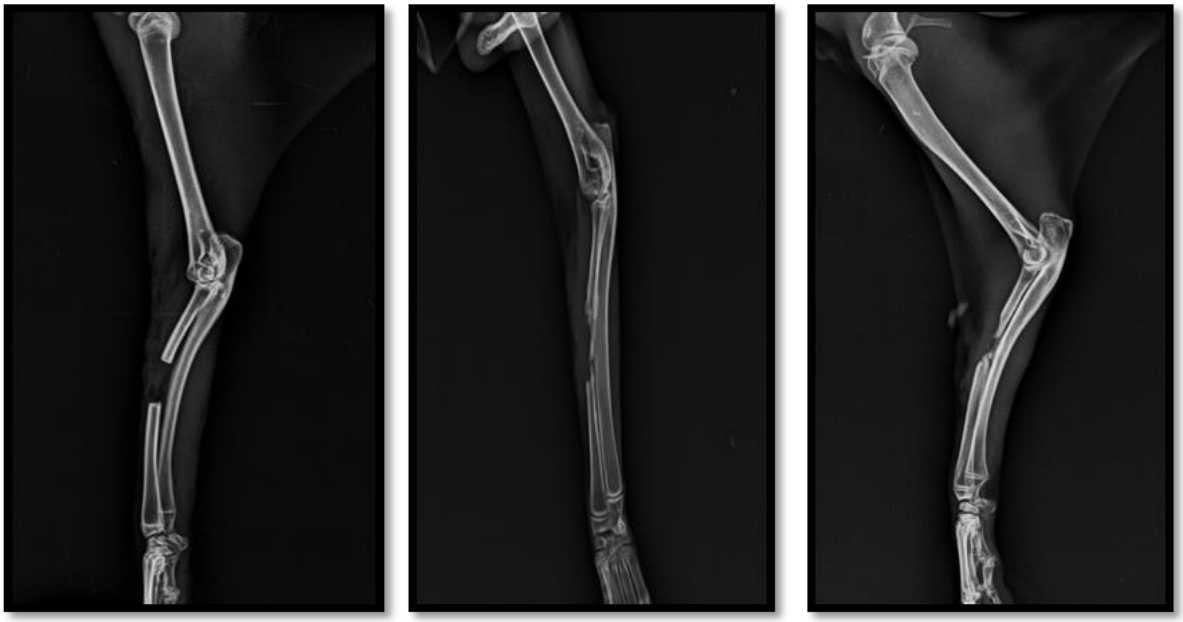

Fig. 1. Group I: Mediolateral radiographs A) 0 day B) 30th day C) 60th day. Decrease in the bone density with small amount of callus between ends on day 30th with little bridging between ends and unrepaired persistent defect on day 60th

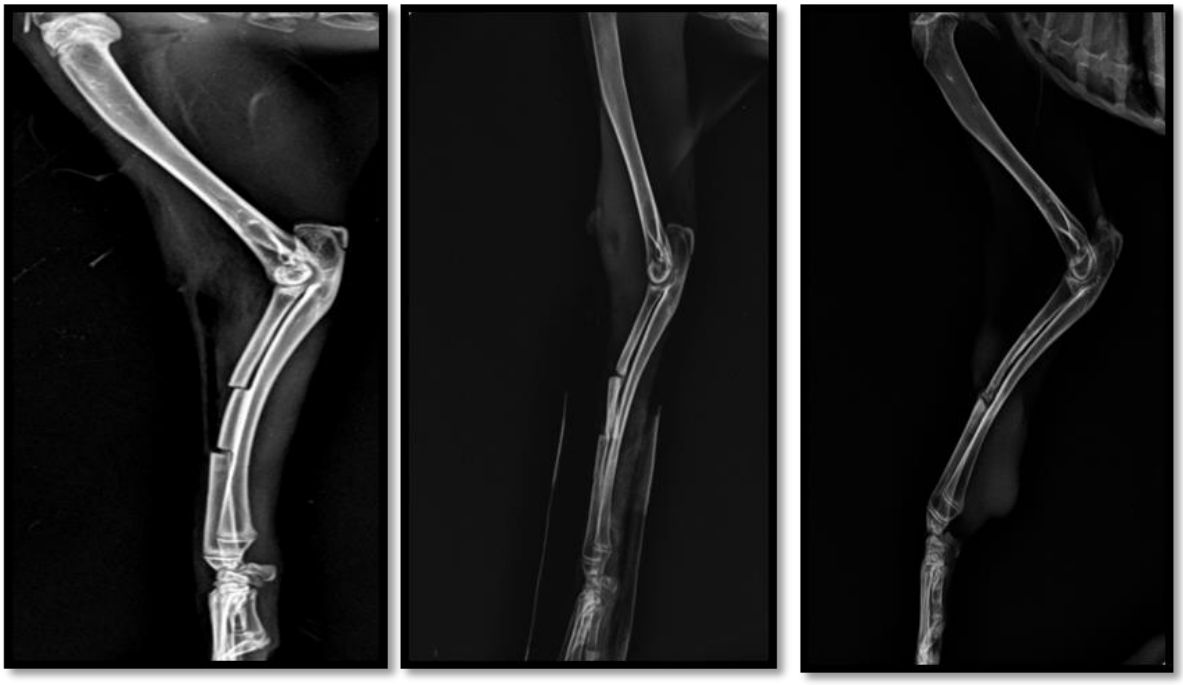

Fig. 2. Group II Mediolateral radiographs A) 0 day B) 30th day C) 60th day. Bridging of the gap with radiopaque callus on day 30th with remodeled callus and union between autologous graft and callus on day 60th

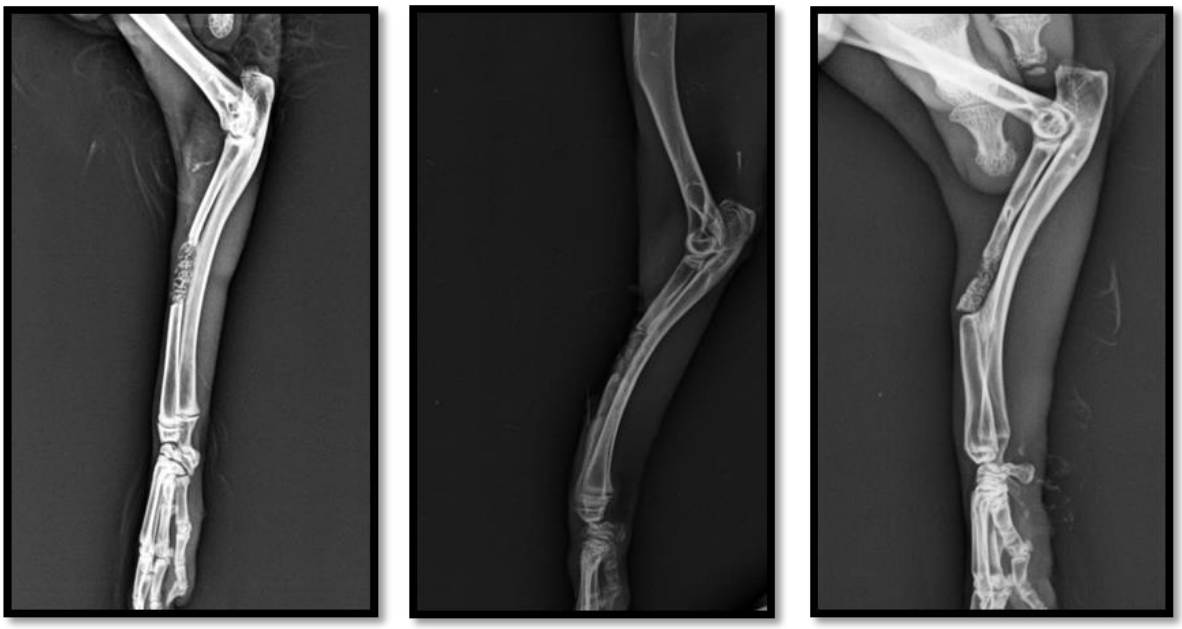

Fig.3. Group III: Mediolateral radiographs A) 0 day B) 30th day C) 60th day. Little amount of implant resorption with little callus formation on day 30thand obvious amount of callus with moderate implant resorption seen on day 60th

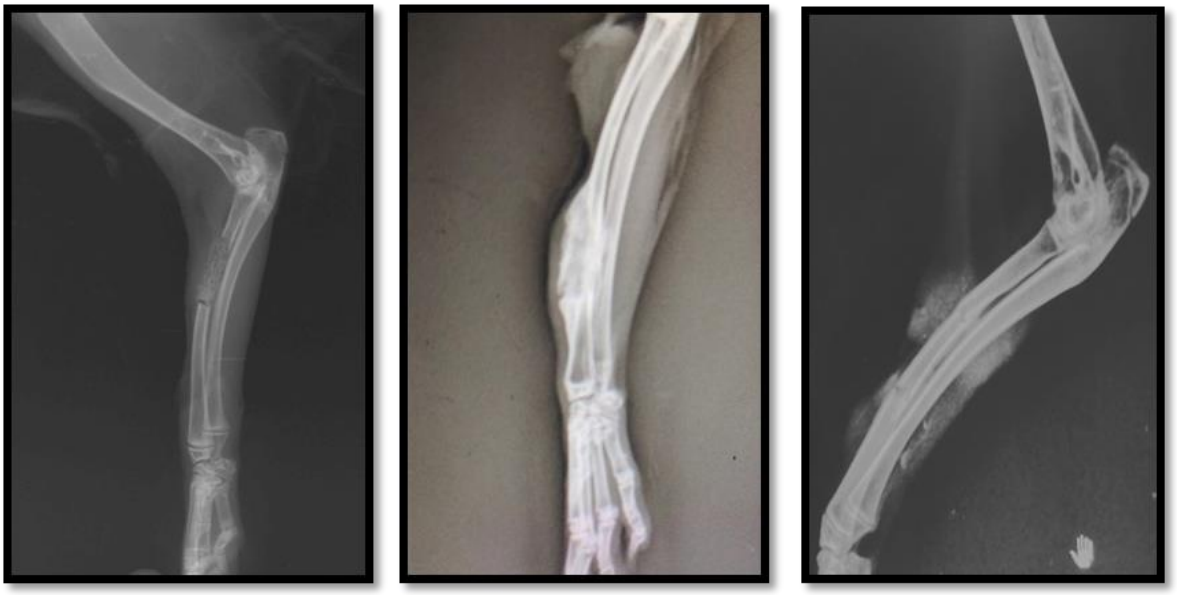

Fig.4. Group IV: Mediolateral radiographs A) 0 day B) 30th day C) 60th day. Large amount of callus with moderate implant resorption on day 30th and complete bone union with remodeled bone at defect site on day 60th

CONCLUSION

The tissue engineered construct involving xenogeneic (bovine) decellularized scaffold along with the mesenchymal stem cells offers a better strategy for healing of critical size bone defects in rabbits.

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