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# Radiographic evaluation of healing of critical size calvarial defect treated with silica-collagen-hydroxyapatite biocomposite graft and homologous platelet-rich plasma in rat model#

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## Abstract

*Seventy two adult male Wistar rats were selected and randomly divided into three groups with 24 rats. Calvarium of each rat was subjected to creation of 4.0 mm critical sized defect under general anaesthesia. The defect was treated with silica-collagen-hydroxyapatite biocomposite in Group I. Combination of silica-collagen-hydroxyapatite biocomposite with 50µL of freshly prepared homologous platelet-rich plasma (PRP) was applied into the defect in Group II. The defect of Group III was kept free of any biomaterial. Dorso-ventral radiographs of the rat skull were taken on the day of surgery, 2nd, 4th, 8th and 12th week to evaluate the healing of the defect. Gray scale of radiographic images were recorded and analysed by using Image J software. In the present study, the PRP treated group exhibited significant enhancement of the gray scale on 12th week compared to Group I and III and hence it was concluded that the silica-collagen-hydroxyapatite biocomposite along with PRP significantly increased the new bone formation in critical sized calvarial defects radiographically.*

**Keywords:** *Silica-collagen-hydroxyapatite, homologous platelet-rich plasma, rat, calvarial defect model, gray scale*

Bone regeneration and repair faced considerable difficulties in craniofacial surgery particularly in addressing calvarial defects that occurred from trauma, congenital disorders, infections or surgical interventions. Preclinical studies utilised animal models frequently, especially rodents to assess the performance of bone grafts, scaffolds and regenerative materials due to their compact size and affordability. The rat calvarial defect model was one of the most established animal models for studying intramembranous bone regeneration due to biological similarities to human bone healing mechanisms. The critical-sized calvarial defect provided an ideal platform for evaluating the osteoconductive and osteoinductive potential of various biomaterials (Gomes and Fernandes, 2011).

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Biomaterials are vital in preclinical studies as they offer both structural integrity and biochemical signals that encourage and direct bone regeneration. Silica-collagen-hydroxyapatite biomaterial was developed and standardised at Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram. The collagen and hydroxyapatite in the biomaterial facilitated the osteoconduction. The silica particles can promote differentiation of osteoblast and stimulate mineralisation in bone defects. Platelet-rich plasma (PRP) has gained widespread attention in orthopaedics and dentistry for its ability to promote bone healing due to the presence of various growth factors which promoted the bone regeneration process (Bacevich *et al.,* 2024).

Radiographic analysis is essential for monitoring bone regeneration in calvarial defect models. Radiographic assessment of bone healing in calvarial defects in laboratory animals is challenging, particularly due to overlapping bone structures and the use of radiolucent biomaterials. Despite of these difficulties, radiographic evaluation remained a cost-effective diagnostic tool for monitoring new bone formation in preclinical studies.

Gray scale analysis was employed as a reliable method for quantifying radiographic density within a specified region. Image analysis was performed using ImageJ software. In the present study, gray scale measurement was utilised to evaluate new bone formation in a rat calvarial defect model treated with a radiolucent silica-collagen-hydroxyapatite composite**.** This method was found effective for statistically analysing the increase in gray scale values, reflecting progressive mineralisation within the defect area.

## Materials and methods

### *Surgical procedure*

The present study was approved by Institutional Animals Ethics Committee (IAEC), College of Veterinary and Animal Sciences, Mannuthy, Thrissur under Kerala Veterinary and Animal Sciences University with project proposal no. CVAS/MTY/IAEC/24/75. A total of 72 adult male Wistar rats were selected for the study. All the rats were provided with one week acclimatisation period before the start of surgical procedure.

The biomaterial selected for the present study included silica-collagen-hydroxyapatite and a combination of silica-collagen-hydroxyapatite and homologous platelet-rich plasma. The silica-collagen-hydroxyapatite biomaterial (95 per cent hydroxyapatite-silica and 5 per cent collagen) was developed and standardised at the Bioceramic Laboratory, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Thiruvananthapuram. It was available in a flexible sheet form with thickness of 0.5 mm and could be easily shaped into a 4.0 mm round piece using a 4 mm biopsy punch. The porosity of the biomaterial was confirmed through scanning electron microscopy (Fig. 1). Whole blood for the preparation of platelet-rich plasma was collected from three rats from the control group on their sacrifice period through intracardiac route with ten per cent sodium citrate as anticoagulant. The pooled whole blood was subjected to soft spin with a force of 160×g for 20 minutes. After the first centrifugation, the plasma with suspended platelets and buffy coat layer were transferred into a sterile falcon tube and subjected to hard spin with a force of 400×g for 15 minutes. Two third portion of platelet-poor plasma was removed and mixed the remaining platelet- poor plasma with platelet pellet to make PRP (Messora *et al.,* 2011).

The rats were premedicated with inj. buprenorphine at a dose rate of 0.05mg/kg body weight subcutaneously as an analgesic five minutes before the induction of anaesthesia. Induction of anaesthesia was done with combination of inj. xylazine and inj. ketamine at a dose rate of 7.0 mg/kg and 70 mg/kg body weight intraperitoneally. The depth of anaesthesia was assessed by the absence of pain reflex to toe pinch. Preoperatively all the rats were administered with 5.0 mL/kg body weight sterile normal saline intraperitoneally (Spicer *et al.,* 2012). Positioned the rats in ventral recumbency and prepared the forehead for aseptic surgery. A one centimetre long incision was made in the calvarial skin from bregma to lamda by using a No: 11 Bard-Parker scalpel blade. The skin and subcutaneous tissue were separated gently. Bleeding from the surgical site was arrested by mechanical pressure using sterile cotton buds. The right parietal bone was isolated for the creation of the calvarial defect. After isolation of the right parietal bone, a four millimetre diameter defect was created by using a burr with four millimetre diameter connected to the contra-angled handheld drill. Sterile normal saline at a temperature of approximately 4-10º C (0.5 to 1 mL) (Isler *et al.,* 2011) was slowly poured into the site during the defect creation to prevent the thermal damage of the bone tissue. Removed the bone flap gently without damaging the underneath dura mater. The preformed (round shape with 4 mm diameter) and moderately porous silica-collagen-hydroxyapatite biomaterial was placed accurately into the created defect by using micro-forceps in Group I (Fig. 2A). In Group II, along with silica-collagen-hydroxyapatite biomaterial, 50µL of freshly prepared platelet-rich plasma was also added by using micro-pipette (Fig. 2B). The defect in Group III was left untreated and considered as control (Fig. 2C). The subcutaneous tissue was apposed in a simple interrupted manner by using polyglactin 910 size-3/0 suture material. The skin incision was closed by using fine nylon in a simple interrupted suture pattern. Each rat was housed individually after the surgical procedure to prevent the mutilation of the surgical site by other rats and monitored their activities regularly. Post-operatively, all the rats were treated with inj. buprenorphine at a dose rate of 0.05 mg/kg body weight subcutaneously for three days and inj. ceftriaxone sodium (Gramocef - 0.25, Micro Animal

Health Care Private Ltd., Bangalore) at a dose rate of 40 mg/kg body weight subcutaneously for five days. After ten days, the sutures were removed upon uneventful healing.

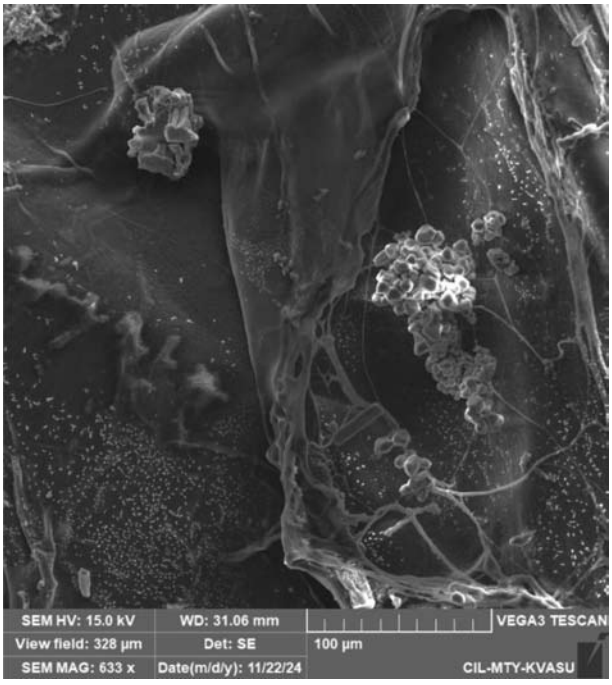


**Fig. 1.** Scanning electron microscopic image of silica-collagen-hydroxyapatite

***Radiographic evaluation and analysis***

Radiographic evaluation of the healing of calvarial defects were performed on day 0, 2nd, 4th, 8th and 12th week under short ether anaesthesia in dorso-ventral position by using fixed X-ray machine (Stationary X-ray generator, Multiphos 15 single tube, Model No. 5602, Siemens LTD., Verna industrial estate, Goa, India) with film focal distance at 100 cm and with exposure parameters of 10 mAs and 48 kVp (Fig. 4). The radiographic density was measured through gray scale (0 - 255) using ImageJ software. After capturing the radiograph, the final images were opened using ImageJ software. The defect area was selected and analysed using the grayscale tool in the software (Fig. 6) (Magat and Sener, 2019).

***Statistical analysis***

The values were analysed by using one-way ANOVA followed by Duncan Multiple range test. Comparison within each group between the observation period was done by using repeated measures ANOVA followed by least significant different test for pair wise comparison.


**Fig. 2A.** Group I with silica-collagen-hydroxyapatite, **2B.** Group II with silica-collagen-hydroxyapatite and homologous PRP, **2C.** Group III - control

**Table 1.** Results of comparison of Gray scale valuebetween groups (n=6)

| Weeks | Group I | Group II | Group III | F-value (p-value) |
|---|---|---|---|---|
| Day 0 | $0.13^{C} \pm 0.00$ | $0.13 \pm 0.00$ | $0.12 \pm 0.00$ | 2.01 (0.167) |
| 2nd week | $0.15^{bC} \pm 0.00$ | $0.17^{a} \pm 0.00$ | $0.13^{b} \pm 0.00$ | 12.85** (0.001) |
| 4th week | $0.19^{BC} \pm 0.02$ | $0.25 \pm 0.08$ | $0.16 \pm 0.02$ | 0.90 (0.427) |
| 8th week | $0.22^{AB} \pm 0.01$ | $0.26 \pm 0.03$ | $0.18 \pm 0.04$ | 1.69 (0.217) |
| 12th week | $0.26^{bA} \pm 0.00$ | $0.35^{a} \pm 0.03$ | $0.19^{b} \pm 0.02$ | 11.78** (0.001) |
| F-value (p-value) | 17.16** (<0.001) | 5.03 (0.057) | 2.07 (0.123) | |

** Significant at 0.01 level
Means having different small letter as super script differ significantly within a row
Means having different capital letter as super script differ significantly within a column

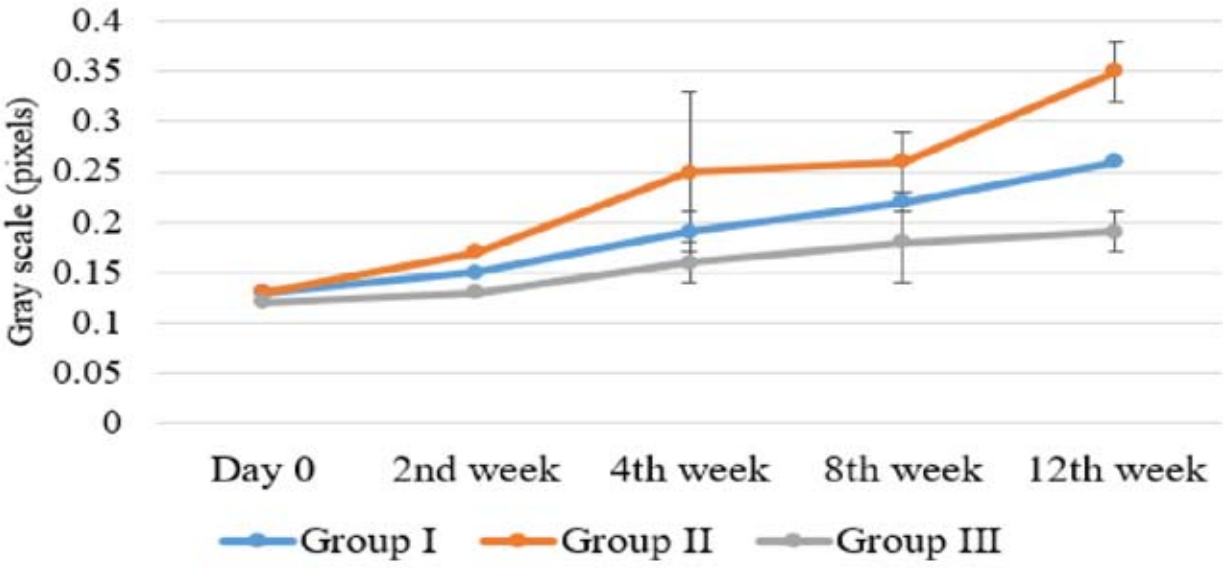


**Fig. 3.** Graphical representation of gray scale value between groups

**Results and discussion**

Platelet-rich plasma was prepared using ten per cent sodium citrate as an anticoagulant and yielded 1.5 mL of PRP with an enrichment factor of 4.25.

In Group I, the mean ± SE gray scale value on day 0, 2nd, 4th, 8th and 12th week were 0.13 ± 0.00, 0.15 ± 0.00, 0.19 ± 0.02, 0.22 ± 0.01 and 0.26 ± 0.00 respectively. Gray scale value was significantly increased from day 0 to 4th week, from 4th week to 8th week and from 8th week to 12th week. In Group II, the mean ± SE gray scale values on day 0, 2nd, 4th, 8th and 12th week were 0.13 ± 0.00, 0.17 ± 0.00, 0.25 ± 0.08, 0.26 ± 0.03 and 0.35 ± 0.03 respectively. Significant difference was not observed between the observation periods but gradual increase in the gray scale values were noted between the observation periods. In Group III, the gray scale values on day 0, 2nd, 4th, 8th and 12th weeks were 0.12 ± 0.00, 0.13 ± 0.00, 0.16 ± 0.02, 0.18 ± 0.04 and 0.19 ± 0.02 respectively. Significant difference was not observed between the observation periods. But increases in the gray scale values were noted between the observation periods (Fig. 3 and 5).

A significant increase in the gray scale value was noted on 2nd and 12th week of Group II compared to Group I and Group III. Significant difference was not noticed between other observation periods (Table 1).

In this study, adult male rats were selected to evaluate bone formation with the aim of eliminating the potential bias introduced by age and sex hormones on bone formation and bone mineral density thereby ensuring uniformity of data. Osteoblasts and osteocytes possessed receptors for both testosterone and oestrogen however testosterone receptors were more predominant in bone cells (Costa *et al*., 2019). Consequently, male animals exhibited greater bone formation compared to females which further justified the selection of adult male rats for this study.

Different types of graft materials are available for the management of calvarial defects such as autografts, allografts and xenografts. Autografts are the ideal choice but the lack of availability in larger amounts and associated complications reduced its widespread use. Therapeutic approaches with allografts and xenografts had drawbacks of limited source availability and host rejection. Synthetic ceramic bone scaffolds like hydroxyapatite alone have limited ability for osteoconduction and osteoinduction. Collagen based scaffolds possess reduced mechanical strength but incorporation of the bioceramic material appears to enhance its performance in bone healing (Scabbia and Trombelli, 2004). Incorporation of silica can promote differentiation of osteoblast and stimulate mineralisation in bone defects (Bosetti and Cannas, 2005). Hence, the combination of these biomaterials seems to be suitable for bone regeneration.

Platelet-rich plasma provided a concentrated supply of growth factors that encouraged cellular growth and differentiation essential for bone repair. These factors were also contributed to the formation of new blood vessels and supported collagen production (Lieshout and Hartog, 2021). There are no standard concentration levels for PRP application. According to Weibrich *et al.* (2004) for the potential effect of PRP, it contained minimum $1\times10^6$ platelets/µL. In the present study the platelet concentration was $2.73\times10^6$ platelets/µL with enrichment factor of 4.25.

Marx (2004) stated that 2-6 fold platelets from the baseline produced significant effect in the healing process.

Healing of calvarial defect was assessed through radiographic images. Gray scale was recorded and analysed from radiographic images and the progressive increase in the radiographic density indicated new bone formation at the defect site. A significant increase in the gray scale value was observed in the 12th week of Group II compared to Group I and III indicated that better new bone density was observed in PRP treated group. Both biomaterial treated groups had high new bone density than control group. Similarly, Kim *et al.* (2014) evaluated the healing of rabbit calvarial defects treated with platelet-rich plasma (PRP), platelet-rich fibrin (PRF) and concentrated growth factor using gray scale analysis. They found that the PRP- and PRF-treated groups showed higher gray scale values at the 12th week compared to the other

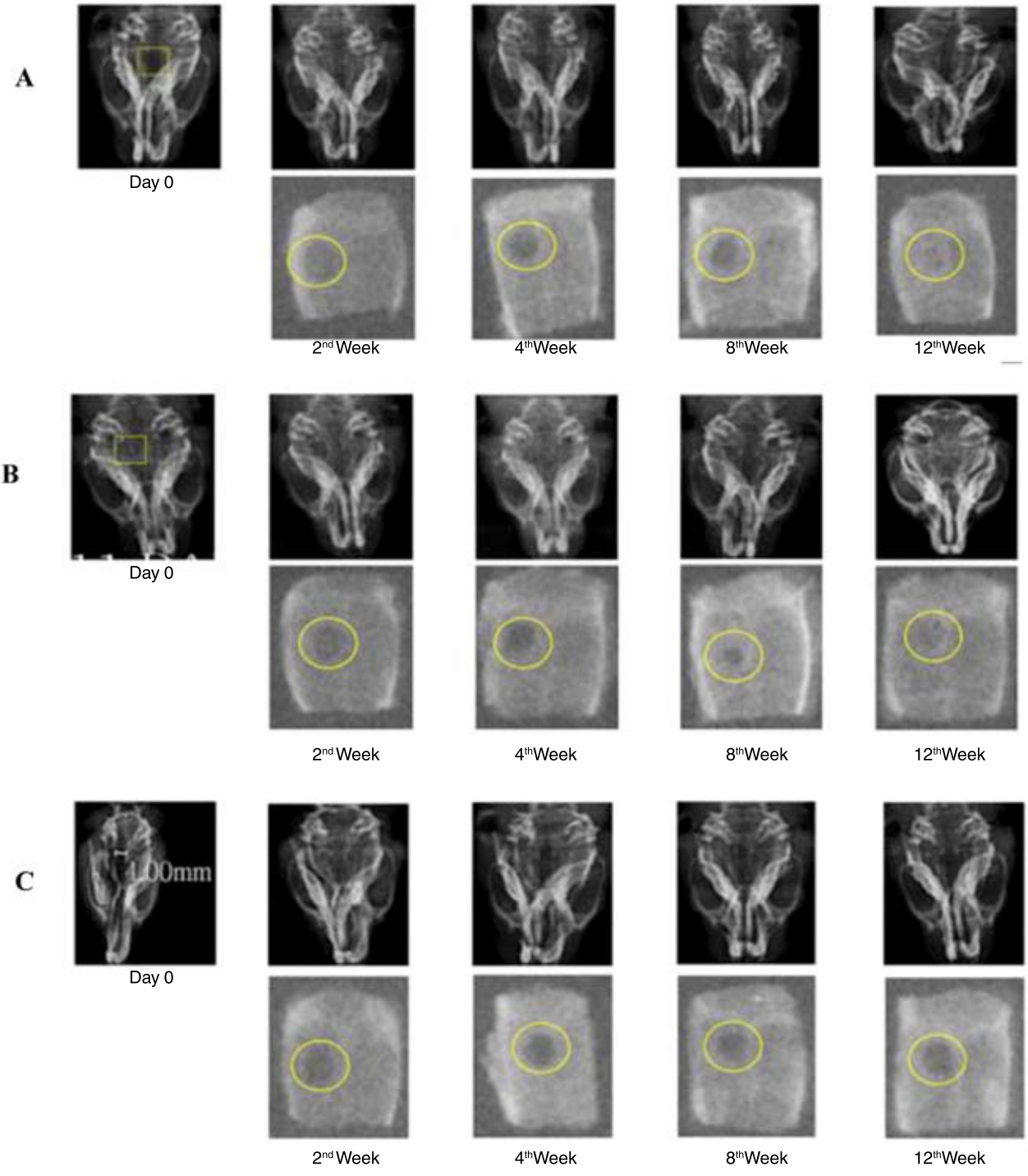


**Fig. 4.** The dorsoventral radiographs of the skull and the defect area highlighted **4A.** Group I, **4B.** Group II and **4C.** Group III

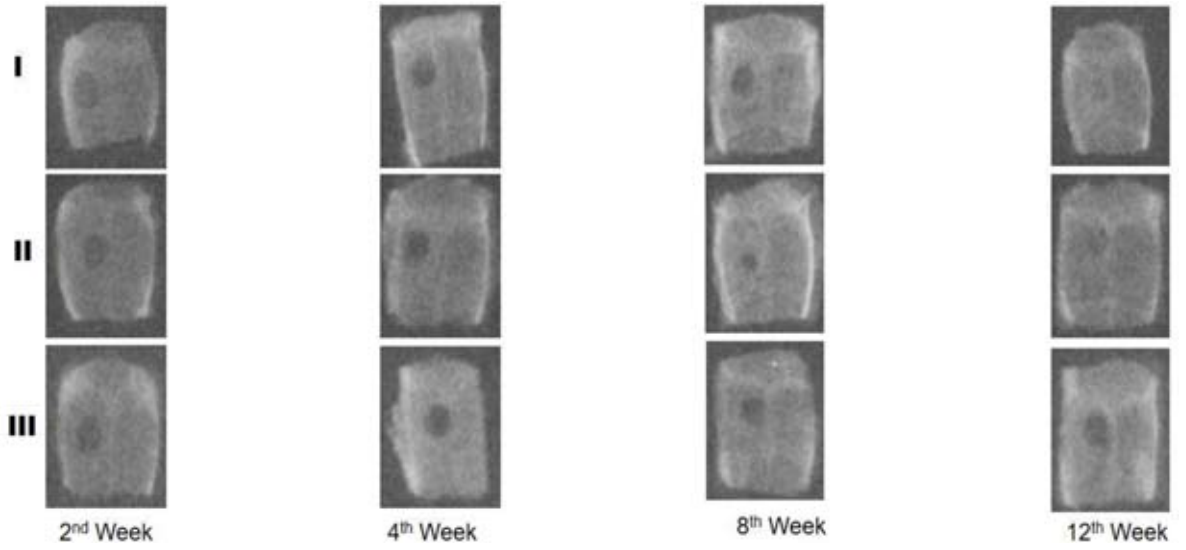


**Fig. 5.** Comparative evaluation of radiographic images – Group I, Group II and Group III

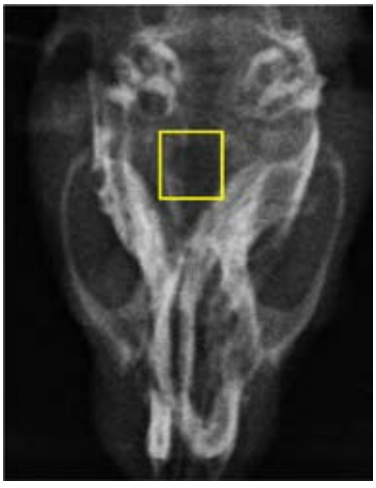

**Fig. 6.** Selected defect region in ImageJ software

groups. Chen *et al*. (2018) also reported increased radio-opacity of the defect in cross linked chitosan treated group compared to control group during the observation periods. Manasa *et al.* (2023) and Dinesh *et al*. (2024) observed progressive increase in the radio-opacity from 2nd week to 12th week in rat calvarial defect treated with decellularised tissue engineered triphasic composite bioceramic and polyvinyl alcohol hydroxyapatite composite respectively. Penteado *et al*. (2013) also reported the higher bone density in radiographic analysis in PRP and bioglass treated group in rabbit calvarial defect model compared to other treatment groups. Dinesh *et al.* (2018) scored the radiographic healing of critical size defects of femur treated with tri-phasic composite bio-ceramic implants in rat model with 0 – 10 point scoring system as developed by Sadegh *et al.* (2015).

## Conclusion

Radiographic evaluation is a practical and valuable approach for assessing bone regeneration in rat calvarial defect models. Despite its limitations in providing detailed three-dimensional insights, its low cost, non-invasive nature and ability to monitor healing over time make it an integral part of many research protocols. To achieve a more thorough understanding of the regenerative process, it is often combined with advanced imaging methods. Moreover, gray scale analysis proves to be an effective tool for measuring increased radio-opacity, especially in defects treated with radiolucent biomaterials.

## Acknowledgements

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## Conflict of interest

The authors declare that they have no conflict of interest.

## References

Bacevich, B.M., Smith, R.D.J., Reihl, A.M., Mazzocca, A.D. and Hutchinson, I.D. 2024. Advances with platelet-rich plasma for bone healing. *Biol. Targets Ther*. **18:** 29-59.https://doi.org/10.2147/BTT.S290341.

Bosetti, M. and Cannas, M. 2005. The effect of bioactive glass on bone marrow stromal cells differentiation. *Biomaterials*. **26:**3873-3879.https://doi.org/10.1016/j.biomaterials.2004.09.059.

Chen, C.K., Chang, N.J., Wu, Y.T., Fu, E., Shen, E.C., Feng, C.W. and Wen, Z.H. 2018. Bone formation using cross-linked chitosan scaffolds in rat calvarial defects. *Implant Dent*. **27:**15-21. 10.1097/ID.0000000000000670.

Costa, K. J., Gala-García, A., Passos, J. J., Santos, V.R., Sinisterra, R. D., Lanza, C. R. and Cortés, M. E. 2019. Testosterone improves the osteogenic potential of a composite in vitro and in vivo. *Cell Tissue Res*. **376:** 221-231. https://doi.org/10.1007/s00441-018-2970-3

Dinesh, P.T., Venugopal, S.K., Martin, J.K.D., Devanand, C.B., Pillai, U.N. and Nair, D. 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.

Dinesh, A., Dinesh, P.T., Fernandez, F.B., Sooryadas, S., Pradeep, M., Anoop, S., Jineshkumar, N.S., Remya, V. and Verma, H.K. 2024. Radiographic observations on healing of critical size calvarial defects treated with polyvinyl alcohol-hydroxyapatite composite ceramic in rat models. *Int. J. Vet. Sci. Anim. Husb*. **9:** 1236-1238.https://doi.org/10.22271/veterinary.2024.v9.i1q.1123.

Gomes, P.S. and Fernandes, M.H. 2011. Rodent models in bone-related research: the relevance of calvarial defects in the assessment of bone regeneration strategies. *Lab. Anim*. **45:** 14-24.https://doi.org/10.1258/la.2010.010085.

Isler, S.C., Cansiz, E., Tanyel, C., Soluk, M., Selvi, F. and Cebi, Z. 2011. The effect of irrigation temperature on bone healing. *Int. J. Med. Sci*. **8:** 704 – 708. 10.7150/ijms.8.704.

Kim, T.H., Kim, S.H., Sándor, G.K. and Kim, Y.D. 2014. Comparison of platelet-rich plasma (PRP), platelet-rich fibrin (PRF) and concentrated growth factor (CGF) in rabbit-skull defect healing. *Arch. Oral Biol*. **59:** 550-558.https://doi.org/10.1016/j.archoralbio.2014.02.004.

Lieshout, E.M.M.V. and Hartog, D.D. 2021. Effect of Platelet-rich plasma on fracture healing. *Injury*. **52:** 58-66.https://doi.org/10.1016/j.injury.2020.12.005.

Magat, G. and Sener, S.O. 2019. Evaluation of trabecular pattern of mandible using fractal dimension, bone area fraction, and gray scale value: comparison of cone-beam computed tomography and panoramic radiography. *Oral Radiol*. **35:** 35-42. https://doi.org/10.1007/s11282-018-0316-1.

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 HAS i in rat models. *J. Vet. Anim. Sci.* **54:** 916-920.

Marx, R.E. 2004. Platelet-rich plasma: evidence to support its use. *J. Oral Maxillofac. Surg*. **62:** 489-496.10.1016/j.joms.2003.12.003.

Messora, M.R., Nagata, M.J.H., Furlaneto, F.A.C., Dornelles, R.C.M., Bomfim, S.R.M., Deliberador, T.M., Garcia, V.G. and Bosco, A.F. 2011. A standardized research protocol for platelet-rich plasma (PRP) preparation in rats. *Rev. Sul-Bras. Odontol*. **8:**299-304.

Penteado, L.A., Colombo, C.E., Penteado, R.A., Assis, A.O. and Gurgel, B.C., 2013. Evaluation of bioactive glass and platelet-rich plasma for bone healing in rabbit calvarial defects. *J. Oral Sci.* **55:** 225-232. https://doi.org/10.2334/josnusd.55.225.

Sadegh, A.B., Karimi, I., Shadkhast, M., Mahdavi, M.H. 2015. Hydroxyapatite and demineralized calf fetal growth plate effects on bone healing in rabbit model. *J. Orthopaed. Traumatol*. **16:**141-149.https://doi.org/10.1007/s10195-014-0323-x.

Scabbia, A. and Trombelli, L. 2004. A comparative study on the use of a HA/collagen/chondroitin sulphate biomaterial (Biostite®) and a bovine-derived HA xenograft (Bio-Oss®) in the treatment of deep intra-osseous defects. *J. Clinic. Periodontol.* **31:** 348-355. https://doi.org/10.1111/j.1600-051X.2004.00483.x.

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. *Nat.Protoc*. **7:**1918-1929. https://doi.org/10.1038/nprot.2012.113.

Weibrich, G., Hansen, T., Kleis, W., Buch, R. and Hitzler, W. E. 2004. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. *Bone*. **34:** 665-671. https://doi.org/10.1016/j.bone.2003.12.010. ■