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Research Article

Effects of Polyvinyl Alcohol a Hydroxyapatite Composite Ceramic on Calvarial Defects with Critical Size in Rat Models

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ABSTRACT

Introduction: Biodegradable composite biomaterials are essential in healthcare, effectively tackling numerous complex challenges. Bone reconstruction is a surgical procedure aimed at remedying segmental bone loss, which is notably complicated and often fails to heal properly. A novel bone graft substitute incorporating polyvinyl alcohol (PVA) and synthetic hydroxyapatite (HA) has been developed and is tested *in vivo* in calvarial defect models of rat. The present study aimed to evaluate the bone regeneration potential of PVA – HA composite bone graft.

Materials and methods: A total of 24 adult male Wistar rats aged 12-15 weeks with an average weight of 150 grams were used in the current study. A 4 mm full-thickness critical-size defect was created on the parietal bone and filled with the pre-sized graft material. Radiography, micro-computed tomography, scanning electron microscopy, histology, and serum biochemical parameters, including alkaline phosphatase and acid phosphatase activity, were utilized to evaluate the healing potential of the graft material. The animals were observed for twelve weeks. An immediate postoperative dorsoventral view of the skull was exposed at day zero and subsequent radiographs were taken periodically at weeks 2, 4, 8, and 12 in a group including 24 animals.

Results: Immediate post-operative radiographs revealed the radiolucent nature of the graft material. Throughout the healing process, it was observed that the graft remained in position and was intact. The values of serum biochemical parameters (alkaline phosphatase and acid phosphatase activity) were haphazard throughout the observation period. In the 8th week, signs of progressive degradation of the graft material and bone regeneration could be seen, particularly on radiography, micro-CT scanning electron microscopy, and histologic examination.

Conclusion: It is concluded that the test graft material successfully accelerated bone regeneration and completely integrated with the host bone at week 12 of the experiment in the rat model.

1. Introduction

The notable increase in bone injuries recorded in recent years, largely due to severe trauma and age-related degenerative diseases, has led to an urgent need for advancements in bone tissue engineering[1]. Although bone tissue has a strong capacity for self -regeneration, the body cannot adequately repair major bone damage. Currently, autografts, allografts, and xenografts are used to treat bone defects. However, these procedures have significant disadvantages, including the requirement for additional surgeries, resulting in discomfort, transmission of diseases, and graft rejection[2]. Tissue engineering, nanotechnology, and veterinary science experts worked closely together to develop bone substitutes that can improve, maintain, or restore bone function and are clinically useful. According to

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Bisht et al.[3] the three O's-Osteoinductive stimuli, Osteoconductive scaffolds, and Osteoprogenitor cell activation are the ideal features of a bone implant that are all met by the implant used in the current study. The current study aimed to evaluate the healing potential of PVA – HA composite ceramic in critical-sized rat defect models using planar radiographs, micro-computed tomography, histology, serum biochemistry, and scanning electron microscopy over an observation period of twelve weeks.

2. Materials and Methods

2.1. Ethical approval

The Institutional Animal Ethics Committee (IAEC) of the College of Veterinary and Animal Sciences, Pookode, Kerala, India, approved the experimental protocols (IAEC/COVAS/PKD/20/1/2023.).

2.2. Study animals

A total of 24 adult male Wistar rats aged 12-15 weeks with an average weight of 150 grams were used in the current study as experimental models. After an acclimatization period of seven days, animals were premedicated with buprenorphine hydrochloride at the dose rate of 0.05mg/kg body weight given subcutaneously 20 minutes before induction[4]. Anesthesia was induced with a combination of Xylazine and ketamine at the dose rates of 7 mg/kg and 70 mg/kg body weight, respectively given intraperitoneally[5]. The parietal bone was approached as per Spicer et al.[6], through a linear skin incision over the scalp from the nasal bone to just the caudal to the bregma. After exposure of the right parietal bone, a 4 mm full-thickness critical size defect was created and the defect was filled with the pre-sized PVA – HA composite ceramic (India). Bone regeneration and host-to-graft integration were analyzed periodically during weeks 2, 4, 8, and 12 using planar radiography, microcomputed tomography, histology, estimation of bone turnover marker activity, and scanning electron microscopy[6].

3. Results and Discussion

Immediate post-operative radiographs revealed that the graft material was radiolucent. As a result, the graft was only faintly visible.

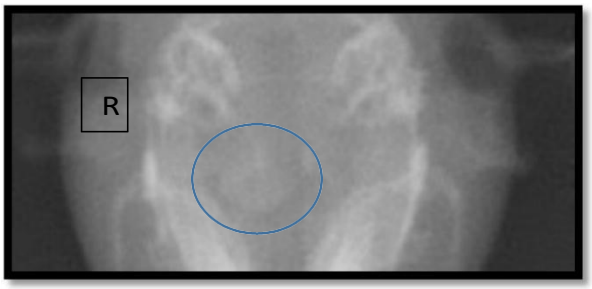

Figure 1. Immediate postoperative radiograph in rats with critical size bone defect filled with the pre-sized PVA – HA, (Marked area is the area of the defect grafted with the bio-material test which is barely visible radiographically)

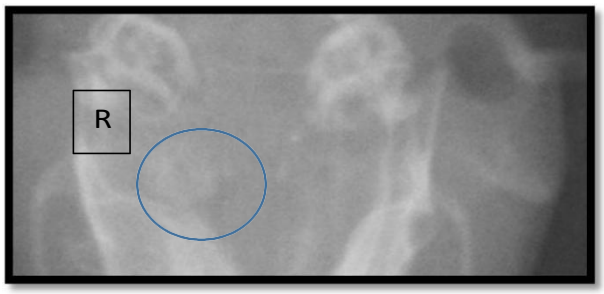

Figure 2. The critical size bone defect filled with the pre-sized PVA – HA, the grafted area (marked) at the second week in a rat. The enhanced radiographic density compared to the previous radiograph.

As the healing progressed, the radiographic density of the material increased for four weeks and decreased thereafter (Figures 2, 3, and 4). At week 12, the graft material was barely visible indicating that the graft was completely integrated with the host bone. The alteration in the radiographic density showed the initial ingrowth of cellular elements into the graft followed by calcification and progressive degradation of the graft material.

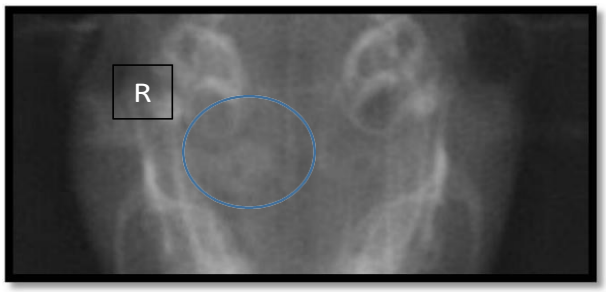

Figure 3. The critical size bone defect filled with the pre-sized PVA – HA, the grafted area (marked) at the fourth week in a rat. The enhanced radiographic density compared to the previous two radiographs

The findings of the present study are in line with the findings of Rao et al.[4], Manasa[5], and Dinesh et al.[7], where the authors observed enhancement of radiographic density of the graft material during the initial phase of bone healing and gradual reduction of the density thereafter. Assessment of healing by planar radiography was difficult due to the radiolucent nature of the graft material. The addition of metallic elements like silver, strontium, and silica improves the radiodensity of the material and makes it radiographically more visible as suggested by Ciobanu and Ciobanu[8]. Moreover, this metal has antibacterial properties also which may be an additional advantage for

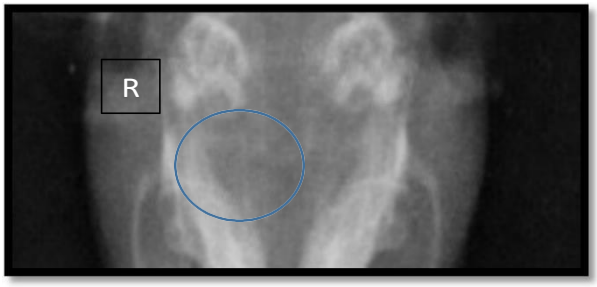

Figure 4. The critical size bone defect filled with the pre-sized PVA – HA, with barely visible graft material (marked area) at week 12 in a rat

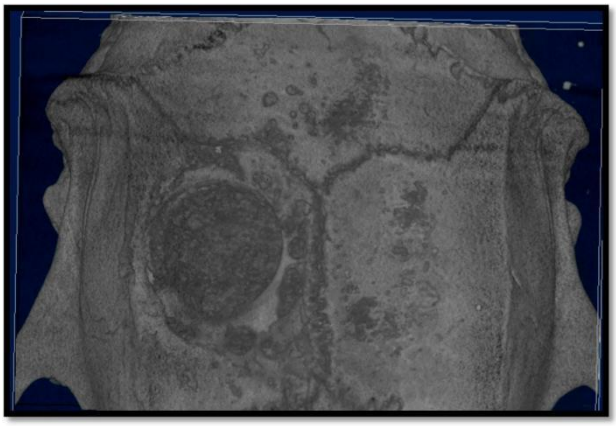

Figure 5. Micro CT at week 2 in rats with critical-size bone defect filled with the pre-sized PVA – HA

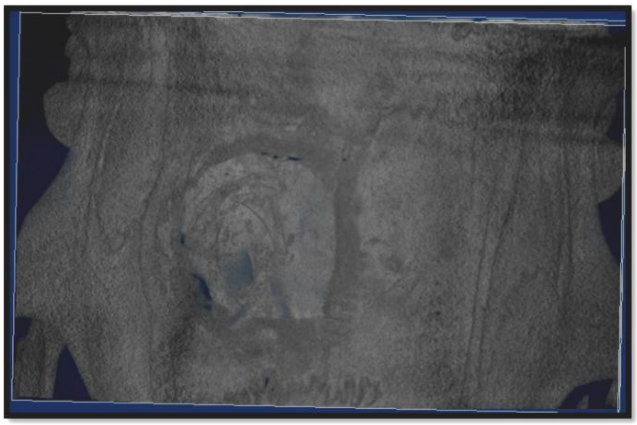

Figure 6. Micro CT at week 4 in rats with critical-size bone defect filled with the pre-sized PVA – HA

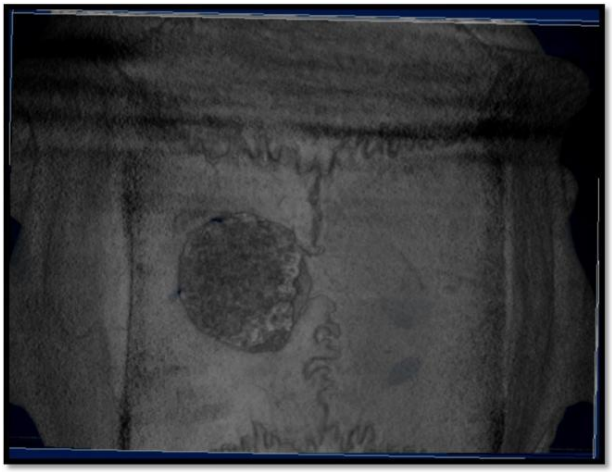

Figure 7. Micro CT at week 8 in rats with critical-size bone defect filled with the pre-sized PVA – HA

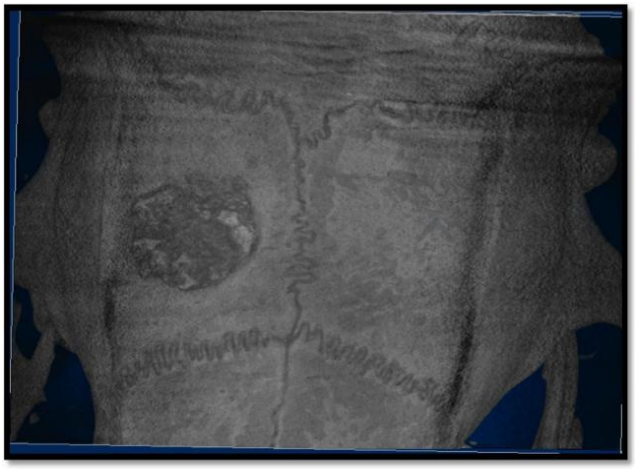

Figure 8. Micro CT at week 12 in rats with critical-size bone defect filled with the pre-sized PVA – HA

the graft material. In the 12th week, the graft material was hardly distinguishable from the host bone radiographically indicating complete integration of the graft with the host bone and healing of the critical size defect (Figure 4).

Micro CT analysis proved significantly more effective than planar radiography in the assessment of bone healing.

Periodic examinations revealed progressive ingrowth of cellular elements into the pores of the graft followed by calcification of the new bone and progressive degradation of the graft material. At the end of the study, the new bone

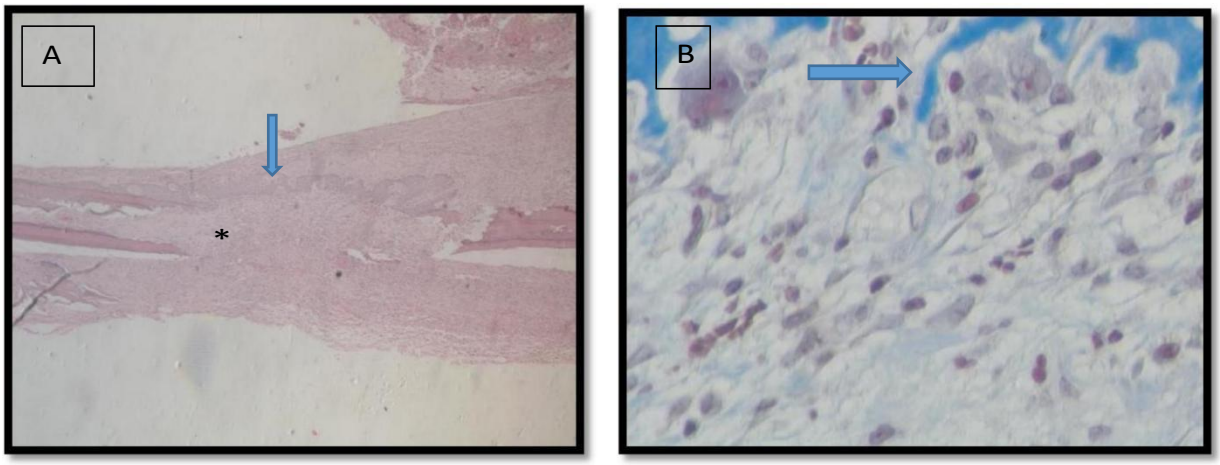

Figure 9. The second week of bone healing in rats with critical size bone defect filled with the pre-sized PVA – HA. **A:** Fibrous tissue (*) filling the defect. Islands of woven bone (arrow) can be seen (H&E, 40x) **B:** Light blue colored fibrous tissue formation (*) and islands of woven bones (arrow) in the defect (Masson's Trichrome, 400x)

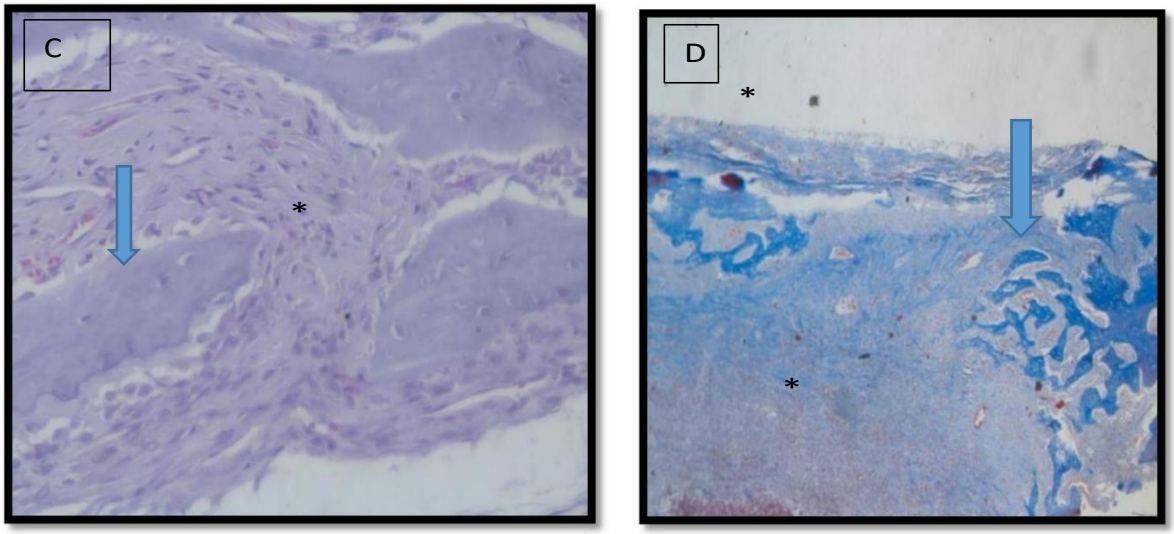

Figure 10. Fourth week of bone healing in rats with critical-size bone defect filled with the pre-sized PVA – HA. **C:** Thin sheets of woven bone (arrow) and fibrous tissue with neovascularization are visible. (H&E, 400x). **D:** Low power view of the defect filled with sheets of woven bones (arrow) and fibrous tissue (*) (Masson's Trichrome, 40x)

was found to be perfectly merged with the host bone indicating complete healing of the critical size defect (Figures 5-8).

Manasa[5] and Xu et al.[9] reported similar findings in different scaffolds. Histological examinations in the 2nd week revealed that the defect was filled with fibrous tissue and a minimal quantity of woven bone with osteoblasts could be identified as small scattered islands within the defection (Figure 9). The incorporation of HA in graft material enhanced host cell migration, adhesion, differentiation, and proliferation as evidenced by the increased cellularity observed in the defection[9]. Similar observations were made by Tontowi et al.[10] who used HA/Gelatin/PVA in Wistar rat femoral defection and observed increased woven bone formation with fibrous connective tissue similar to the findings of the present study.

By the 4th week, thin sheets of woven bone and a moderate amount of fibrous tissue could be observed in the defect. Moderate neovascularization could be observed without infiltration of inflammatory cells (Figure 10). This was similar to the findings of Xu et al.[9] who observed fibrous connective tissue at the defect site in the 4th week when PVA/β tricalcium phosphate composite scaffold containing icariin was grafted in rat calvarium. The findings of the present study were in accordance with the findings of Linh et al.[11], who observed increased bone formation in rat models within the 2nd and 4th weeks when PVA/gelatin-containing nanoparticles of

By the 8th week, the defect was laid down by mixed (lamellar and woven) type of bone. Fibrosis was observed towards the periphery of the defect with islands of woven bone (Figure 11). This was similar to the findings of Xu et al.[9], Lin et al.[12], and Abazari et al.[13], who observed woven bone formation at the end of the 8th week in rat calvarial defect models.

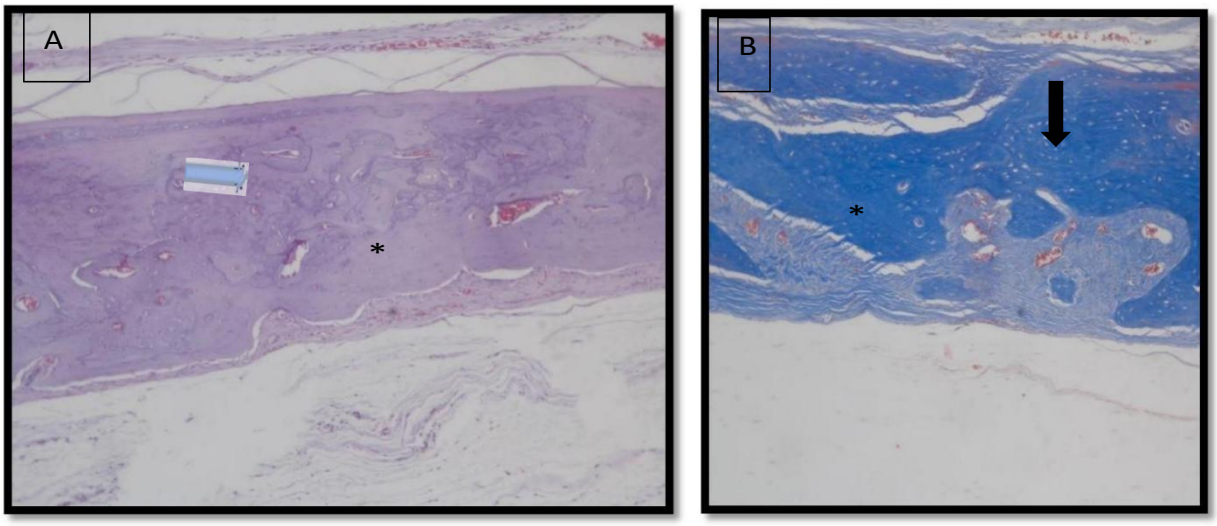

Figure 11. The eighth week of bone healing in rats with critical size bone defect filled with the pre-sized PVA – HA. **A**: Defect partially covered with woven bone (arrow) with lamellar bone (*) (H&E, 100x). **B**: Filling of defect with bone (arrow) with marginal fibrous tissue (*) (Masson's Trichrome, 400x)

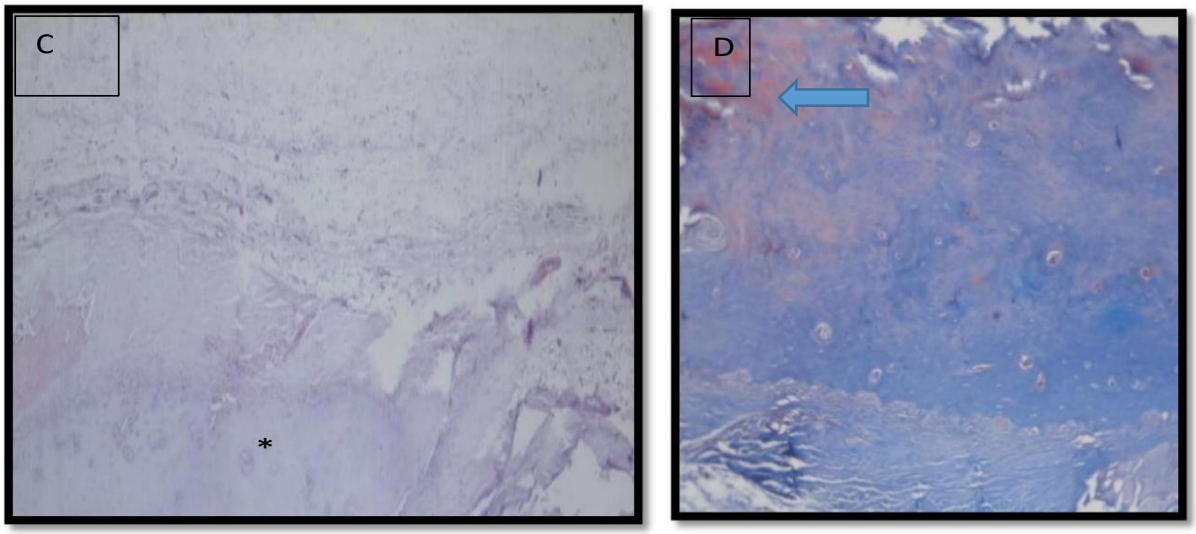

Figure 12. Twelfth week of bone healing in rats with critical size bone defect filled with the pre-sized PVA – HA. **C:** Complete filling of defect with lamellar bone (*) (H&E, 100x). **D:** Lamellar bone with areas of mineralization (arrow) is visible in the filled-up defect area (Masson's Trichrome, 400x)

At week 12, the defect was not identifiable. Complete healing with lamellar bone containing osteocytes and bone marrow formation was observed in the defect site (Figure 12). This was in line with the findings of Lytkina et al.[14], who used cryo-structured materials based on PVA and HA for osteogenesis and observed the overgrowth of osteocytes in the areas of bone defect in histological studies when used in rat tibia defect models on the day 80. Manasa[5] also observed that the calvarial defect in the rat model had completely healed by the 12th week.

Histomorphometric scoring was performed based on the amount and type of new bone formation, fibrosis, inflammatory reaction, vascularity, and edge-bone integration[15]. Inflammatory reaction was completely absent during the entire period of observation of 12 weeks (Figures 9,10,11, and 12). Fibrous tissue proliferation was evident until the 8th week and was absent thereafter. The appearance of scattered islands of woven bone was evident along with fibrous tissue in the second week, thin sheets of woven bone appeared in the fourth week, and the formation of mixed bone was observed in week 8. In week 12, the defect was completely healed with mature lamellar bone and a haversian system embedded with osteocytes. Graft resorption was also evident by the 12th week.

Scanning electron microscopy images of the second week indicated the presence of minor areas of granulation tissue at the periphery of the implant area (Figure 13). Minor areas of graft placement in distraction from the bony bed can be visualized. Crystalline structure and porosity were evident. The 4th-week images indicated the presence of peri-implant tissues with definite traces of connective fibers and intense cellular activity that generated a rich cover over the graft material (Figure 14). The eighth week micrographs indicated rough areas bordering the graft and elevated cellular coverage of graft to bone bridge areas.

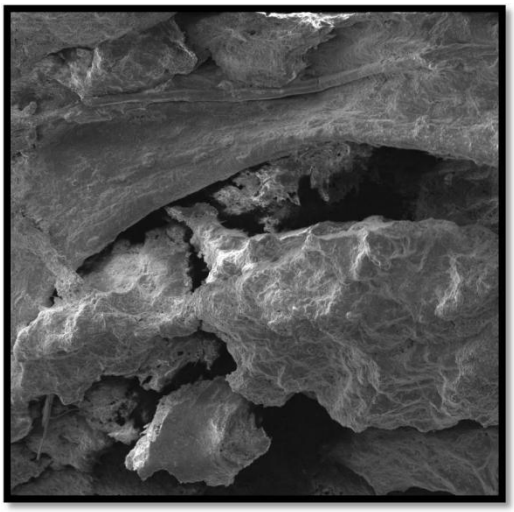

Figure 13. Scanning EM in the second week in rats with critical-size bone defects filled with the pre-sized PVA – HA. The minor areas of granulation tissue at the periphery of the implant area

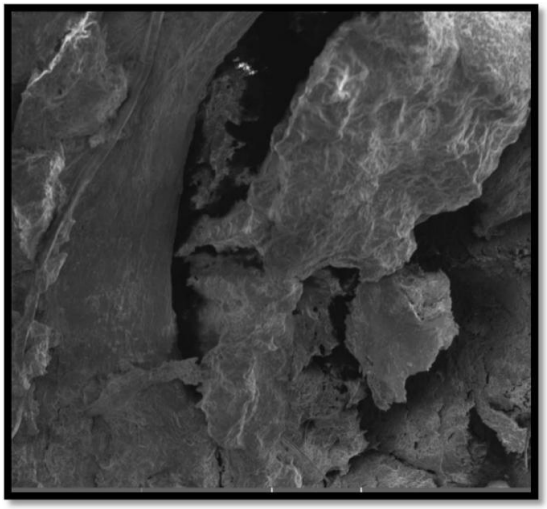

Figure 14. Scanning EM in the fourth week in rats with critical-size bone defect filled with the pre-sized PVA – HA. The presence of peri-implant tissues with definite traces of connective fibers and intense cellular activity that is generating a rich cover over the graft material

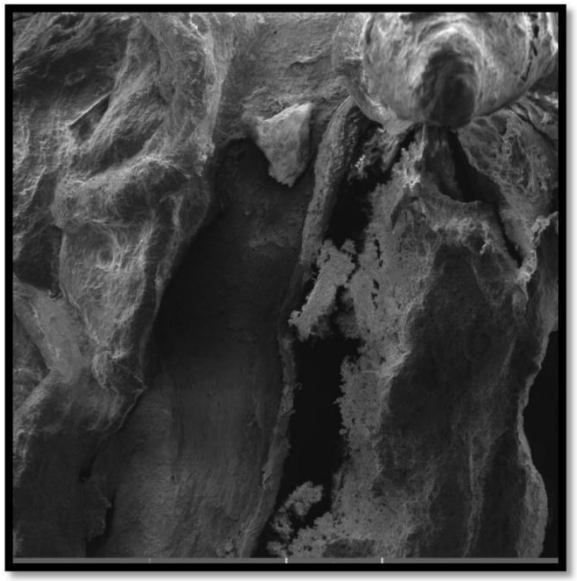

Figure 15. Scanning EM in the fourth week in rats with critical-size bone defect filled with the pre-sized PVA – HA. The rough areas bordering graft and elevated cellular coverage of graft to bone bridge areas. Biodegradation of the graft material was also evident which permitted tissue ingrowth

Biodegradation of the graft material was also evident which permitted tissue to grow (Figure 15). The week 12 indicated the close apposition of surfaces which indicated bone deposition and complete integration (Figure 16).

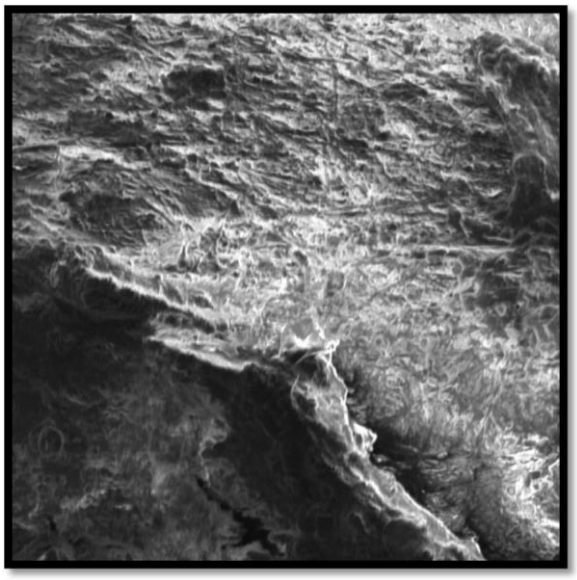

Figure 16. Scanning EM at week 12 in rats with critical-size bone defect filled with the pre-sized PVA – HA. The close apposition of surfaces indicates bone deposition and complete integration

4. Conclusion

The critical-sized segmental defects could be successfully treated using PVA-HA composite graft in rat critical-size calvarial defect models. The graft material facilitated swift bone healing by utilizing the pores, which contributed to an accelerated recovery of the fracture. No gross failure of the graft material was noticed in this study. Radiographic evaluation revealed the radiolucency of the material. Degradation of graft material was radiographically evident by 12 weeks. The histological evaluation proved high levels of osteoconduction, osteoinduction, and osteointegration between the graft and the host bone. Assessment of the activity of bone turn markers showed that the activity of serum ALP and ACP was haphazard throughout the entire period of observation of 12 weeks. Micro-computed tomographic analysis revealed enhanced densification, indicating potential new bone formation, alongside a loss of internal architecture that suggests graft resorption. The results from scanning electron microscopy indicated that the surfaces were in close contact, which implies the occurrence of bone deposition.

Declarations

Competing interests

The authors declare that there is no conflict of interest in the research work.

Authors' contributions

Anu Dinesh Conducted the research, Gathered the data, B. Fernandez Prepared the graft material, conducted the research, and gathered the data. T. Dinesh Conducted the research, gathered the data Sooryadas- Conducted research, gathered the data Pradeep - Conducted the research and gathering the data S. Anoop – Conducted the research and gathered the data N. S. Jinesh Kumar Conducted the research and gathering the data V. Remya Conducted the research and gathered the data H. K. Verma Prepared the graft material, conducted the research, and gathered the data. All authors read and approved the final version of the study.

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Ethical considerations

The article is written originally based on the collected data from present research and it is submitted for first time to this journal.

Availability of data and materials

The data are available according to a reasonable request.

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