ISSN: 03875547
Volume 44, Issue 05, October, 2021

# Histologic and Histomorphometric Evaluation of Bone Healing Following Growth Factor Enhanced Matrix (GEM 21S) in Rabbit Calvaria

Malak Shoukheba[1], Omneya Wahba[2], Walid Aly Elamrousy[3]

Ass. Professor of Oral Medicine, Periodontology, Oral Diagnosis and, Dental Radiology Department, Faculty of Dentistry, Tanta University, Egypt[1]
Ass. Professor of Oral Pathology Department, Faculty of Oral and, Dental Medicine, Kafr El-Sheikh University, Egypt[2]
Lecturer of Periodontology, Oral Medicine, Oral Diagnosis and, Dental Radiology, Faculty of Oral and Dental Medicine, Kafr El-Sheikh University, Egypt[3]

**Keywords:**
growth factors enhanced matrix (GEM21s), beta-tricalcium phosphate bone substitute, COX-2 immunohistochemical.

**ABSTRACT**

This study explored the process of bone regeneration of growth factor enhanced matrix (GEM21s) in rabbit calvaria compared to beta-tricalcium phosphate (β-TCP). Twelve rabbits weighing between 3.500 - 4.000 g were subjected to three standard 8 mm in diameter defects at the parietal bone. The 36 defects in the 12 rabbits were categorized into 3 groups (each of 12 defects). Group 1: defects have been left unfilled with any substitute. Group II: defects were packed with β-TCP. Group III: defects were filled with GEM 21S. After 3 weeks, 6 rabbits (18 defects 6 from each group) were sacrificed, and the rest of the rabbits will be sacrificed at 6 weeks. The samples were prepared for histologic and histomorphometric analysis. Results of the present work have revealed that group II and Group III showed bone formation at both 3, and 6 weeks with a significant new bone formation in Group III (GEM21S) in comparison to group II, P< 0.001. Both groups showed a fast rise in the formation of new bones from 3 weeks to 6 weeks in comparison to group I. Immunohistochemical analysis showed higher COX-2 expression in the control group than group II, whereas, minimal expression was observed in GEM 21s treated defects. GEM21s demonstrated excellent biocompatibility and declared additional bone growth following a phase of healing of 6 weeks and it has the superior potential of bone reconstruction compared to β-TCP alone.



## 1. INTRODUCTION

The primary objective of periodontal therapy is to eliminate the inflammatory phase, avoid the disease progression and enhance the periodontal regeneration [1]. Many trials are made to regenerate the damaged periodontal tissues, such as bone grafts and guided tissue regeneration (GTR), although these approaches have shown promise in the regeneration of the periodontal tissues, handful of them that have the ability to

stimulate angiogenesis directly to the healing area [2].

Many commercial bone graft products are limited in that they provide osteoconduction only. This may be addressed by incorporating growth factors and biomaterial agents that induce mitogenic and chemotactic responses to promote regeneration such as platelet- derived growth factor, platelet-rich plasma, enamel matrix derivative, bone morphogenic protein, and other growth factors [3], [4].

Polypeptide growth factors are of a group of natural biological mediators that control the major cellular processes of tissue healing. Platelet- derived growth factor (PDGF) is the extensively researched periodontics growth factor [5].

Recently, for clinical usage, Growth factor enhanced matrix (GEM 21S) is a new and improved wound healing and bone regeneration method that is currently available. This graft material is composed of a concentrated pure solution of recombinant human platelet-derived growth factor (rh-PDGF-BB), the synthetic form of the body's most important natural wound healing stimulant PDGF-BB, and an osteoconductive (bone scaffold) matrix composed of beta-tricalcium phosphate (β-TCP) that has been approved for human use by the FDA [6], [29- 33]. This grafting is a very porous, resorbable matrix that offers a bone ingrowths structure, helps to avoid soft tissue collapse and improves blood clot stability [7].

Several clinical and radiographic studies revealed that GEM 21S is an effective bone material for mild to severe periodontitis treatment in under six months [7], [8]. Maroo and Murthy, [7] assessed the adjunctive use of rh-PDGF-BB to β-TCP in regenerative management of intrabony defects compared to only β-TCP clinically and radiographically. The rh-PDGF-BB group showed improved radiological and clinical markers significantly over the control group at both six and nine months. Accordingly, the adjunctive use of rh-PDGF-BB to β-TCP provides promising results in the intrabony defects cure.

Recently, a double-blind randomized controlled clinical trial was performed for assessment of collagen membrane efficacy with and without (GEM 21S) combined with coronally advanced flap in covering denuded root surfaces in Miller Class I or II gingival recession combined with buccal bone defects for 6 months period. The results revealed, from both esthetic and clinical views, a favorable response to GEM 21S with Collagen membrane in the form of covering the denuded root surfaces and increase in the thickness of gingival tissues, CAL gain, and PD reduction [6].

Due to the lack of histological evidence of the influence of GEM 21S on bone regeneration, the present study was conducted to evaluate the efficacy of platelet-derived growth factor (rh-PDGF-BB) along with β-TCP to establish periodontal regeneration in rabbits with bone defects.

## 2. Material and Methods

Twelve adult rabbits (five-month-old) of 3.5 – 4.0 kg in weight were included in the study. The process was conducted at the Faculty of Medicine, Tanta University Animal House. Animals were maintained under conventional conditions with availability of water and food. The methods fulfilled the ethical standards of the Research Ethics Committee of Faculty of Dentistry, Tanta University and with the Helsinki Declaration.

The rabbits were intramuscularly anaesthetized with a combination of ketamine hydrochloride (Ketanes®, Alke, Turkey) and xylazine (Rompun®, Bayer, Leverkusen, Germany) for total of 0.59 ml/kg.

The operation was conducted with sterility protocols. In order to prevent bacterial infection, the surgical area

was shaven and scrubbed with iodide solution. A midline incision on the skull was cut, and both the temporalis muscle and the periosteum were reflected laterally by a periosteal elevator, then the periosteum was removed, and two distinct and yet identical bicortical cranial rounded defects with a diameter of 8 mm were made in the parietal bones, every side of the midline of each animal had one defect (a total of 36 defects) with an internal diameter of 8mm using a slow-speed electric handpiece of a trephine drill (Komet Inc., Lemgo, Germany) under copious irrigation with sterile saline solution. Fig (1)

The 36 defects in the 12 rabbits were categorized into three groups (12 defects each). Group 1 comprised of 12 defects were left unfilled. While defects in group 2 were filled with β-TCP. Group III included 12 defects that were filled with GEM 21S (Biomimetic Therapeutics Inc, Franklin, TN, USA). 4/0 resorbable sutures (Vicryl®, Johnson & Johnson, Brussels, Belgium) were used to suture the surgical area.

An antibiotic (cefazoline 25 mg/kg) and a painkiller (diclofenac sodium) were given intramuscularly, 2 times daily for 4 days, to prevent postoperative infection and discomfort. The sutures were checked daily for the sign of swelling, redness, or suture cutting and disinfected daily with betadine solution. Animals underwent normal diet programs in their cages (fig 1).

***2.1 Tissue preparation for histological analysis***

Three weeks after the operation, 6 rabbits (18 defects, 6 defects from each group) were anaesthetized by general anesthesia technique and intracardially injected with 100 mg/kg of sodium thiopental (Pental®, Bilim, Istanbul, Turkey) and the other 6 rabbits (18 defects) were sacrificed 6 weeks afterward, the calvarial bones were obtained and bone blocks were collected for histopathologic analysis in boxes labelled with numbers and documented by study group (fig 2).

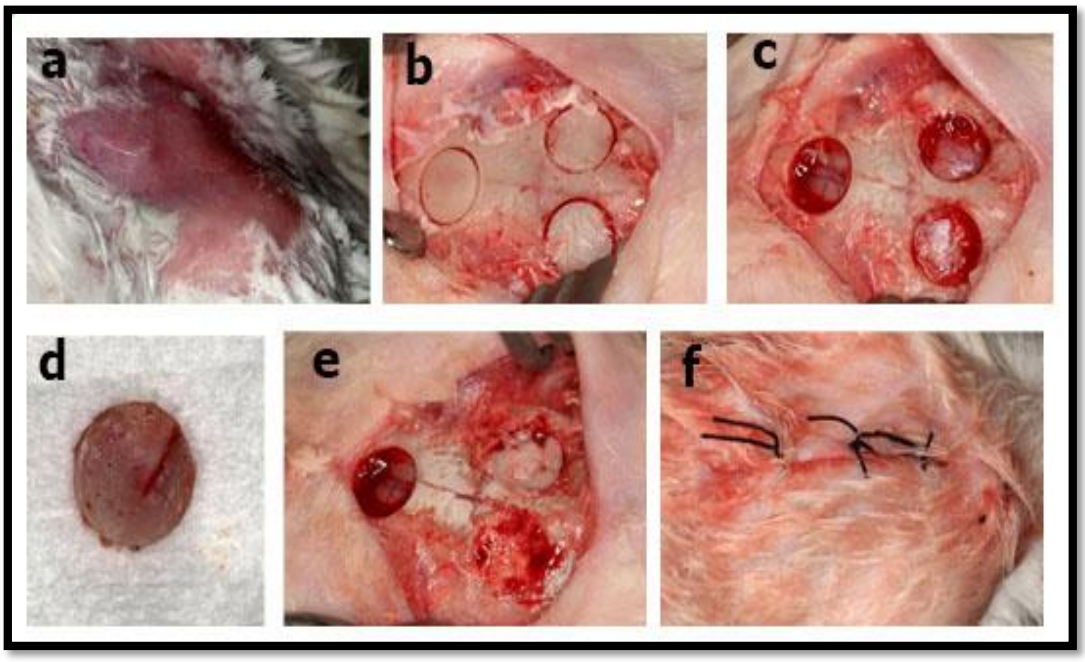


**Fig (1):** (a) Clinical photograph showing shaving the surgical area. (b) The trephined defects in the rabbit calvarias. (c) The three identical bony defects. (d) The trephinzed bone. (e) The defect filled with treatment material, one defect filled with GEM21S, the second with β-TCP, and the third one left empty (as a control). (f) Sutures in place

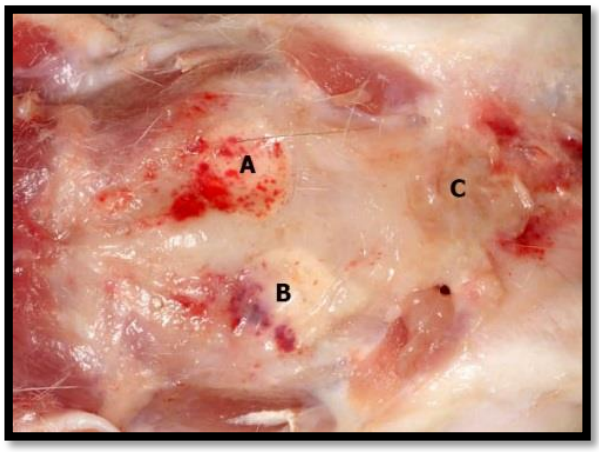


**Fig (2):** Cranial gross appearance of the defects after healing at 6 weeks, showing A: βTCP filled defect, B: GEM filled defect, and C: Control defect (unfilled).

Then, dissection of the parietal bones was performed, set in 10% formalin, and treated with EDTA to prevent decalcification and then ascending concentrations of alcohol (70% -100%) for dehydration and xylene to clear. The samples were then integrated into paraffin wax. 5 micron sections perpendicular to the bone surface were cut, put on glass slides, deparaffinized, hydrated, and stained with hematoxylin and eosin (H&E) stain and Masson`s trichrome staining for histological evaluation and histomorphometric analysis.

The sections were studied with a light microscope (Olympus BX60, Tokyo, Japan) at power x20 magnification. The microscope is connected to the digital camera (LEICA ICC50 HD Camera system) via software LAS EZ version 3.0.0. The percentage area of bone that newly formed was assessed on the image as follow:

The cursor has been used to highlight newer bone trabeculae regions, and these regions became covered by a computer-measurable binary blue color. The software calculated the area of newly formed bone in relation to the total defect area found in the digitized histopathological photos.

The percentage size of bone that newly formed in each group was measured in five consecutive defect sections, where region 3 denotes the defect center and the mean values were taken as showing in the schematic diagram in fig (3).

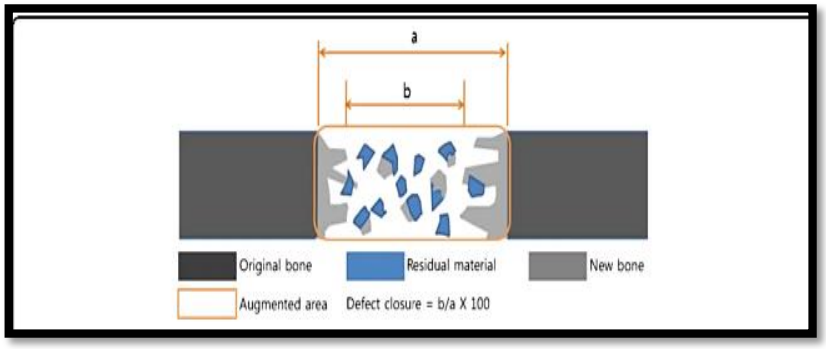


**Fig (3):** Schematic diagram of histomorphometric analysis

***2.1.1 Immunohistochemical Staining***

To stain immunohistochemically, 4 µm sections of each paraffin block were produced and deparaffinized in xylene solution, followed by dehydration in a graded alcohol series. Hydrogen peroxide (3%), in a phosphate buffer solution, was used to block the internal peroxidase activity. Then, in a microwave oven (Panasonic 1380W, USA) antigen retrieval was carried out for 10 minutes, under almost 2 atm pressure at 120°C. Additional incubations using prediluted ready-to-use primary rabbit monoclonal antibody anti-COX2 (monoclonal antibodies DakoCytomation Norden A/S, Glostrup, Denmark; dilution 1: 50). Evaluation of COX-2 immunostaining: any cytoplasmic brown staining was considered a positive result, we classified COX-2 immunostaining into high and low immunostaining with semiquantitative scoring by two independent pathologists to record the percentage of positive cells for COX-2.

### *2.2 Statistical analysis*

Data were analyzed using Statistical Program for Social Science (SPSS) version 20.0. Quantitative data were expressed as mean± standard deviation (SD). Mann-Whitney (Z) test for non-parametric data is used to compare between 3 and 6weeks periods for the three treated groups, and ANOVA test for intergroup comparison. P values < 0.05 were considered statistically significant.

## 3. Results

Throughout the duration of the study, all animals remained healthy. No substantial inflammatory response, necrosis, or foreign body responses were detected in any of the specimens.

### *3.1 Group (1) defects unfilled (control group)*

H&E stained slices of the control groups' centers of body defects showed the presence of fibrous tissue and thin irregular osteoid bone. Few osteocytes in their lacunae were scattered in the bone area. Mild chronic inflammatory cells were noted within the fibrous tissue. The osteoid bone after 6 weeks was slightly observed more than after 3 weeks but the inflammatory cell's response was more considered after 6 weeks of the study (fig 4)

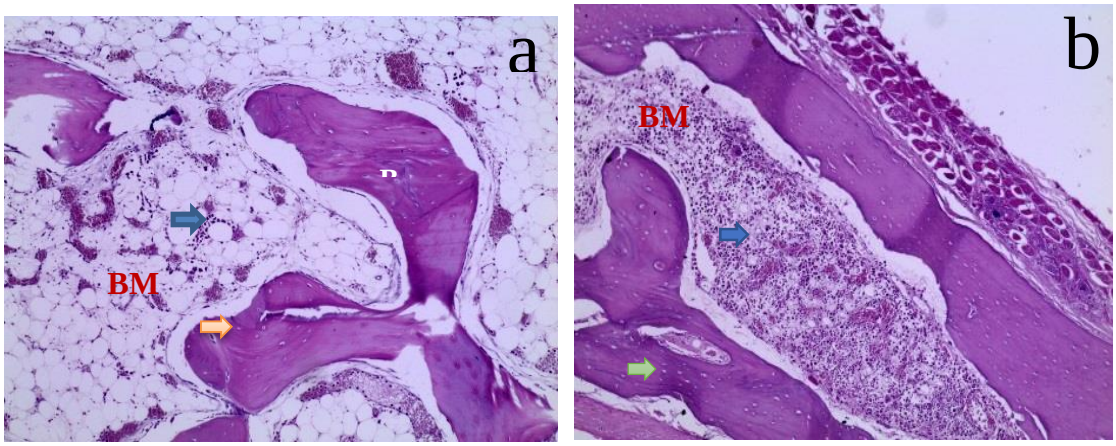


**Fig (4):** Hematoxylin and Eosin-stained photomicrographs of the control group; a. after 3 weeks, b. after 6 weeks. (B) osteoid bone, (BM) bone marrow. The green arrow is pointing at lacunae containing osteocytes, the blue arrow at inflammatory cells.(OMX 200)

### *3.2 Group (2) defects filled with β-TCP*

H&E stained sections obtained from the defects that filled with β-TCP revealed the presence of dense osteoid bone with fibrovascular bone marrow. The newly formed bony trabeculae were significant ($p < 0.005$), table (1) comparing after 3 and 6 weeks. Lacunae containing osteocytes and osteoblasts bordering the bony defects

were noted. Moderate chronic inflammatory cells infiltration was observed in bone marrow spaces after 3 weeks more than 6 weeks (fig 5).

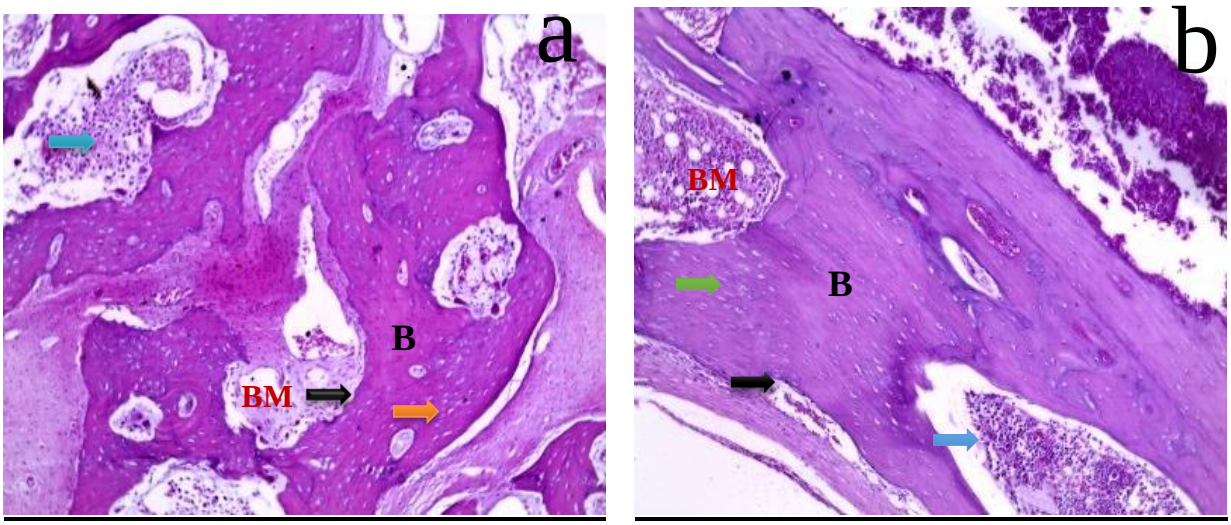


**Fig (5):** Hematoxylin and Eosin-stained photomicrographs group 2; a. after 3 weeks, b. after 6 weeks. (B) osteoid bone, (BM) bone marrow. The green arrow is pointing at lacunae containing osteocytes, the blue arrow at inflammatory cells, and the black arrow at osteoblasts (OM X 400).

### *3.3 Group (3) defects filled with GEM 21S*

H&E stained sections obtained from the defects that filled with GEM 21S revealed the presence of denser osteoid bone than the above two groups with significant difference ($p = 0.001$), table (1). Lacunae containing osteocytes and osteoblasts bordering the bony defects were also noted with minimal chronic inflammatory cells infiltration obviously observed at the end of 6 weeks (fig 6).

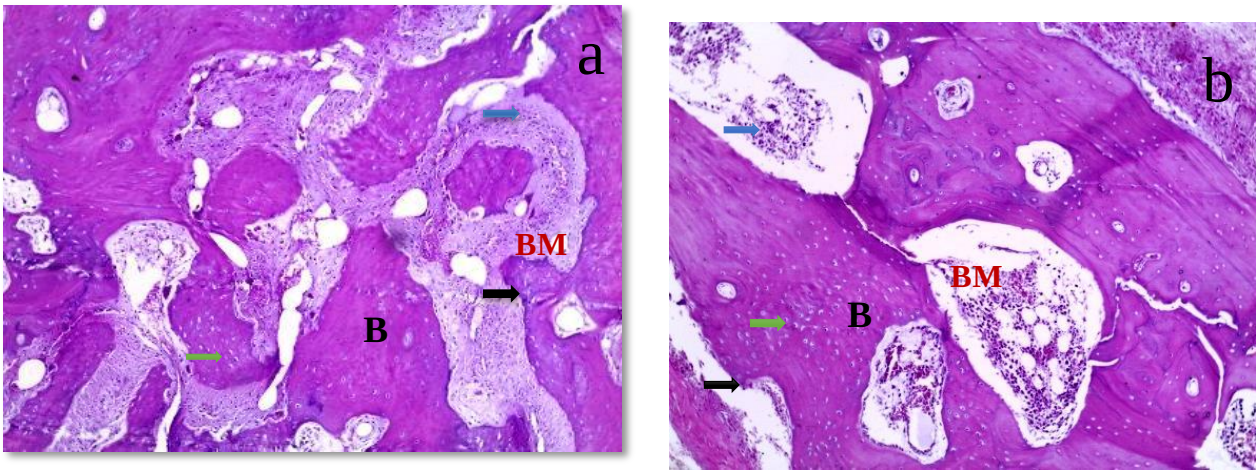


**Fig (6):** Hematoxylin and Eosin-stained photomicrographs group 3; a. after 3 weeks, b. after 6 weeks. (B) osteoid bone, (BM) bone marrow. The green arrow is pointing at lacunae containing osteocytes, the blue arrow at inflammatory cells and the black arrow at osteoblasts. (OM X 400)

### *3.4 Masson`s trichrome staining*

The microstructure of the tissues in the defect area was observed using histological analysis including Masson`s trichrome with the conventional H&E staining. The blue color indicated the regenerated bone,

ISSN: 03875547
Volume 44, Issue 05, October, 2021

collagen fibers or osteoid, whereas the color red indicated the mature bone. There was a significant increase in the area of new bone formed between control, defects filled with β-TCP and defects filled with GEM 21S ($p < 0.005$), table (1), fig (7).

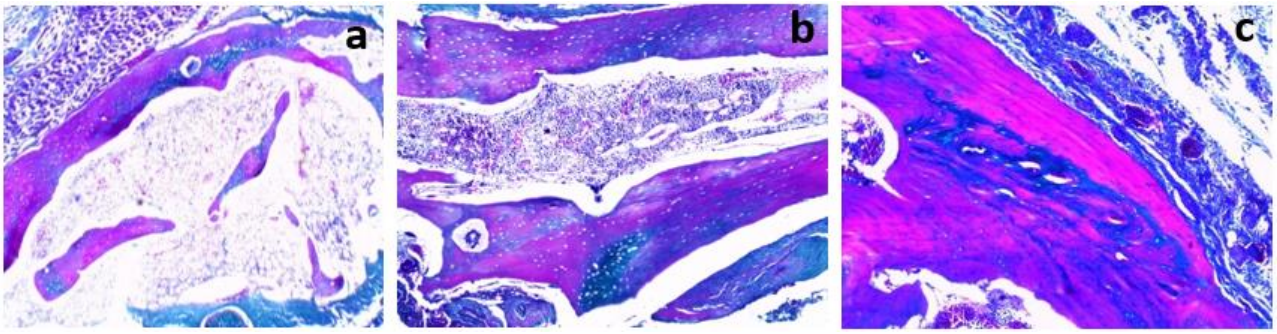


**Fig (7):** Masson`s trichrome stained photomicrographs of test groups; a. control group, b. Group (2) defects filled with β-TCP, c. Group (3) defects filled with GEM (OM X 400).

### *3.5 Immunostaining of cyclooxygenase (COX2)*

COX-2 immunostaining was detected as brown staining in the cytoplasm of bone marrow tissue, endothelial cells, osteocytes, and osteoblasts. There was an obvious difference in COX-2 expression between the groups of the study. The higher COX-2 expression was observed in the control group, with a slight decrease in defects filled with β-TCP although the expression was low in defects filled with GEM 21S fig(8).

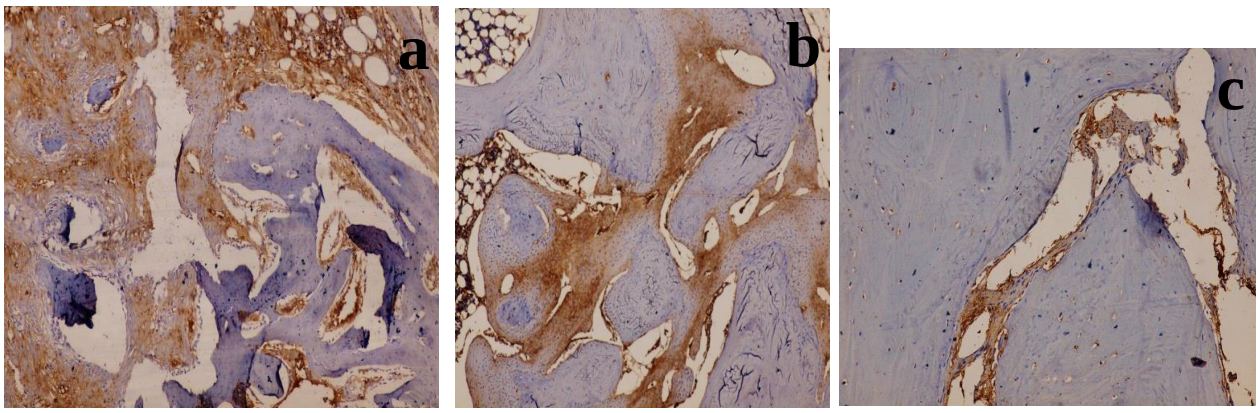


**Fig (8):** COX-2 stained photomicrographs of the treated groups; a. control group (group I), b. Group (II) defects filled with β-TCP, c. Group (III) defects filled with GEM 21S (OM X 400)

**Table 1:** comparison between Mean ± SD of the % area occupied by newly formed bone trabeculae in all the treated groups at 3- and 6-weeks evaluation periods

| **% area of bone formation** | | **3 weeks** | **6 weeks** | **Z. test** | **P. value** |
|---|---|---|---|---|---|
| **Group I (control group)** | **Range** | 10 – 20 | 20 – 35 | 5.221 | 0.001* |
| | **Mean ± SD** | 13.33 ± 4.08 | 27.50 ± 5.24 | | |

| | | | | | |
|---|---|---|---|---|---|
| **N-6** | | | | | |
| **Group II (β-TCP treated) N=6** | **Range** | 33.9 – 45.3 | 50.10 – 65.90 | 3.523 | 0.001* |
| | **Mean ± SD** | 39.97 ± 4.61 | 58.53 ± 6.30 | | |
| **Group III (GEM21S treated) N=6** | **Range** | 45.8 – 70.3 | 75 – 88 | 7.351 | 0.001* |
| | **Mean ± SD** | 55.7 ± 10.34 | 81.12 ± 5.15 | | |
| | **f. test** | 57.031 | 139.151 | | |
| | **P. value** | 0.001* | 0.001* | | |
| | **Group I vs Group II** | 0.001* | 0.001* | | |
| | **Group I vs Group III** | 0.001* | 0.001* | | |
| | **Group II vs Group III** | 0.001* | 0.001* | | |

Z= Mann-Whitney, P= level of significance at 0.05, n= number of specimens, β-TCP = beta- tricalcium phosphate, GEM21S= growth factor enhanced matrix, SD = standard deviation, f = ANOVA test

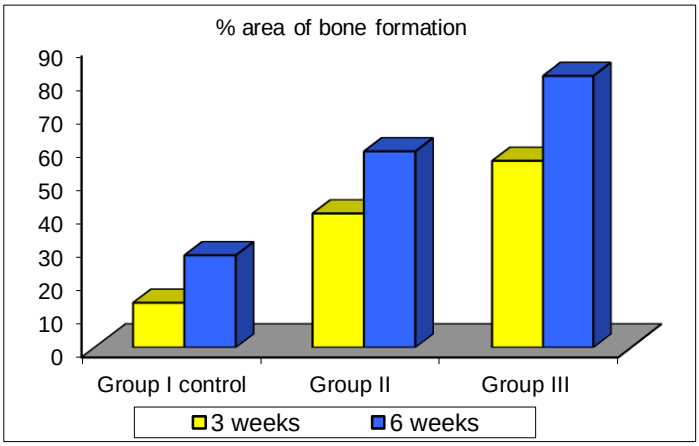

**Fig (9):** Column chart representing a mean percentage of new bone formation and the differences between the studied groups at the study evaluation periods.
Group II: (β-TCP treated) Group III(GEM21S treated)

**4. Discussion**

Due to the limitations of many bone grafts in reconstructing critical bone defects, [9] Growth factor enhanced matrix (GEM 21S), a revolutionary innovative wound healing and bone regeneration technology, has been approved for clinical usage. Additionally, due to the very limited histologic studies of the efficacy of GEM21S on the healing of bone defects and bone regeneration. As a result, the current study was designed to determine the effectiveness of GEM 21S. in treating bone defects in calvarias of rabbits.

GEM 21S is consisting of a concentrated solution of pure recombinant human platelet-derived growth factor (rh-PDGF-BB) and an osteoconductive (bone scaffold) matrix which is beta-tricalcium phosphate (β-TCP) [10]. Hence it was compared to (β-TCP) that was used as a positive control group in this study.

The rabbit has been regarded an appropriate experimental animal for evaluating biomaterials' osteogenic potential [11]. By creating bilateral defects, help in comparing the efficacy of the two treatment within the same animal. Additionally, several advantages of using the rabbits model such as their short regeneration time, small size, lower cost, easy access, and handling compared to monkeys and dogs [12].

When evaluating bone healing in an animal model utilizing novel regenerating material, it is essential to determine if the material adheres to the critical-size defect (CSD) concept, which is defined as a small diameter osseous defect that doesn't heal spontaneously [13], [14]. Hollinger and Kleinschmidt 1990 demonstrated that the use of standard flaws with a diameter of 8 mm in the parietal bones of rabbit calvaria resulted in a significant increase in their contact with bone graft materials [15]. Hence, in this study, full-thickness defects with a diameter of 8 mm were generated to assess bone healing histomorphometrically.

Additionally, the choice of the parietal bones of rabbit calvaria is ideal for evaluating osteogenesis encouraged by biomaterials because it has comparable morphology and embryological origin to the maxilla, it is a zone with less mechanical stress and a higher degree of relative stability for surrounding structures [16].

Three and six weeks following surgery, rabbits were sacrificed to evaluate bone development during the early phases of healing and to provide a clearer indication of the new bone's capacity to grow over the bone defects [17].

Results of the present study showed that healing proceeded without any evidence of inflammation, infection, or fibrosis developed between the particles of the biomaterial and the regenerated bone, confirming the biocompatibility of GEM 21S and β-TCP.

These findings are consistent with [18] who reported that rhPDGF-BB in combination with bone allograft promotes robust periodontal regeneration in both Class II furcations and interproximal intrabony defects with no clinical or histological adverse effects.

Similarly, [19] presented mild side effects such as postoperative edema and discomfort that seemed to be unrelated to the β-TCP.

The results of the present study showed that group II and Group III resulted in bone formation at 3 weeks but

Group III (GEM21S) elicited pronounced statistically significant new bone formation (55.7± 10.34%) compared to group II (β-TCP) (39.97±4.61%) P<0.007, thus GEM21S notably promoted bone formation, especially during the early healing phase.

While at 6 weeks, both groups II and III showed a high proportion of newly produced bone tissue. However, histomorphometric analysis showed that in group III the total of novel bone redevelopment (81.12±5.15%) was statistically significantly higher than that in group II (58.53±6.30%) P< 0.001. Both groups demonstrated a significant increase in new bone growth from 3 to 6 weeks, in comparison to group I. (the control group).

Results indicated that GEM 21S -treated defects had a greater new bone ratio than in defects treated with $\beta$-TCP alone. This can be explained by the presence of rh-PDGF-BB that initiate connective tissue healing by promoting the proliferation of fibroblasts and osteoblasts, and also, it upregulates the expression of VEGF that supports angiogenesis of wound healing [20]. Moreover, the presence of β-TCP which is considered as an effective delivery system entrapping rh-PDGF-BB within its microspores prolonging their action [21].

However, the less amount of bone formed in the β-TCP group than in group III can be explained by the fact that β-TCP degrades slowly and requires a long time to be replenished by new bone tissue, and this is confirmed by the notable amount of bone that formed at 6 weeks in group II.

These results corroborate with [22], who found that combining platelet-rich fibrin with beta-tricalcium phosphate may significantly speed bone repair compared to using either substance alone. Combining PRF and β-TCP for bone healing may provide a quicker healing option to utilizing these biomaterials alone. This may be explained by the fact that β-TCP is a biodegradable ceramic that is often employed in bone tissue engineering due to its inorganic structural resemblance to bone [23].

Material deterioration was also seen in this study, as new bone developed in the area left by the transplant's resorption without any graft remnants remaining. This is referred to as "creeping substitution," and it occurs with effective graft materials [24].

Additionally, the current study's findings are partly contradictory with those of [25] who reported that β-TCP proved to be a biocompatible, osteoconductive, and bioresorbable bone graft substitute.

Our findings suggest that β-TCP/ GEM21s may promote new bone growth while also dissolving the biomaterial. Additionally, histological examinations revealed that each group had a distinct pattern of new bone development and maturation. This indicates that the inclusion of rh-PDGF-BB, which has osteoinductive properties, may enhance the efficacy of β-TCP.

It is worthy to note that Inflammation is an essential component of wound healing, but it may impede tissue regeneration if it continues. As a result, the development of immunomodulatory methods to enhance periodontal regeneration results may be of interest [26].

The current study, we investigated immunohistochemically expression of COX-2 as an inflammatory mediator involved in the conversion of arachidonic acid to prostaglandins that plays a critical role in periodontal tissue inflammation, [27] which plays a critical function in the first phases of bone healing and also acts as an osteoblast differentiation stimulator [28].

Results of the present study showed that the control group (Group I) had a significantly higher expression in

comparison to groups II and III where group III showed the least amount of Cox-2 expression. This indicated that Cox-2 expression could be significantly correlated positively to the inflammation. These results agree with histological data that showed the presence of inflammatory cells infiltration in group I more than group II and III and group III showed the least amount of inflammatory cells infiltration. This clarifies the clinical safety of GEM21S to be used in bone tissue healing and regeneration.

## 5. Conclusions

The histological examination of rabbit cranial bone defects treated with GEM21s revealed great biocompatibility and significant new bone formation following a 6-week healing period, indicating that it has a greater potential for bone restoration than β-TCP alone.

Additional research using a more difficult model, such as a beagle canine, may be more suitable for assessing and clarify the exact mechanism and results of GEM21s on bone healing process and periodontal regeneration. Moreover, further radiographic evaluation investigating the bone gain after GEM21s by cone-beam radiograph.

## 6. References

[1] Villar CC, Cochran DL. Regeneration of periodontal tissues: guided tissue regeneration. Dent. Clin. 2010;54(1):73–92.

[2] Lin Z, Rios H F, Cochran D.L. Emerging regenerative approaches for periodontal reconstruction: a systematic review from the AAP Regeneration Workshop. J Periodontol. 2015;86(2 Suppl): S134–S152.

[3] Nishimura F, Terranova VP. Comparative study of the chemotactic responses of periodontal ligament cells and gingival fibroblasts to polypeptide growth factors. J Dent Res. 1996; 75:986–992

[4] Takayama S , Murakami S, Miki Y, Ikezawa K, Tasaka S, Terashima A, Asano T, et al. Effects of basic fibroblast growth factor on human periodontal ligament cells. J Periodontal Res. 1997; 32:667–675.

[5] Lynch SE, Williams RC, Polson AM, Howell TH, Reddy MS, Zappa UE, et al. A combination of platelet-derived and insulin-like growth factors enhances periodontal regeneration. J Clin Periodontol. 1989 Sep; 16(8):545-8.

[6] Singh P, and Suresh D K. Clinical evaluation of GEM 21S® and a collagen membrane with a coronally advanced flap as a root coverage procedure in the treatment of gingival recession defects: A comparative study. J Indian Soc Periodontol. 2012 Oct-Dec; 16(4): 577–583.

[7] Maroo S, Murthy KR. Treatment of periodontal intrabony defects using β-TCP alone or in combination with rhPDGF-BB: a randomized controlled clinical and radiographic study. Int J Periodontics Restorative Dent. 2014;34(6):841-847

[8] Avula J, Rajababu P, Rohini S, Babu B, Colleges A, Naveen A et al . Multi-centre, randomized clinical trial on the efficacy and safety of recombinant human platelet-derived growth factor with β-tricalcium phosphate in human intra-osseous periodontal defects. J Clin Periodontol. 2011; 38(2):163-72.

[9] Wang W, and Yeung K. Bone grafts and biomaterials substitutes for bone defect repair: A review. Bioact Mater, 2017; 2(4):224-247.

[10] Dandu SR, Murthy KR. Multiple Gingival Recession Defects Treated with Coronally Advanced Flap and Either the VISTA Technique Enhanced with GEM 21S or Periosteal Pedicle Graft: A 9-Month Clinical Study. Int J Periodontics Restorative Dent. 2016 Mar-Apr;36(2):231-7.

[11] Li Y, Chen SK, Li L, Qin L, Wang XL, Lai YX.. Bone defect animal models for testing efficacy of bone substitute biomaterials. J Orthop Translat. 2015 Jun 16;3(3):95-104.

[12] Madden E, Caton G. Animal models for periodontal disease.Methods in Enzymology.1994;235:106-119.

[13] Hollinger JO, Kleinschmidt JC. The critical size defect as an experimental model to test bone repair materials. J Craniofac Surg 1990;2:237–293.

[14] Cooper GM, Mooney MP, Gosain AK, Campbell PG, Losee JE, Huard J. Testing the critical size in calvarial bone defects: revisiting the concept of a critical-size defect. Plast Reconstr Surg. 2010; 125(6):1685-1692.

[15] Hollinger JO, Kleinschmidt JC. The critical size defect as an experimental model to test bone repair materials. .J Craniofac Surg. 1990;1(1):60-8.

[16] Borie E, Fuentes R, del Sol M, Oporto G, Engelke W. The influence of FDBA and autogenous bone particles on regeneration of calvaria defects in the rabbit: A pilot study. Ann Anat 2011;193:412-417.

[17] Wong R.W.K., Rabie A.B.M. Statin collagen grafts used to repair defects in the parietal bone of rabbits. Br. J. Oral Maxillofac. Surg. 2003;41:244–248.

[18] Nevins M, Camelo M, Nevins ML, Schenk RK, Lynch SE. Periodontal regeneration in humans using recombinant human platelet-derived growth factor-BB (rhPDGF-BB) and allogenic bone. J Periodontol, 2003;74:1282- 1292.

[19] Stavropoulos A, Windisch P, Szendröi-Kiss D, Peter R, Gera I, Sculean A. Clinical and histologic evaluation of granular Beta-tricalcium phosphate for the treatment of human intrabony periodontal defects: a report on five cases. J Periodontol. 2010; 81(2):325-34.

[20] Travelli L, Ravida A, Barootchi S, Chambrone L, Giannobile W. Recombinant human platelet-derived growth factor: a systematic review of clinical findings in oral regenerative procedures. JDR Clin Trans Res. 2021;6(2):161-173.

[21] Thakare K, Deo V. Randomized controlled clinical study of rhPDGF-BB+ β-TCP versus HA+ β-TCP for the treatment of infrabony periodontal defects: clinical and radiographic results. Int J Periodont Rest Dent. 2012;32:689-96.

[22] Yilmaz D, Dogan N, Ozkan A, Sencimen M, Eren Ora B, Ibrahim Mutlu I. Effect of platelet rich fibrin and beta tricalcium phosphate on bone healing. A histological study in pigs. Acta Cir. Bras. 2014; 29:59-65.

[23] Jeong J, Kim JH, Shim JH, Hwang NS, Yeong Heo C. Bioactive calcium phosphate materials and applications in bone regeneration. Biomater Res.2019; 23(4): 3-11.

[24] Khan SN, Cammisa FP, Jr., Sandhu HS, Diwan AD, Girardi FP, Lane JM. The biology of bone grafting. J Am Acad Orthop Surg. 2005;13:77–86.

[25] Leventis MD, Fairbairn P, Dontas I, Faratzis G, Valavanis KD, Khaldi L, et al. Biological response to β-tricalcium phosphate/calcium sulfate synthetic graft material: an experimental study. Implant Dent. 2014;23(1):37-43.

[26] Landen NX, Li D, Stahle M. Transition from inflammation to proliferation: a critical step during wound healing. Cell Mol Life Sci. 2016;73(20):3861–85.

[27] Lipsky PE, Brooks P, Crofford LJ, DuBois R, Graham D, Simon LS, et al. Unresolved issues in the role of cyclooxygenase-2 in normal physiologic processes and disease. Arch Intern Med. 2000,10;160(7):913-20.

[28] Zhang X, Schwarz EM, Young DA, Edward Puzas J, Rosier RN, et al. (2002) Cyclooxygenase-2 regulates mesenchymal cell differentiation into the osteoblast lineage and is critically involved in bone repair. J Clin Invest 109(11): 1405-1415.

[29] Vakili-Samiani, S., Jalil, A. T., Abdelbasset, W. K., Yumashev, A. V., Karpisheh, V., Jalali, P., ... & Jadidi-Niaragh, F. (2021). Targeting Wee1 kinase as a therapeutic approach in Hematological Malignancies. DNA Repair, 103203.

[30] NGAFWAN, N., RASYID, H., ABOOD, E. S., ABDELBASSET, W. K., Al-SHAWI, S. G., BOKOV, D., & JALIL, A. T. (2021). Study on novel fluorescent carbon nanomaterials in food analysis. Food Science and Technology.

[31] Marofi, F., Rahman, H. S., Al-Obaidi, Z. M. J., Jalil, A. T., Abdelbasset, W. K., Suksatan, W., ... & Jarahian, M. (2021). Novel CAR T therapy is a ray of hope in the treatment of seriously ill AML patients. Stem Cell Research & Therapy, 12(1), 1-23.

[32] Jalil, A. T., Shanshool , M. T. ., Dilfy, S. H. ., Saleh, M. M., & Suleiman, A. A. . (2021). HEMATOLOGICAL AND SEROLOGICAL PARAMETERS FOR DETECTION OF COVID-19. Journal of Microbiology, Biotechnology and Food Sciences, e4229. https://doi.org/10.15414/jmbfs.4229

[33] Abosaooda, M., Majid, W. J., Hussein, E. A., Jalil, A. T., Kadhim, M. M., Abdullah, M. M., ... & Almashhadani, H. A. (2021). Role of vitamin C in the protection of the gum and implants in the human body: theoretical and experimental studies. Int. J. Corros. Scale Inhib, 10(3), 1213-1229.