Radiographic observations on healing of critical size calvarial defects treated with polyvinyl alcohol-hydroxyapatite composite ceramic in rat models

Anu Dinesh, PT Dinesh, FB Fernandez, S Sooryadas, M Pradeep, S Anoop, NS Jineshkumar, V Remya and HK Verma

DOI: https://doi.org/10.22271/veterinary.2024.v9.i1q.1123

Abstract
Biodegradable composite bio-materials play a pivotal role in the healthcare and address many challenging issues. Bone refurbishment is a surgical procedure that repair segmental bone loss that are extremely complex which fail 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 rat calvarial defect models. The novel material is structurally and biomechanically similar to that of a natural bone. This study, focused on exploring the newer scaffold for bone regeneration which will positively eliminate the current issues in healing bone defects treated with a variety of other bone grafts. Radiography was utilised to evaluate the healing potential of the graft material. An immediate postoperative dorsoventral view of the skull was taken at day zero and subsequent radiographs were taken periodically at second, fourth, eighth and twelfth weeks. Immediate post operative radiograph revealed the radiolucent nature of the graft material. Throughout the healing process, it was observed that the graft remained in position and was intact. By eighth week, signs of progressive degradation of the graft material and bone regeneration could be seen. The test material was found to be completely integrated with the host bone by 12 weeks. It could be concluded that the test graft material successfully accelerated bone regeneration, and the PVA-HA composite could be used as an alternative to the available conventional bone grafts.

Keywords: PVA-HA composite ceramic, critical size calvarial defect model, osteoconductivity

Introduction
An urgent need for bone tissue engineering has arisen due to the huge increase in bone injuries over the past few years, which are mostly due to severe trauma and age-related degenerative disorders. Despite the fact that bone tissue has a strong capacity of self-regeneration, the body cannot adequately repair major bone damages. Currently, autografts, allografts and xenografts are used to treat bone defects. However, these procedures have significant disadvantages, including requirement for additional surgeries, resulting in discomfort, transmission of diseases and graft rejection. 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 Bisht et al. (2021) [11] the three O's-Osteoinductive stimuli, Osteoconductive scaffolds, and Osteoprogenitor cells activation—are the ideal features of a bone implant that are all met by the implant used in this study. The goal of the current study was to evaluate the healing potential of PVA-HA composite ceramic in critical sized rat defect models using planar radiographs over an observation period of twelve weeks.

Materials and Methods
The Institutional Animal Ethics Committee (IAEC) of College of Veterinary and Animal Sciences, Pookode approved the experimental protocols (IAEC/COVAS/PKD/20/1/2023). 24 adult male wistar rats 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. Anaesthesia 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. The parietal bone was approached as per Spicer et al. (2012) [6], through a linear skin incision over the scalp from the nasal bone to just 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 graft material. Bone regeneration and host to graft integration was analysed periodically during second, fourth, eight and 12th week using planar radiography.

**Results and Discussion**

Immediate post operative radiograph revealed that the graft material was radiolucent. As a result the graft was only faintly visible. (Fig. 1) As the healing progressed, the radiographic density of the material increased till four weeks and decreased thereafter. The alteration in the radiographic density indicated initial ingrowth of cellular elements in to the graft followed by calcification and progressive degradation of the graft material (Fig. 2-5). The findings of the present study are supported by the findings of Dinesh et al. 2018 [3], Rao et al. 2021 [5] and Manasa et al. 2022 [1]. Assessment of healing by planar radiography was difficult due to the radiolucent nature of the graft material. Addition of metallic elements like silver, strontium or silica will improve the radiodensity of the material and make it radiographically more visible as suggested by Ciobanu and Ciobanu, (2021) [2]. Moreover these metal has antibacterial properties also which may be an additional advantage for the graft material. By 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.

**Conclusion**

From the observations of the current study, it could be inferred that critical sized segmental defects could be successfully treated with the use of PVA-HA composite graft. Being porous, it aided in rapid bone healing through the pores helping in faster healing of the fracture. No gross failure of the graft material was noticed in any of the animals. Radiographic evaluation revealed radiolucency of the material. Degradation of graft material was radiographically evident by 12 weeks. The present study has proved the successful use of PVA-HA bone substitute for the treatment of critical sized segmental defects in the rat calvarium.

**Acknowledgement (If any)**

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