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Radiographic evaluation of polymethylmethacrylate in combination with β -tricalcium phosphate and platelet-rich plasma for critical size bone defects in rabbits

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Abstract

In the present study the efficacy of polymethylmethacrylate (PMMA) alone and in combination with β -tricalcium phosphate (β -TCP) and platelet-rich plasma (PRP) was evaluated for the management of critical size bone defects in rabbit model. The experimental study was conducted on six healthy adult male New Zealand White rabbits and were treated with PMMA in combination with β -Tricalcium Phosphate and Platelet Rich Plasma (PRP). PRP was prepared using a standardized double centrifugation technique and was administered immediately after surgery and subsequently on 7th and 15th postoperative days. A 0.5 cm diaphyseal defect was surgically created in the radius bone of each rabbit under aseptic conditions. Postoperative care was attentively maintained and included administration of enrofloxacin at a dose of 5 mg/kg intramuscularly to prevent infection, and meloxicam at 0.5 mg/kg body weight intramuscularly for analgesia and anti-inflammatory effects and application of a Robert johnes bandage for external support. Radiographic evaluation was conducted preoperatively and on 15th, 30th, 45th and 60th postoperative days and assessment included reduction in defect size, radiographic density, callus formation and status of implant. The combined use of PMMA, β -TCP and PRP produced synergistic effects, yielding superior structural repair, mineralization, osteoblastic activity, radiographic density and overall functional recovery in critical size bone defects and emphasized the clinical potential of using a multimodal approach in improving bone regeneration and healing in the management of critical size bone defects in rabbits.

Keywords: Critical size bone defect, polymethylmethacrylate, β -tricalcium phosphate, platelet-rich plasma, bone regeneration, rabbit

Introduction

Critical-size bone defects (CSBDs) are defined as osseous defects that fail to heal spontaneously during the lifetime of an animal (Liu *et al.*, 2016) [2]. Such defects often result from trauma, infection, tumor resection, or congenital abnormalities and represent a major challenge in orthopedic surgery. Inadequate healing of these defects may lead to delayed union, malunion, or non-union, necessitating the use of bone grafts or substitute biomaterials (Al Ruhaimi, 2000; Lu *et al.*, 2021) [1, 13]. Various bone substitutes, including polymethylmethacrylate (PMMA) biologically inert and lacks osteogenic and osteoconductive properties yet is a widely used synthetic bone cement that provides immediate mechanical stability and structural support at the defect site (Nandi *et al.*, 2010) [15]. β -Tricalcium phosphate (β -TCP) is a biocompatible and bioresorbable bioceramic with excellent osteoconductive characteristics. Its porous structure promotes vascularization, cellular infiltration, and new bone formation while undergoing gradual resorption and replacement by native bone tissue (Erbe *et al.*, 2001; Bai *et al.*, 2010; Wang *et al.*, 2013) [5, 2, 19]. Platelet-rich plasma (PRP), an autologous concentration of platelets, contains numerous growth factors and cytokines that enhance angiogenesis, osteogenesis, and tissue regeneration. The regenerative potential of PRP has been reported to be greater when combined with osteoconductive scaffolds such as β -TCP (Vardhan *et al.*, 2017) [18]. To evaluate and compare the healing efficacy of polymethylmethacrylate (PMMA) alone and in combination with β -tricalcium phosphate (β -TCP) and platelet-rich plasma (PRP) in critical-size bone defects in rabbits.

Materials and Methods

Location/place of work

The work was conducted in the Department of Veterinary Surgery and Radiology, College of Veterinary Science and Animal Husbandry, Rewa, N.D.V.S.U., Jabalpur (M.P.) approximately for a period of six months. The study was conducted on six healthy adult male rabbits of New Zealand White breed. The animals were procured from certified animal house and maintained on standard diet and *ad libitum* water. Acclimatization period of seven days was provided to the animals prior to the initiation of the experimental procedure. Animals of different groups were subjected to different treatment protocol for a period of 8 weeks.

Preparation of autologous platelet rich plasma from the test animals

PRP was prepared according to the method reported by Jeong *et al.* (2020) [7] with slight modifications. PRP was produced by a two-step gradient centrifugation technique using centrifugation machine (REMI Laboratory Centrifuge R-4C). Five ml of blood was collected intracardially using a sterile syringe and transferred into separate vial containing 0.8 ml of acid citrate dextrose (ACD) Solution A anticoagulant (Plate 7b). In first stage, blood samples were centrifuged at 1600 RPM for a period of 10 min at room temperature. This resulted in the formation of a gradient that has three main layers: an inferior layer, an intermediate layer and a superior layer that represents red blood cells, white blood cells and plasma, respectively. Following initial centrifugation, the collection tubes were uncapped and the plasma (upper layer) was transferred into another tube for further processing. In second stage, the transferred plasma was again centrifuged at 4000 RPM for another 10 min to obtain two layers: the upper 2/3rd layer of the column constituted platelet-poor plasma (PPP) and the lower 1/3rd layer of the column constituted platelet rich plasma (PRP). The PPP was initially aspirated and removed for preventing mixing with the PRP and the remaining contents were gently mixed to re-suspend the platelets thereby producing PRP syringe (Xu *et al.*, 2020) [20]. 1 ml of prepared PRP was injected immediately after surgery (group B and D) and at 7th and 15th postoperative day into the defect site with the help of sterile syringe (Jee *et al.*, 2016 and Singh *et al.*, 2020) [6, 17].

Surgical procedure

The rabbits were anesthetized with an intramuscular injection of xylazine at a dose rate of 5 mg/kg body weight. After sedation ketamine was injected at a dose rate of 50 mg/kg body weight intramuscularly (Khan *et al.*, 2022) [11] and maintenance of anesthesia was done with combination of xylazine and ketamine (1:1 ratio). Eye lubricant (Refresh tears) was instilled into both eyes until palpebral reflexes returned in order to prevent the drying of the cornea. The rabbits were secured in lateral recumbency. An area of forelimb between elbow and knee joints was

prepared for aseptic surgery. The selected limb was disinfected with 10% povidone-iodine solution. A 3 cm long skin incision was taken over the antero-medial part of the radius from the medial epicondyle of the humerus to the styloid process of radius (Milovancev, 2004 and Yadav *et al.*, 2016) [14, 21]. Following incision, soft tissues were dissected and radius was exposed by gentle retraction of the muscles. Under aseptic condition 0.5 cm diaphyseal section of radius was removed using orthopedic wire saw. Cold sterile normal saline solution was continuously poured at the surgical site, while removing the bone segment, to avoid thermal necrosis (Bhadane *et al.*, 2018 and Karki, 2022) [3, 10]. The bone void was filled with polymethylmethacrylate (PMMA) together with beta-tricalcium phosphate granules and platelet rich plasma. After completion of surgical process, muscles were sutured in continuous pattern followed by skin suture in simple interrupted pattern. Immediately after surgery, antiseptic dressing with povidone iodine solution and application of ointment mupirocin was done followed by application of Robert Johnes bandage to immobilize and protect the limb. After recovery, the animals were allowed free access to feed and water in their individual cages. Post-operatively the rabbits were treated with antibiotic enrofloxacin at the dose of 5 mg/kg body weight IM every 24 hours for 5 days (Cabanes *et al.*, 1992) [4] and anti-inflammatory agent meloxicam at the dose of 0.5 mg/kg body weight IM every 24 hours for 3 days (Kahn, 2005) [8]. A daily application of liquid betadine and mupirocin ointment were used to treat the wound. Skin sutures were removed on seventh post-operative day.

Radiographic examination

Radiographic evaluation include reduction in bone defect, radiographic density, remodelling of bone and status of implant was done to evaluate the process of new bone formation at the induced defect site. The radiograph of surgical site on medio-lateral view was taken using factors of 45kVp, 8mAs and FFD 90 cm, immediately after surgery and on 15th, 30th, 45th and 60th postoperative day. Radiographic evaluation showed the highest reduction in bone defect size, increased radiographic density, distinct fracture-line bridging, and marked implant resorption shows a significant difference in healing from day 30 onward. By day 60, indicating enhanced bone regeneration. These findings suggest that the combined application of PMMA, β -TCP, and PRP produced the most favourable radiographic outcome. (Kalita *et al.*, 2022) [9] reported that the bone defect appeared more radio-dense on day 20 compared to day 0, which was attributed to the osteogenic properties of PRP and β -TCP. Furthermore, they observed complete bridging of the defect by day 60, with markedly higher bone density compared to the initial phase of remodeling (Oryan *et al.*, 2018) [16] reported that in the PMMA group, the implant was not completely degraded up to 8 weeks. However, in the PMMA-PG group, the defect was filled with fibrous tissue or cartilage, although complete bone union was still not achieved.



Radiographs at day 0, day 15, day 30, day 45 and day 60

Conclusion

The animals treated with combined therapy (PMMA + β -TCP + PRP) exhibited comparatively faster and better functional recovery in critical size bone defect in rabbits. Radiographic evaluation revealed improved reduction in bone defect, bone density, fracture bridging and resorption of implant in animals treated with PMMA along with beta-TCP and platelet-rich plasma. PMMA alone provided structural stability, however its combination with β -TCP and PRP showed osteoconductive and osteogenic potential.

References

1. Al Ruhaimi KA. Effect of adding resorbable calcium sulfate to grafting materials on early bone regeneration in osseous defects in rabbits. *Int J Oral Maxillofac Implants*. 2000;15(6):.
2. Bai F, Wang Z, Lu J, Liu J, Chen G, Lv R, *et al*. The correlation between the internal structure and vascularization of controllable porous bioceramic materials *in vivo*: a quantitative study. *Tissue Eng Part A*. 2010;16(12):3791-3803.
3. Bhadane B, Maiti S, Mohan D. Role of embryonic stem cell-hydroxyapatite construct with growth proteins for osteogenesis in the repair of bone defects in rabbit model. *J Stem Cell Res Ther*. 2018;4(3):67-80.
4. Cabanes A, Arboix M, Anton JM, Reig F. Pharmacokinetics of enrofloxacin after intravenous and intramuscular injection in rabbits. *Am J Vet Res*. 1992;53(11):2090-2093.
5. Erbe E, Marx J, Clineff T, Bellincampi L. Potential of an ultraporous β -tricalcium phosphate synthetic cancellous bone void filler and bone marrow aspirate composite graft. *Eur Spine J*. 2001;10(Suppl 2).
6. Jee CH, Eom NY, Jang HM, Jung HW, Choi ES, Won JH, *et al*. Effect of autologous platelet-rich plasma application on cutaneous wound healing in dogs; 2016.
7. Jeong W, Kim YS, Roh TS, Kang EH, Jung BK, Yun IS. The effect of combination therapy on critical-size bone defects using non-activated platelet-rich plasma and adipose-derived stem cells. *Childs Nerv Syst*. 2020;36(1):145-151.
8. Kahn CM. The Merck veterinary manual. 2005. Available from: <http://www.merckvetmanual.com>.
9. Kalita S, Nath PJ, Singh CK, Nath NC, Dutta B. Application of platelet rich plasma (PRP) with β -tricalcium phosphate (β -TCP) and demineralized bone matrix (DBM) in healing of bone tissues in rabbits. *J Anim Res*. 2022;12(3):389-400.
10. Karki N. Osteosynthesis in induced tibial segmental defect using co-precipitated hydroxyapatite gel and titanium mesh cage in rabbits [PG thesis]. Pantnagar, Uttarakhand: G.B. Pant University of Agriculture and Technology; 2022.
11. Khan S, Pawde AM, Manjusha KM, Banu SA, Kalaiselvan E, Kumar R, *et al*. Effect of adipose-derived stromal vascular fraction and non-activated platelet-rich plasma (nPRP) on hydroxyapatite-tricalcium phosphate (HA-TCP) based management of atrophic non-union fractures in rabbit model. In: Proceedings of the 44th Annual Congress of Indian Society for Veterinary Surgery and National Symposium on Recent Trends in Surgical and Imaging Techniques for Enhancement of Productivity and Health Status of Farm and Pet Animals; 2022 Feb; Uttarakhand. Indian Society of Veterinary Surgery; 2022.
12. Lei W, Wu Y, Yuan H, He P, Wu J, Chen J, *et al*. Establishing rabbit critical-size bone defects to evaluate the bone-regeneration potential of porous calcium phosphate ceramics. *Front Bioeng Biotechnol*. 2025;12:1524133.
13. Lu H, Zhou Y, Ma Y, Xiao L, Ji W, Zhang Y, *et al*. Current application of beta-tricalcium phosphate in bone repair and its mechanism to regulate osteogenesis. *Front Mater*. 2021;8:698915.
14. Milovancev M, Ralphs SC. Radius/ulna fracture repair. *Clin Tech Small Anim Pract*. 2004;19(3):128-133.
15. Nandi SK, Roy S, Mukherjee P, Kundu B, De DK, Basu D. Orthopaedic applications of bone graft and graft substitutes: a review. *Indian J Med Res*. 2010;132(1):15-30.
16. Oryan A, Alidadi S, Bigham-Sadegh A, Moshiri A. Healing potentials of polymethylmethacrylate bone cement combined with platelet gel in the critical-sized radial bone defect of rats. *PLoS One*. 2018;13(4).
17. Singh R, Chandrapuria VP, Shahi A, Swamy M, Bhargava MK, Shukla PC. Guided tissue regeneration with β -tricalcium phosphate and platelet-rich plasma for fracture repair in dogs using internal fixation. *J Anim Res*. 2020;10(1):25-31.
18. Vardhan KH, Prasad VD, Sreenu M, Syaamsundar N. Histopathological evaluation of polymethyl methacrylate and hydroxyapatite implants for fracture healing in rabbits. *J Vet Sci Technol*. 2017;6(3):3-6.
19. Wang Z, Guo Z, Bai H, Li J, Li X, Chen G, *et al*. Clinical evaluation of β -TCP in the treatment of lacunar

- bone defects: a prospective, randomized controlled study. *Mater Sci Eng C*. 2013;33(4):1894-1899.
20. Xu J, Gou L, Zhang P, Li H, Qiu S. Platelet-rich plasma and regenerative dentistry. *Aust Dent J*. 2020;65(2):131-142.
21. Yadav GU, Sarkate LB, Lokhande DU, Khandekar GS. Long bone fractures and its repair in dogs: a study of 10 patients. *Intas Polivet*. 2016;17(1):6-9.