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Histopathological Evaluation of Polymethyl Methacrylate and Hydroxyapatite Implants for Fracture Healing in Rabbits

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Abstract

Experimentally created mid diaphyseal fractures in 24 rabbits were treated by intramedullary pinning with K-wires, polymethyl methacrylate (PMMA) implants and intramedullary pinning after stuffing with hydroxyapatite (HA) paste and the histopathological changes were recorded. Healing process was not progressive, in control group, as indicated by presence of only a few osteocytes in the matrices, discontinuity in the epithelial layer and extensive vacuolation with occasional presence of giant cells. In PMMA group, healing pattern by endochondral method of ossification was evident by day 30 and day 60, normal structure of bone with remarkably high osteoid formation and osteocytes proliferation were observed. In HA group, vacuolation and discrete presence of osteocytes separated by interlacing trabeculae when compared to the other two groups were more pronounced. By day 60, endosteal vascularization could be depicted under high power. To conclude, of all the three groups healing was better in PMMA group when compared to the other two groups.

Keywords: *polymethyl methacrylate (PMMA), hydroxyapatite (HA), endosteal vascularization, endochondral ossification, vacuolation*

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INTRODUCTION

Fracture is defined as a discontinuity in hard tissues such as bone and cartilage. The fracture in animals invariably causes pain and suffering to them, apart from loss of function of' the affected limb. The methods to address fractures in different species vary significantly. In the recent years, there has been a trend to use biodegradable and bio-absorbable implants for fracture healing in animals. As polymethyl methacrylate (PMMA) can be molded to different shapes and sizes, it was used for the repair of fractures. Hydroxyapatite (HA) is a biocompatible implant material having osteoconductive capacity and is known for its passive support for neovascularization. In the present paper, the efficacy of PMMA and HA was shown in terms of histopathological features.

MATERIALS AND METHODS

The experiment was conducted in 24 rabbits divided into three groups of eight each. In all the animals, diaphyseal fractures were created using a circular wire saw and were immobilized with K-wires (n=8; Group- I), PMMA implants (n=8; Group, II) and HA quoted implants (n=8; Group, III). The stainless-steel K-wires of 1–2 mm diameter were employed to immobilize the fracture fragments through standard protocol. In the second group, the PMMA implants resembling K-wires were prepared by mixing the powder with liquid copolymer and were used to immobilize the fracture fragments (Figure 1). It was smeared in the groove of a mold under low temperature and the implant was used for immobilizing the fracture fragments. In the third group, HA paste was stuffed into the medullary cavity of the fragments and K-wires were used for fixation (Figure 2). Two rabbits from each group were euthanized using Thiopentone Sodium by intravenous route till effect and the fractured femur was collected for gross and histopathological evaluation. The bone samples were made free from the soft tissue. After total decalcification, bone samples were subjected for routine paraffin embedding technique and the sections were stained by the Hematoxylin-Eosin staining technique as per the method of Singh and Sulochana [1].

RESULTS AND DISCUSSION

Healing process was not progressive after 15 days, in control group, as indicated by complete loss of normal histopathological structure with only a few osteocytes in the matrices discontinuities of the epithelial layer and extensive vacuolation with occasional presence of giant cells were recorded (Figure 3). In one animal, that developed osteomyelitis the corresponding sections exhibited edema of the dense connective tissue with vacuolation (Figure 4). In PMMA group, on day 15, sections revealed dense connective tissue with comparatively less number of osteocytes and intact layer of fibrous part of periosteal layer (Figure 5). Healing pattern by endochondral method of ossification was evident by day 30 (Figure 6). There was an exaggerated growth of epithelium with matrices in the bone cortex containing chondrocytes. At high power, active osteocyte proliferation was also evident subperiosteally. By day 60, sections revealed normal structure of bone with remarkably high osteoid formation and osteocytes proliferation (Figure 7). In HA group, vacuolation and discrete presence of osteocytes separated by interlacing trabeculae when compared to the other two groups were more pronounced (Figure 8). By day 60, osteogenic activity through endochondral ossification was found in one section with complete epithelialization with endosteal vascularization could be depicted under high power (Figure 9).

At 30 days, perfect union of fracture fragments was not achieved, and the callus was rubber like without mineral deposition in the control group. In PMMA group, healing was superior with the presence of a fibrous callus around the fracture line. In HA group, though there was presence of a primary callus, periosteal layer was not seen. These findings were contradicting with Na *et al.* [2] and Nakagawa *et al.* (2006) [3], who observed that, the fracture line could not be identified by 4th week after experimental creation of metaphyseal fracture. At 60 days, cicatrization was noticed at the fracture site in the control group and unresorbed callus was found. In PMMA group, the healed bone sample almost resembled the normal bone. In HA group, excessive cicatrization and excessive connective tissue growth were found. These results were in accordance with those of Saraf *et al.* [4], who observed incomplete union and soft callus four weeks after surgery. In PMMA group, there was epithelialization and fibrous tissue formation in the periosteal layer which was in accordance with the findings of Miller *et al.* [5] and Ito [6]. In PMMA group, histopathological sections clearly revealed healing pattern by endochondral method of ossification with presence of large number of osteocytes. Figueiredo *et al.* [7] reported the differentiation of connective tissue cells into chondroblasts and osteoblasts in implants of demineralized bone matrix.

Contrary to the findings of Flately *et al.* [8], complete healing with osteoid formation was seen in half of the control animals. In PMMA group, concentric layers of bone lamellae with giant cells indicative of inflammatory reaction were noted as recorded by Togawa *et al.* [9] and Verlaan *et al.* [10]. No giant cells were found in HA group which is in accordance with the findings of Kato *et al*. [11], Holmes [12], Braz *et al.* [13] and Carlo *et al*. [14]. Absence of fibrous tissue between implant and bone in HA group was in accordance with findings of Uchida *et al.* [15] but contradicting with the findings of Alibadi *et al.* [16]. Total bone formation was more in HA group when compared to control group which is in accordance with the findings of Lima *et al.* [17]. To conclude, of all the three groups healing was better in PMMA group when compared to the other two groups.

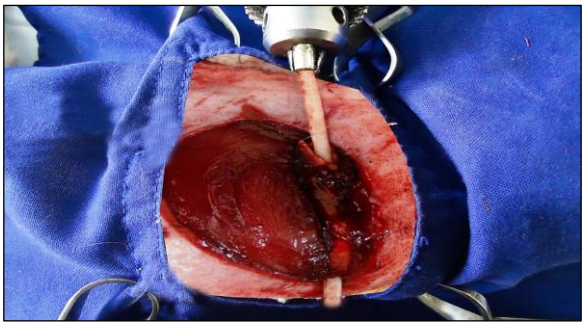

Fig. 1: *Note PMMA Implant Immobilizing the Fracture Fragments.*

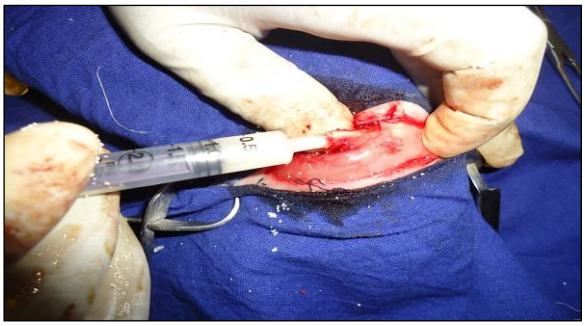

Fig. 2: *Note HA Paste Stuffed into the Medullary Cavity of Bone Fragments.*

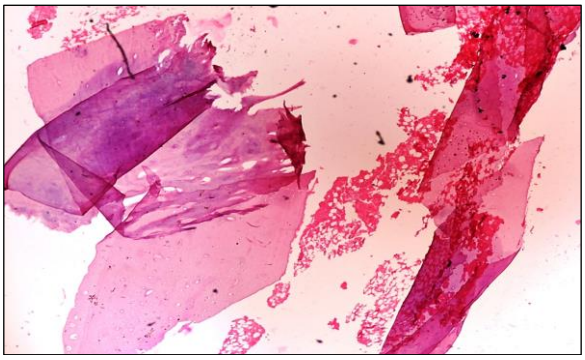

Fig. 3: *Note Unorganized Osteoid and a Very Few Osteocytes in Control Group, Day 30 (H&E, 10X).*

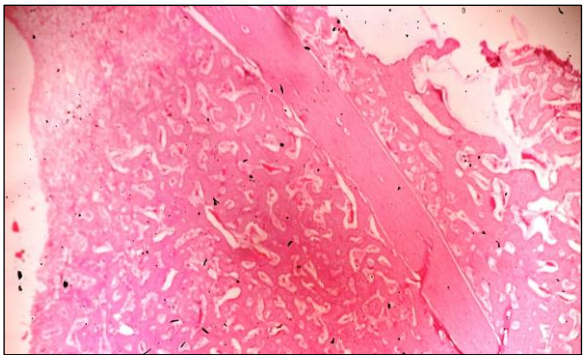

Fig. 4: *Note Osteomyelitis with Edema of Dense Connective Tissue (H&E, 10X).*

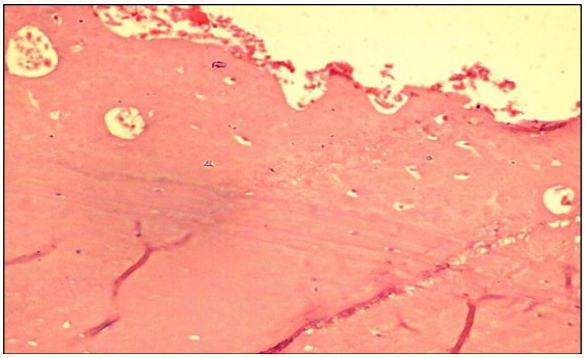

Fig. 5: *Note Intact Layer of Fibrous Portion of Periosteal Layer in PMMA Group, Day 30 (H&E, 10X).*

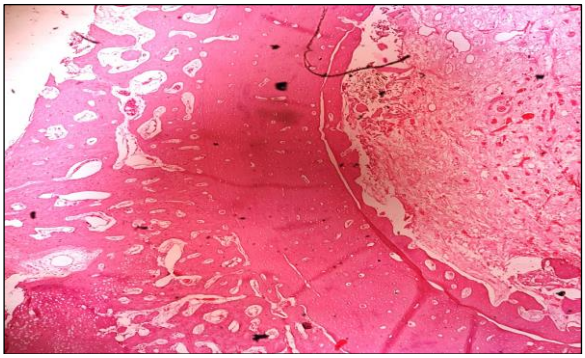

Fig. 6: *Note Endochondral Method of Ossification in PMMA Group, Day 30 (H&E, 10X).*

Fig. 7: *Note Near Normal Bone Structure in PMMA Group, Day 60 (H&E, 10X).*

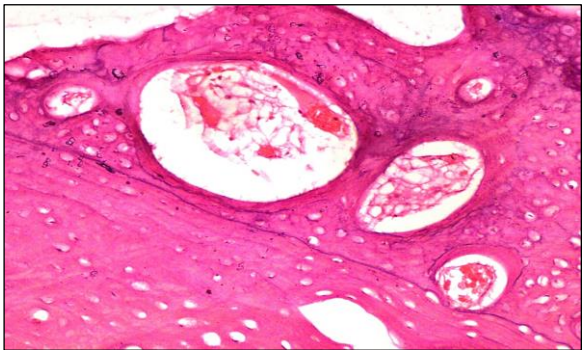

Fig. 8: *Note Pronounced Vacuolation and Predominant Osteocyte Formation in HA Group, Day 30 (H&E, 10X).*

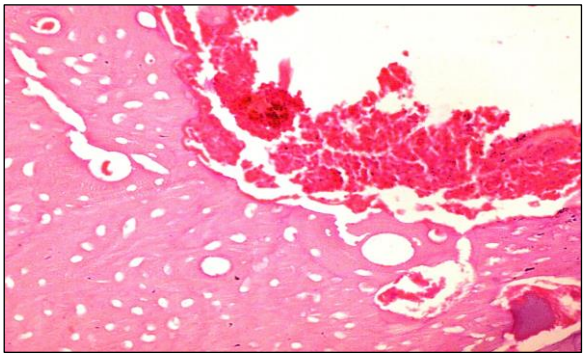

Fig. 9: *Photograph Showing Endosteal Vascularization in HA Group, Day 60.*

SUMMARY

K-wires, PMMA and HA implants were employed to treat experimentally created mid-diaphyseal fractures in rabbits and were sacrificed at different time intervals to judge their suitability histopathologically.

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