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Comparison of fracture healing efficiency using decellularized caprine periosteum treated with or without undifferentiated bone marrow stem cells in New Zealand white rabbits

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Abstract
Caprine periosteum was decellularized with 5% *Sapindus mukorossi* and 1.5 cm of radial bone defect in New Zealand White rabbits was created in 2 groups of 6 rabbits in each. Scaffold in group I (control) and group II (treated with bone marrow stem cells) compared for fracture healing efficiency. Radiographically, at 12 weeks, there was newly formed bone in the bone defect site in group II only. Histologically, Group II showed woven bone formation which was evident of bone healing. Group II showed no significant difference in ALP.

Keywords: Decellularization, fracture, graft, periosteum, bone healing

Introduction
Critical sized bone defects due to cancer, trauma or congenital deformity can be cured early by application of tissue engineering for bone regeneration. An ideal bone scaffold should be made of such materials that mimic the architecture and properties of natural bone extra cellular matrix. In this study, *in vivo* investigation was done to evaluate tissue engineered decellularized caprine periosteum efficiency, which was seeded with autologous bone marrow stem cells isolated from rabbit iliac crest. It was observed that modified composite scaffold could restore bone defects of critical size in rabbits in shorter time interval. In the previous studies of this research work, a biomimetic tissue-engineered periosteum was obtained by decellularization of caprine periosteum which can be used to promote bone development via endogenous repair mechanisms. Oryan *et al.* (2018) [6] demonstrated that the bone marrow stem cells have the potential to drastically increase the bone regeneration ability in rats. Udehiya *et al.* (2013) [8] regenerated bone in rat calvarial defect by seeding bone marrow-derived cell onto composite scaffold made of acrylated hyaluronic acid.

Materials and methods
Periosteum tissues were obtained from caprine femur and decellularized using *Sapindus mukorossi* aqueous extract (5 wt %) according to the protocol developed by Goyal *et al.* (2021) [4].

5 ml of bone marrow derived undifferentiated mesenchymal stem cells were isolated from iliac crest of New Zealand White rabbits (*Oryctolagus cuniculus*) using Jamshidi needle according to the protocol reported by Vaz *et al.* (2010) [9]. The isolated material was homogenized, and a sample of 50 micro liters was separated for the cell count. *In vivo* study was done between two groups, group I and group II (six rabbits in each), after prior approval from Institutional Animal Ethics Committee (IAEC/CVSc/2/P-05/2020/15). The animals were anesthetized by intramuscular injection of ketamine hydrochloride (60 mg/kg of body weight) and xylazine hydrochloride (6 mg/kg of body weight) (Amarpal *et al.*, 2010). 1.5cm critical sized bone defect in radius was created in both experimental groups randomly "Fig.1"according to the surgical procedure performed by Udehiya *et al.* (2013) [8]. In group I, bone defect was implanted with decellularized periosteal scaffolds as considered as control group. In group II, bone defect was implanted with decellularized periosteal scaffolds alongwith 0.5ml ($3.45x10^6$ cells/ml) of bone marrow aspirate was injected directly into the bone defect using a micropipette.

Evaluation of fracture healing efficiency of periosteum graft was done with radiological assessment, histological analysis, gross observation and biochemical parameter. Fracture healing was radio graphically assessed at 55 kVp, 6.5 mAs and 85 cm FFD. Mediolateral view of the operated site was taken just after operation and subsequently on 6th week and 12th week post-surgery according to the procedure adopted by Udehiya *et al.* (2013) [8]. Blood samples (2 ml) was collected in morning 1 to 2 hrs before surgery (time 0) and at 3, 6 and 12 weeks after surgery for evaluation of ALP levels. New Zealand White rabbits from both groups were sacrificed and their reconstructed radiuses were harvested and completely cleared from the soft tissues. Gross observation of the harvested limb was done at 12th week. Bone healing was observed similarly to Zhao *et al.* (2016) [10]. Specimens from graft sites were collected at 6th and 12th week for hematoxylin and eosin histomorphology according to procedure done by Stubbs *et al.* (2004) [7].

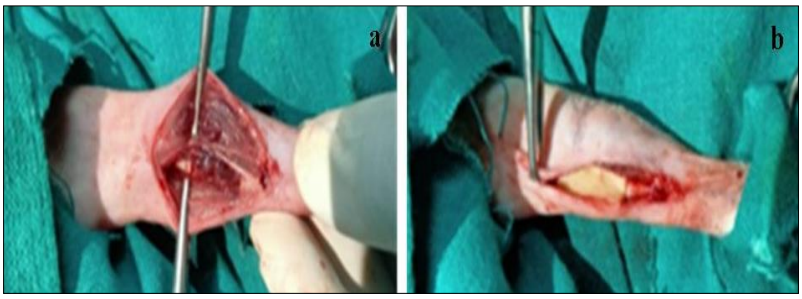

Fig 1: Creation of critical sized bone defect in the radius bone (a) and implantation of decellularized periosteal scaffold at the defect site (b)

Results and discussion

1. Radiographic Examination

Radiographic images of group I and group II animals at 0, 6 and 12 weeks interval after implantation is shown in "Fig 2".

In Group I (control), low density calluses were observed in the bone margins of the defect area at 6 weeks post-operation. At 12 weeks, more calluses were observed and the volume of newly-formed bone and the bone mineral density were both increased as compared to 6 week interval. After 12 weeks, the scaffold was absorbed further with some new bone formation. In Group II, the high callus amount was seen at 6 weeks postoperative. At 12 weeks, there was much more newly formed bone in the bone defect area. The scaffold was partially degraded and new bone formation was observed under X-ray inspection. The bone mineral density was also greatly increased. Newly-formed bone was substituted by degraded periosteal grafts. Regeneration of critical size bone defect of group I was poor and there was minimum callus formation. This decellularized periosteum alongwith undifferentiated bone marrow stem cells induce better new bone formation which might be due to the biomimetic action of the decellularized periosteum scaffolds and osteogenic property of the bone marrow stem cells. The positive effect of MSCs in the treatment of bone fractures was demonstrated by Ghasroldasht *et al.* (2019) [3].

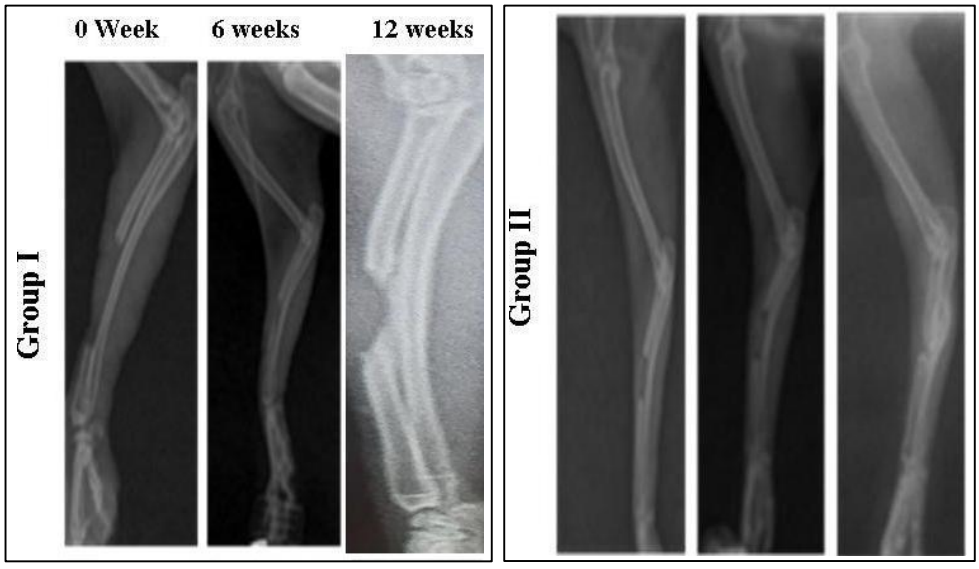

Fig 2: Radiographic images of group I (decellularized periosteal scaffolds), group III (decellularized caprine periosteum with undifferentiated bone marrow stem cells) animals at 0, 6 and 12 weeks interval after implantation.

2. Biochemical parameter

In group I, ALP levels showed significant difference than 0th day levels which was due to poor bone healing at 12th week. ALP levels showed no significant difference throughout bone healing in group II at 12th week as shown in "Table 1." Aljumaily (2010) [1] observed ALP levels for bone healing of right ulna in male New Zealand White rabbits. All rabbits had normal levels of serum alkaline phosphatase (11.8±2.8 IU/dl) at time of osteotomy. After five weeks the level was 12.2±4.6 IU/dl in healed bone. Over all, ALP levels before fracture and after complete healing should be similar, although rise in ALP levels after fracture (from 0th day to 3rd day) may rise due to periosteal damage which is a rich source of ALP in serum.

Table 1: Mean± SE of serum alkaline phosphatase (KA units) of rabbits in group I and group II at different time intervals.

	Group	Time interval (Weeks)	
		0	12
Serum ALP	I	4.46±0.08[a]	1.67±0.13[*a]
	II	6.97±0.24[b]	4.50±0.08[*b]

* Differ significantly from day 0 value.
[a b c] Values differ significantly between the groups at particular time interval.

3. Gross observation

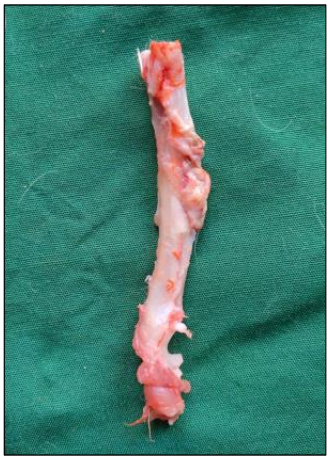

Fig 3: Macroscopic view of bone defects repair in Group II at 12 weeks interval

Gross observation of new bone formation was done at 12th week in Groups II. Macroscopic view "Fig. 3" of critical size bone defect exhibited complete absorption of the periosteum scaffold. It was noticed that both the borders and centers of the defects were covered by a newly formed bone along with irregularly shaped callus. Zhao *et al.* (2016) [10] standardized the minimum size of critical defect in radial bone in New Zealand White rabbits. Five groups of rabbits with different sizes (1.0, 1.2, 1.4, 1.7 and 2.0 cm) of complete periosteal defects were created under anesthesia. It was observed that in all 5 groups, with gradual increase in defect size, bone growth was significantly decreased, at 12th week.

4. Histological analyses

Histological figures of H & E staining in Group I and group II animals at 6 and 12 weeks interval after implantation are showed in "Fig 4." It was observed that in group I there was least amount of bone regeneration. Callus and granulation tissues were not prominent, vasculature was interrupted and bone marrow was damaged. In group II, new woven bone in the defect was formed in layers around the periphery. There was early callus which contain cells similar to chondrocytes and fibroblasts in group II. Presence of inflammatory cells suggests that bone regeneration had taken place, but there was only minimal graft-bone interaction and minimal fibro-cartilaginous tissue observed within the gap spaces. There was more osteoblastic activity in group II animals that suggests bone healing similar to Kanthan *et al.* (2011) [5].

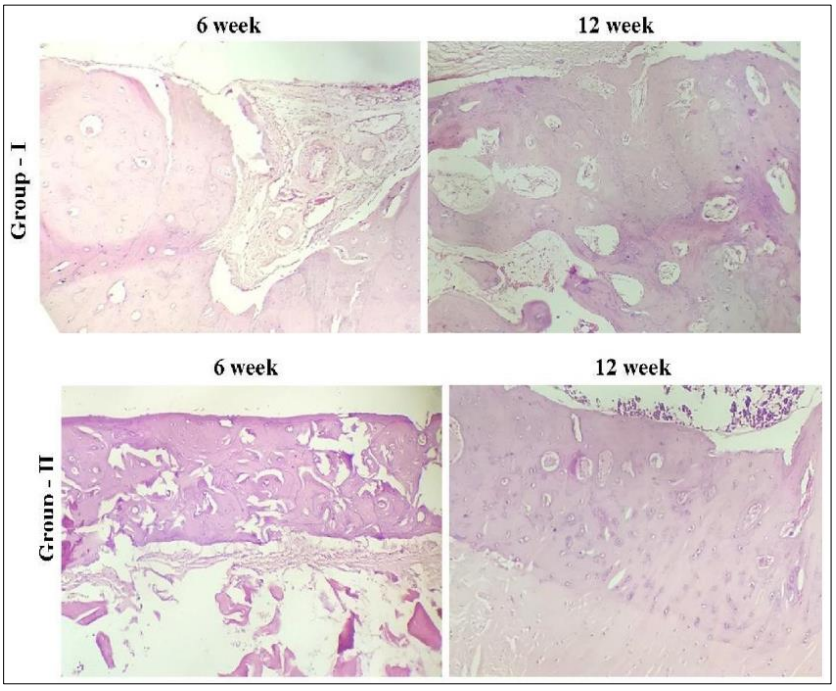

Fig 4: Histological evaluation of H&E staining in Group I (decellularized periosteal scaffolds) and group II (decellularized caprine periosteum with undifferentiated bone marrow stem cells) animals at 6 and 12 weeks interval after implantation.

Conclusions

It was concluded that fracture healing efficiency using 5% *Sapindus mukorossi* extract decellularized caprine periosteum treated with undifferentiated bone marrow stem cells in rabbits can efficiently heal fracture early whereas untreated decellularized caprine periosteum cannot heal fracture with in stipulated time. The bone marrow treated periosteal scaffolds could serve as a xenogeneic biomaterial for reconstruction of critical size bone defects.

Conflict of interest

The Author(s) declare(s) that there is no conflict of interest".

Acknowledgment

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References

1. Aljumaily MA. Open osteotomy of ulna as a model for fracture healing studies in rabbits. Journal of the faculty of medicine Baghdad. 2010;52(3):259-262.
2. Amarpal X, Kinjavdekar P, Aithal HP, Pawde AM, Singh J, Udehiya R. Evaluation of xylazine, acepromazine and medetomidine with ketamine for general anaesthesia in rabbits. Scandinavian Journal of laboratory animal sciences. 2010;37(3):223-229.
3. Ghasroldasht MM, Matin MM, Mehrjerdi HK, Naderi-Meshkin H, Moradi A, Rajabioun M, *et al.* Application of mesenchymal stem cells to enhance non-union bone fracture healing. Journal of biomedical materials research Part A. 2019;107(2):301-311.
4. Goyal RP, Khangembam SD, Gangwar AK, Verma MK, Kumar N, Ahmed P, *et al.* Development of decellularized aortic scaffold for regenerative medicine using Sapindus mukorossi fruit pericarp extract. Micron. 2021;142:102997.
5. Kanthan SR, Kavitha G, Addi S, Choon DSK, Kamarul T. Platelet-rich plasma (PRP) enhances bone healing in non-united critical-sized defects: A preliminary study involving rabbit models. Injury. 2011;42(8):782-789.
6. Oryan A, Eslaminejad MB, Kamali A, Hosseini S, Moshiri A, Baharvand H. Mesenchymal stem cells seeded onto tissue-engineered osteoinductive scaffolds enhance the healing process of critical-sized radial bone defects in rat. Cell and tissue research. 2018;734:63-81.
7. Stubbs D, Deakin M, Chapman-Sheath P, Bruce W, Debes J, Gillies RM, *et al. In vivo* evaluation of resorbable bone graft substitutes in a rabbit tibial defect model. Biomaterials. 2004;25(20):5037-5044.
8. Udehiya RK, Aithal HP, Kinjavdekar P, Pawde AM, Singh R, Sharma GT. Comparison of autogenic and allogenic bone marrow derived mesenchymal stem cells for repair of segmental bone defects in rabbits. Research in veterinary science. 2013;94(3):743-752.
9. Vaz CES, Guarniero R, Santana PJD. Evaluation of centrifuged estrogenic bone marrow in fracture consolidation in rabbits. Acta Ortopédica Brasileira. 2010;18(6):321-326.
10. Zhao MD, Huang JS, Zhang XC, Gui KK, Xiong M, Yin WP, *et al.* Construction of radial defect models in rabbits to determine the critical size defects. Plos one. 2016;11(1):0146301.