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Evaluation of Microcrystalline Hydroxyapatite Cement in a Rat Femoral Unicortical Defect Model

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Abstract - Bone graft substitutes are increasingly used in orthopaedic, maxillofacial, and reconstructive surgery to manage osseous defects resulting from trauma, tumour resection, congenital deformities, or delayed union. Among synthetic biomaterials, hydroxyapatite-based grafts have gained considerable attention because of their close resemblance to the mineral phase of natural bone. Microcrystalline hydroxyapatite (mHA) cement is particularly valuable due to its injectability, moldability, biocompatibility, and osteoconductive behaviour. The present study evaluated the biological performance of microcrystalline hydroxyapatite cement used as the control graft in a rat femoral unicortical defect model. Twenty-one adult male Wistar rats were subjected to standardised femoral cortical defects, and the healing response following graft placement was assessed at 2, 6, and 12 weeks through gross examination, radiography, histopathology, and histomorphometric scoring.At 2 weeks, the graft remained stable within the defect and demonstrated early fibrovascular infiltration with immature woven bone formation. By 6 weeks, complete bridging of the cortical defect with substantial lamellar transformation was observed. At 12 weeks, the defect was remodeled with restoration of cortical continuity and mature bone architecture. Histomorphometric scores significantly improved ($p < 0.05$) over time.These findings confirm that mHA cement functions as an effective osteoconductive scaffold capable of supporting progressive bone regeneration and remodeling.

Key Words- Microcrystalline hydroxyapatite (mHA), Bone graft substitute, Femoral defect model, Osteoconduction, Bone regeneration.

INTRODUCTION

Bone is a highly specialised connective tissue composed of an organic collagenous matrix reinforced by inorganic calcium phosphate crystals, predominantly hydroxyapatite. Continuous remodelling through the coordinated activities of osteoblasts, osteocytes, osteoclasts, and osteoprogenitor cells maintains skeletal integrity and facilitates repair following injury (Clarke, 2008; Brown, 2009).

Large bone defects resulting from trauma, infection, tumor excision, or congenital abnormalities frequently exceed the natural regenerative capacity of bone and therefore require grafting procedures. Autogenous bone grafts remain the gold standard because they possess osteogenic, osteoinductive, and osteoconductive properties. However, their use is limited by donor-site morbidity, prolonged surgical time, limited availability, and postoperative complications (Khan et al., 2005; Roberts and Rosenbaum, 2012).

The limitations associated with autogenous grafting have stimulated interest in synthetic bone substitutes. Among these, hydroxyapatite (HA) has gained widespread acceptance because of its close chemical resemblance to the

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mineral phase of native bone and its excellent biocompatibility and osteoconductive characteristics (Bohner, 2000; Mondal et al., 2023). Hydroxyapatite constitutes the principal inorganic component of bone and provides rigidity and structural support to the skeleton (Young, 2003).

Calcium phosphate cements capable of forming hydroxyapatite after implantation provide a bioactive matrix that supports osteoblast attachment, vascular infiltration, and new bone deposition (Xu et al., 2017). Numerous investigations have demonstrated the ability of hydroxyapatite-based materials to promote osseointegration, support cellular proliferation, and facilitate regeneration of osseous defects (Ielo et al., 2022; Mondal et al., 2023; Fendi et al., 2024).

Microcrystalline hydroxyapatite cement represents a clinically useful formulation because it can be molded to conform to defect morphology while providing immediate structural support. Despite its widespread use, further experimental evaluation remains important for understanding its biological performance during bone regeneration.

Therefore, the present study was undertaken to evaluate the healing response of microcrystalline hydroxyapatite cement in experimentally induced unicortical femoral defects in rats.

Materials and Methods

Experimental Animals

Twenty-one healthy adult male Wistar rats weighing approximately 200 g were used in the study. Animals were housed under standard hygienic laboratory conditions with free access to food and water. All procedures were conducted under institutional ethical approval.

Experimental Defect Model

A standardized unicortical defect measuring 2 × 6 mm was created in the diaphyseal cortex of the femur using a dental bur under aseptic surgical conditions. This model is widely accepted for evaluating biomaterials intended for cortical bone regeneration. (figure 1)

Graft Placement

The femur of each rat was filled with microcrystalline hydroxyapatite cement. (figure 1)

Postoperative Care

Animals received analgesics and antibiotics following surgery and were monitored regularly. Seven rats each were euthanized at 2, 6, and 12 weeks for evaluation.

Methods of Assessment

Healing was assessed using:

- Gross morphological examination
- Radiographic imaging
- Histopathology using H&E and Masson’s Trichrome stains
- Histomorphometric scoring systems evaluating inflammation, vascularity, fibrosis, new bone formation, and integration

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Results

Gross Morphological Evaluation

At two weeks post-implantation, the hydroxyapatite cement remained visible within the defect and exhibited complete circumferential integration with surrounding host bone. No evidence of graft displacement, infection, or adverse tissue reaction was observed (figure 2).

At six weeks, the defect area was completely occupied by newly formed bone. Grossly identifiable graft material was absent, indicating progressive replacement of the implanted cement by regenerating osseous tissue (figure 2).

By twelve weeks, complete remodeling of the defect was evident. The repaired area exhibited structural continuity with adjacent cortical bone and was grossly indistinguishable from surrounding normal tissue. (figure 2)

Radiographic Evaluation

Immediate postoperative radiographs confirmed proper creation of the unicortical defects and accurate placement of the hydroxyapatite cement.

During subsequent evaluations, interpretation of healing was limited because the radiopacity of hydroxyapatite cement closely resembled that of native cortical bone. Although progressive reduction in defect visibility was observed, radiographs alone could not reliably differentiate residual graft material from newly formed bone. Similar limitations have been reported for hydroxyapatite-based biomaterials by Chang et al. (2015), Ciobanu and Ciobanu (2017), and Anu (2023).

Histological Evaluation

Two Weeks

Histological sections revealed fibrovascular connective tissue occupying the defect region. Numerous blood vessels, inflammatory cells, and immature woven bone extending from defect margins were observed (figure 3). These findings correspond to the inflammatory and proliferative phases of secondary bone healing described by Gerstenfeld et al. (2003) and Granero (2009).

Six Weeks

Newly formed bone bridged the defect gap. Woven bone predominated within the central regions of the defect, while increasing amounts of lamellar bone were observed. Inflammatory cell infiltration and vascularity were markedly reduced compared with the earlier evaluation period (figure 3). These findings indicate progression toward hard callus formation and early remodeling as described by Szczesny (2015).

Twelve Weeks

The defect site was almost completely occupied by mature lamellar bone. Well-organized trabeculae, numerous osteocytes within lacunae, and restoration of cortical continuity were observed. Minimal fibrous tissue and inflammatory infiltration remained (figure 3). These findings are consistent with the remodeling phase of bone healing described by Ai-Aql et al. (2008).

Histomorphometric Analysis

Histomorphometric evaluation demonstrated progressive improvement in all healing parameters.

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Inflammatory activity progressively declined throughout the healing period, reflecting successful resolution of the inflammatory phase.

Vascularity was greatest at two weeks and subsequently decreased, consistent with maturation of repair tissue and reduced dependence on neovascularization (Granero, 2009).

New bone formation progressed from immature woven bone at two weeks to mature lamellar bone at twelve weeks. Edge integration scores increased steadily, indicating successful incorporation of regenerated tissue into surrounding host bone.

Total histological healing scores increased from 6.33 ± 0.33 at two weeks to 15.67 ± 0.88 at twelve weeks, demonstrating continuous progression toward complete healing.

Quantitative healing scores improved significantly over time:

Table 1. Histologic healing scores (Mean ± SE)

Parameter	2nd Week	6th Week	12th Week
Inflammation	0.33 ± 0.33	2.33 ± 0.33	2.67 ± 0.33
Vascularity	2.67 ± 0.33	1.33 ± 0.33	1.33 ± 0.33
Fibrosis	0.33 ± 0.33	2.33 ± 0.33	2.67 ± 0.33
New Bone Type	1.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
New Bone Quantity	1.00 ± 0.00	2.67 ± 0.33	3.00 ± 0.00
Edge Integration	1.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
Total Score	6.33 ± 0.33	14.67 ± 0.33	15.67 ± 0.88

These values indicate progressive reduction of inflammation and corresponding increases in bone quantity, bone maturity, and graft-host integration.

Scanning Electron Microscopy

SEM examination at two weeks revealed a relatively smooth surface morphology with limited tissue deposition. By six weeks, evidence of woven bone deposition and mineralized tissue formation was apparent.

At twelve weeks, the hydroxyapatite cement largely maintained its structural integrity, exhibiting only mild surface erosion and pitting. No evidence of catastrophic degradation was observed. These findings indicate gradual remodeling while maintaining mechanical stability throughout healing (figure 4).

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Discussion

Hydroxyapatite has long been recognized as one of the most effective synthetic bone substitutes because of its close resemblance to native bone mineral and its excellent osteoconductive characteristics (Bohner, 2000; Mondal et al., 2023).

The early fibrovascular infiltration observed in the present study corresponds with the osteoconductive behavior described by Wahl and Czernuszka (2006), Xu et al. (2017), and Mondal et al. (2023). Effective osteoconduction requires vascular and cellular migration into the implanted scaffold, enabling subsequent bone deposition.

The gradual replacement of graft material by newly formed bone observed during the study reflects the process of creeping substitution described by Flynn (2011). Similar observations have been reported for hydroxyapatite-based biomaterials used in orthopedic and craniofacial reconstruction (Xu et al., 2017).

The transition from fibrovascular tissue and woven bone at two weeks to mature lamellar bone at twelve weeks followed the classical sequence of secondary bone healing described by Gerstenfeld et al. (2003), Granero (2009), and Ai-Aql et al. (2008). Progressive reduction in inflammatory infiltration and vascularity further supported advancement toward tissue maturation.

The inability of radiographs to clearly differentiate residual hydroxyapatite from newly formed bone has been documented previously (Chang et al., 2015; Ciobanu and Ciobanu, 2017). Consequently, histological and ultrastructural evaluations remain essential for accurate assessment of healing when hydroxyapatite-based grafts are used.

The preservation of structural integrity observed by SEM is consistent with the known behavior of hydroxyapatite ceramics. Although hydroxyapatite undergoes slow biological resorption, it provides prolonged structural support during bone regeneration (Bohner, 2000).

Overall, the findings confirm that microcrystalline hydroxyapatite cement functions as an effective osteoconductive scaffold capable of supporting cellular infiltration, vascularization, bone formation, and eventual remodeling into mature osseous tissue.

Conclusion

Microcrystalline hydroxyapatite cement demonstrated excellent biocompatibility, osteoconductivity, and graft-host integration in experimentally induced femoral defects in rats. Progressive bone regeneration, successful defect bridging, and remodeling into mature lamellar bone were observed throughout the study period. Histological, histomorphometric, and SEM findings confirmed effective osseous regeneration and restoration of structural continuity. The results support the continued use of microcrystalline hydroxyapatite cement as a reliable synthetic bone graft substitute for orthopedic reconstruction and bone defect repair.

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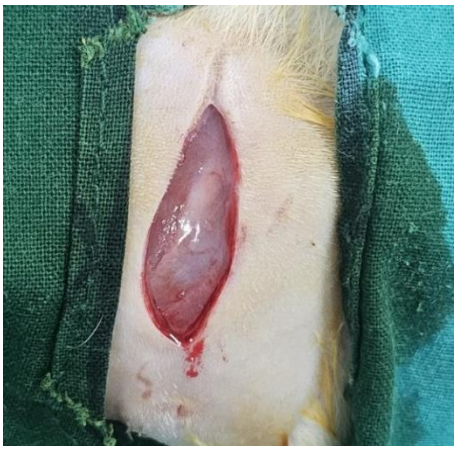
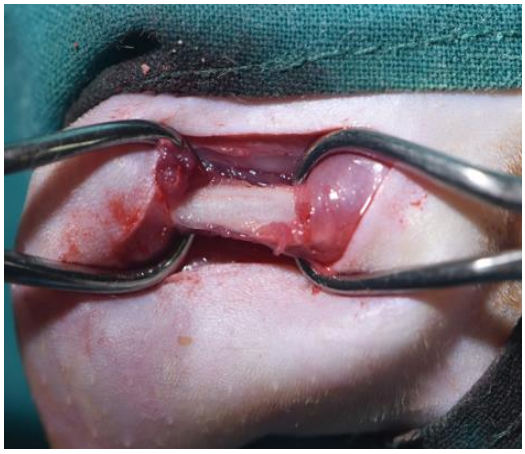
Figure 1. Surgical proce

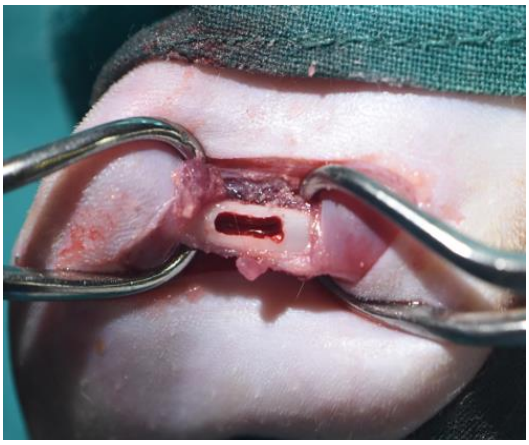
Surgical site



Skin incision

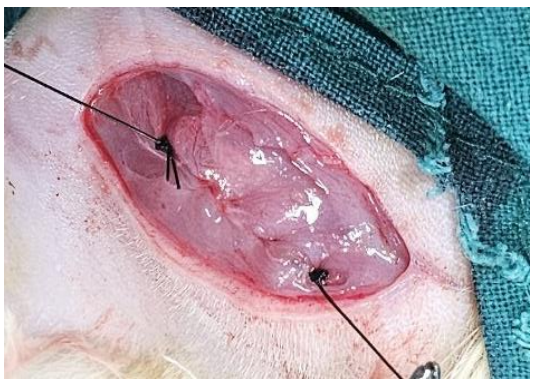


Exposure of the femur



Unicortical defect

Grafted defect



Muscle sutured

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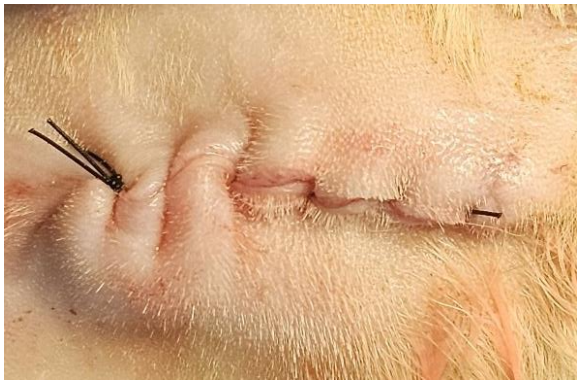
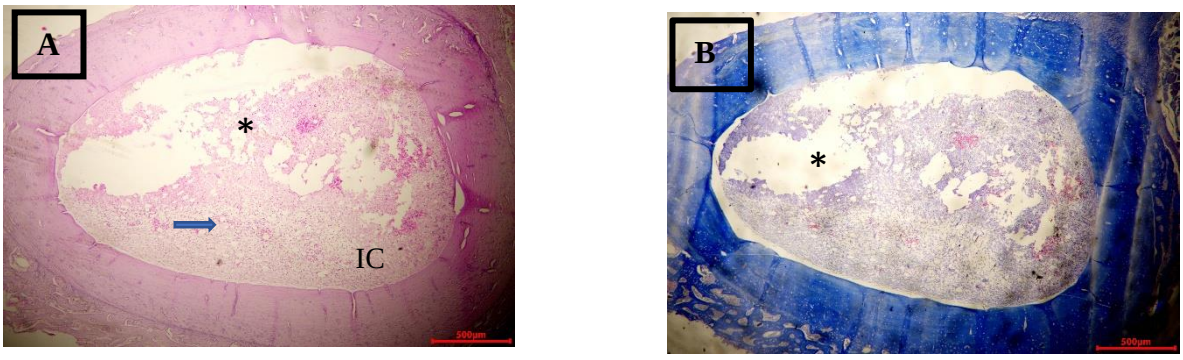


Skin sutured

Figure 2 . Gross evaluation of bone after harvesting

2nd week **6th week** **12th week**

Figure 3. Histopathological evaluation



2nd week of bone healing: A. Defect (*) predominantly filled with fibrous connective tissue consistent with the early reparative phase of bone healing. Blood vessels (arrow) and inflammatory cell infiltrate (IC) are also present. (x40, H&E) **B**. Defect (*) predominantly filled with fibrous connective tissue and collagen deposition

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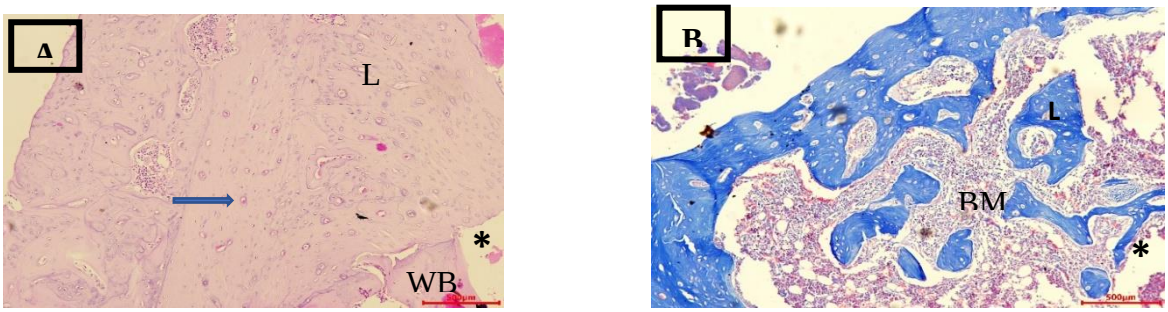
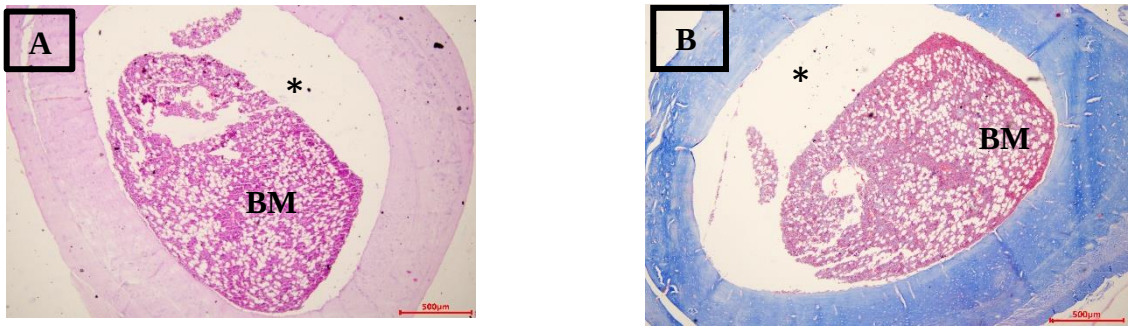
(blue) with minimal immature woven bone formation at the margins, consistent with the early reparative phase of healing. (x40, Mason's Trichrome)

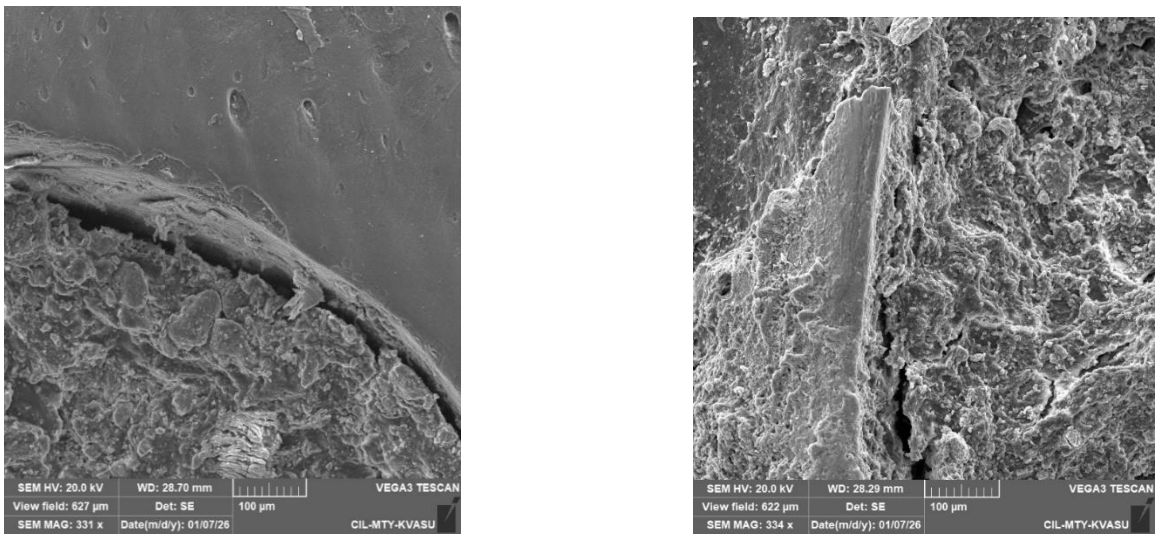


6th week of bone healing: A. Defect (*) filled by lamellar bone (L) with osteocytes in lacunae (arrow) (x100, H&E). **B.** Lamellar bone (bright blue colour) and bone marrow formation at defect site (*) (x100, Mason's Trichrome).



12th week of bone healing: **A.** Defect (*) predominantly filled with marrow tissue (BM) containing adipocytes and hematopoietic elements(x40, H&E). **B**. Defect (*) predominantly occupied by marrow tissue (x40, Mason's Trichrome)

Figure 4. scanning electron microscopy



2nd week observation group **6th week observation**

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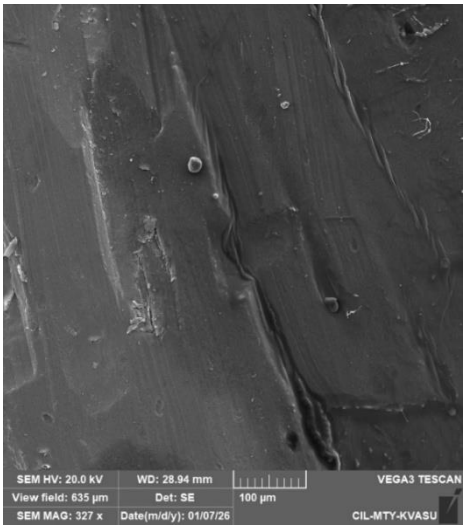

12th week observation

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