RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2023.54.4.916-920

Radiographic evaluation of healing of critical size calvarial defects treated with decellularised tissue engineered HASi in rat models#

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Citation: Manasa M., Dinesh P.T., Fernandez F.B., Sooryadas S., Palekkodan H., Anoop S., Jineshkumar N.S., Remya V. and Varma H.K. 2023. Radiographic evaluation of healing of critical size calvarial defects treated with decellularised tissue engineered HASi in rat models.
J. Vet. Anim. Sci. **54**(4):916-920
DOI: https://doi.org/10.51966/jvas.2023.54.4.916-920

Received: 17.12.2022 *Accepted: 21.12.2022* *Published: 31.12.2023*

Abstract

Extra cellular matrix (ECM) incorporated calcium hydroxyapatite bound with triphasic silica is a custom made bone graft with hybrid properties which is used for bone regeneration. A promising new technique for preparing natural matrices for bone regeneration now in trend is decellularisation. According to reports, the ECM activates mesenchymal stem cells, speeding up bone healing. Although research employing hard tissues like bone are scarce, successful decellularisation has been done using a variety of tissues including skin, cartilage and tendon. The study was carried out in rat calvaria; critical size defects were created and later grafted with decellularised tissue engineered HASi bioceramic (test) and plain HASi bioceramic (control) on left and right side respectively. Radiography was used to assess changes in the graft, the rate of its degradation and the degree of bone repair. Postoperative dorsoventral view of the skull was taken immediately after surgery and thereafter on 2nd, 4th, 8th and 12th week post-surgery. The grafts were found to remain stable throughout the healing period. By 4th week onwards signs of gradual degradation of material with regeneration of bone could be noticed especially on test side compared to control. Test material

 # Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

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was found to be completely integrated with host bone by eight weeks whereas control graft took 12 weeks. At twelve weeks, both the test and control grafts showed complete integration with host bone. It was concluded that the test graft material successfully hastened bone regeneration, making it a promising alternative to conventional bone grafts.

Keywords: *Decellularised tissue engineered HASi bioceramic, critical size calvarial defect, extra cellular matrix*

Over the past few years there is a dramatic rise in bone injuries which mainly resulted from traumatic injuries and age-related degenerative diseases, creating an urgent demand for bone tissue engineering. Although bone tissue demonstrates significant healing ability, the body is incapable to effectively mend large defects in the bone (Einhorn *et al.,* 2015). Mesenchymal stem cells, biochemical signalling and extracellular matrices (ECM) act in concert to heal the bones quickly. Bone defects are currently treated with autografts derived from the same patient, allografts which come from a donor of same species and synthetic biomimetic materials. However, these treatments have serious drawbacks, such as the need for additional surgeries, the need to manipulate bone size and shape, the associated pain and graft rejection. Close collaboration amongst scientists of different areas such as tissue engineering, nanotechnology and veterinary science, lead to the creation of hybrid bone substitutes that are clinically applicable and can restore, maintain or enhance bone function.The design of test graft used in the study meets all the ideal characteristics of bone implantas described by Bisht *et al.*(2021) and they are three O's, namely, osteoinductive stimuli, osteoconductive scaffolds and osteoprogenitor cells activation. The objective of the present study was to assess the healing potential of plain and decellularised tissue engineered HASi in critical sized bone defects in rat calvarium using radiographs, over a period of twelve weeks.

Materials and methods

The experimental procedures were approved by the Institutional Animal Ethics Committee (IACUC) of College of Veterinary and Animal Sciences, Pookode of Kerala Veterinary and Animal Sciences University (No. IAEC/COVAS/PKD/19/2021)

The study was conducted in eighteen adult male Wistar rats under general anaesthesia using xylazine and ketamine (at the dose rate of 7mg/kg and 70mg/kg body weight respectively) which was administered intraperitoneal 20 min after buprenorphine premedication. The parietal bone was approached as per Spicer *et al.* (2012) through a linear skin incision made over the scalp down to periosteum extending from the nasal bone to just caudal to the bregma. After exposure, four-millimetre diameter defects were created on the parietal bone and the critical sized defect was bridged with pre-sized test and control graft on left and right side respectively.

Immediate postoperative radiographs were taken to assess the position of the grafts. Healing of the defect was assessed radiographically by dorso-ventral view of the skull under light anaesthesia using isoflurane (three per cent) in oxygen during 2nd, 4th, 8th and 12th week postoperatively.Three radiographs each at different stages of healing were given to six persons who were unaware of the stage of healing and scoring was done. The scores ranged from 0 to 4 where "0" denoted "No-healing" and "4" denoted "Complete healing" (Table 1).

Table 1. Criteria for radiographic scoring

Sl. no.	Criteria	Score
1.	No healing*	0
2.	25% of defect healed	1
3.	50% of defect healed	2
4.	75% of defect healed	3
5.	100% of defect healed**	4

*Presence of radiolucent line observed between the defect and graft all around

**Absence of radiolucent line observed between the defect and the graft

Result and discussion

All the rats were subjected to radiographic evaluation for assessing healing of the defect immediately after surgery

and thereafter at 2nd, 4th, 8th and 12th week postoperatively. Immediate postoperative radiographs showed that the grafts were in position and a radiolucent line was observed at the interface between graft and host bone all around the defect. The radio density of both the biomaterials (test and control) was higher than host bone radio density. But, the radiographic density of the test material was found to be higher than that of the control (Fig. 1). In the second week radiograph, it was observed that almost 30 per cent of the test material was integrated with the host bone which was indicated by absence of radiolucent line between host bone and the graft. But in case of control graft, the host bone graft integration was only less than 20 per cent (Fig. 2). Rao *et al*. (2021) opined that absence of radiolucent line between the host and the graft could be taken as an indicator for assessing the graft-host bone integration.

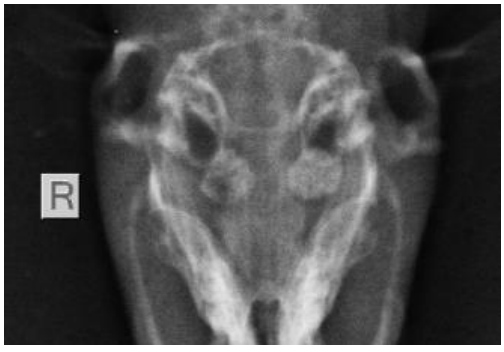

Fig. 1. Immediate postoperative radiographs

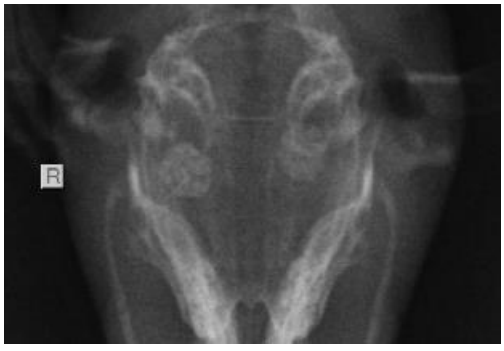

Fig. 2. Radiograph at 2nd week

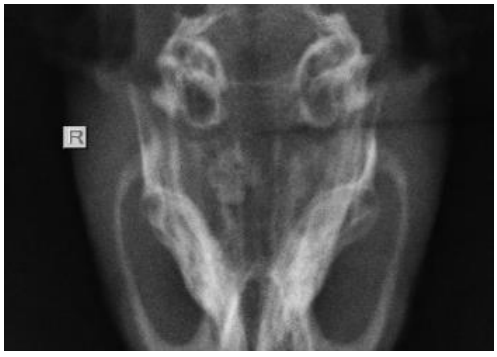

Fig. 3. Radiograph at 4th week

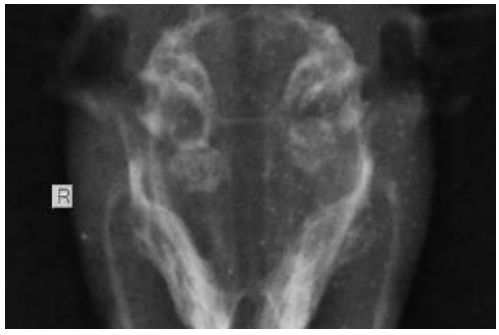

Fig. 4. Radiograph at 8th week

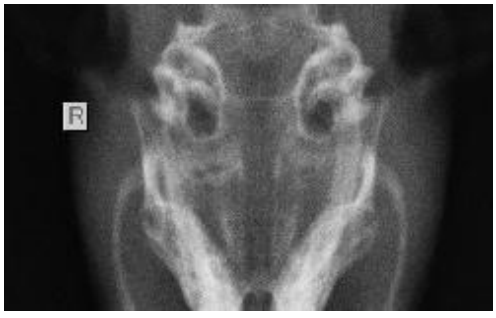

Fig. 5. Radiograph at 12th week

By 4th week, 80 per cent of the test graft and 50 per cent of control graft was found integrated with the host bone. It was found that the radio density of the test material was decreased when compared to the control. Nair *et al.*, (2009) opined that reduction in the density of the biomaterial indicated osteogenesis into the pores of the graft and degradation of the material (Fig. 3). This was noted in the 8th week assessment also. Similar observation was noted by Suresh *et al.* (2017) that decreased radio opacity of the graft was a sign of material degradation and osteogenesis occurring within its pores. Authors were able to contemplate that the graft material gradually degraded along with osteogenesis and bone remodelling.

At eight weeks, it could be noticed that the test graft got completely integrated with host bone which was indicated by absence of

differentiation between the graft and the host bone. The control graft showed 75per cent of integration with host bone (Fig. 4). On the contrary, in the study carried out by Dinesh *et al.* (2018), the authors observed complete integration of the graft and host by fourth week in critical size femoral defects in rat models treated with HASi which is used as the control graft in the present study. By 12th week it was observed that both the defects healed completely and both test and control grafts was integrated fully with the host bone radiographically (Fig. 5).

Radiographs were scored for healing by six blind folded people who were experienced in assessing radiographic healing and were unaware of the stage of healing (Table 2). The radiographic healing score was 2.45 ± 0.04 for test and 1.8± .05 in control at two weeks, 2.92 ± 0.05 in test and 2.35 ± 0.05 in control at four weeks, 3.18 ± 0.16 for test and 2.95 ± 0.11 for control at eight weeks and 3.98 ± 0.01 in test and 3.76 ± 0.04 in control at 12 weeks in a score card in which maximum point was four. The radiographic healing scores differed significantly between the test and control at each observation except at second week.

Table 2. Radiographic healing scores (Mean±SE)

Stage of healing	Control	Test
2 weeks	1.86 ±0.05^{a}	2.45 ± 0.04^{a}
4 weeks	2.35 ± 0.05^{b}	2.92 ± 0.05^{c}
8 weeks	2.95 ± 0.11^{d}	3.18 ± 0.16^{e}
12 weeks	3.76 ± 0.04^{f}	3.98 ± 0.01^{g}

P ≤ 0.01means with different superscript differ significantly

Hence, the present study has proved that test biomaterial could be successfully employed in clinical conditions for treating critical size bone defects. In conclusion, defects treated with the test biomaterial significantly improved bone regeneration compared to the control biomaterial. The test biomaterial possesses hybrid properties and all ideal characteristics of a bone graft, making it a potential bone substitute in the field of bone regeneration.

Acknowledgement

The authors are thankful to the Dean, college of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala for providing the facilities to carry out the work.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Bisht, B., Hope, A., Mukherjee, A. and Paul, M. K. 2021. Advances in the fabrication of scaffold and 3D printing of biomimetic bone graft. *Annals of Biomed. Engineering*, **49:**1128-1150.

Dinesh, P.T., Syam K. Venugopal, John Martin K. D., Devanand C. B., Usha N. Pillai and N.D Nair. 2018. Radiographic evaluation of healing of critical size defects of femur treated with tri-phasic composite bio-ceramic implants in rat models. *Ind.J.Nat.Sci.* **9**:15504-15507.

Einhorn,T.A.andL.C.Gerstenfeld.2015.Fracture healing: mechanisms and interventions. *Nat. Rev. Rheumatol.***11**:45–54.

Nair M. B. 2009. Bone reconstruction of goat femur segmental defects using tissue engineered bioceramic scaffolds with osteogenically induced mesenchymal stem cells and platelet rich plasma. Ph.D. Thesis, Sri ChithiraThirunal Institute for Medical Science and Technology, Trivandrum, 235p

Rao, R., Dinesh, P.T., Sooryadas, S., Chandy, G. and Mathew, M. 2021. Successful utilization of triphasic silica containing ceramic coated hydroxyapatite (HASi) for the treatment of comminuted tibial fracture in a goat: a case report. *J. Vet. Anim. Sci.* **52**: 200-203

Spicer, P.P., Kretlow, J.D., Young, S., Jansen, J.A., Kasper, F.K. and Mikos, A.G. 2012. Evaluation of bone regeneration using

the rat critical size calvarial defect. *Nature protocols.* **7:**1918-1929.

Suresh, D., Syam, K.V., Chandy, G., Sooryadas, S. and Deepa, P.M. 2017. Radiographical evaluation of Sr-HASi bioceramic substitute for critical size bone defect in a goat–Clinical study. *J. Vet. Anim. Sci*. ***48***: pp.40-42. ■