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REGENERATIVE POTENTIAL OF CONCENTRATED GROWTH FACTOR (CGF) MEMBRANE IN AN INDUCED BONE DEFECT MODEL IN RABBITS: A LIGHT MICROSCOPIC AND HISTOMORPHOMETRIC STUDY

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ABSTRACT

Background: There is an increasing demand for innovative biomaterials and techniques in dental practices to improve regenerative therapies. Researchers have employed guided bone regeneration (GBR) using biologically compatible membranes to repair bone defects. These membranes promote bone healing by ensuring biocompatibility and mechanical stability, and they address issues like edentulous ridges and extraction sockets. This study evaluates the effectiveness of concentrated growth factor membrane (CGF) as a regenerative material for bone defects in rabbits, using histochemical and histomorphometric analysis to assess bone matrix maturation.

Materials and methods: The study included 36 male New Zealand rabbits, each with a one wall bone defect created near the lower left first molar. The rabbits were divided into two groups: Group I (defects without CGF treatment) and Group II (defects treated with a CGF membrane). Each group was further divided into three subgroups (a, b, and c) based on time intervals of 7, 30, and 45 days. One-way ANOVA was used to analyze and compare the data.

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Results: The highest statistically significant mean value was observed in Group II, Subgroup C (48.31 ± 10.36), while the lowest values were seen in both Group I and Group II, Subgroup A, with mean values of 0.00 ± 0.00, indicating no significant difference between these subgroups.

Conclusions: CGF membrane demonstrated great potential for GBR, significantly accelerating bone formation in one-wall defect compared to controls, particularly at 45-days interval. It shows potential for clinical applications improving outcomes for patients undergoing GBR therapies.

KEYWORDS: Concentrated Growth Factor (CGF), Guided Tissue Regenerative Biomaterials, Bone Regeneration, Light Microscopy and Histomorphometry.

INTRODUCTION

Recently, there is an increasing demand for innovative biomaterials and advanced methods in guided tissue regeneration in dental practices to improve the success of treatments for patients receiving regenerative therapies. Guided bone regeneration (GBR) is one of these approaches that enhances bone regeneration by preventing connective tissue infiltration. This approach is beneficial because it supports osteopromotion, maintains biocompatibility, and provides mechanical stability while blocking non-osteogenic tissues from entering the defect area[1].

Biological membranes used in guided tissue regeneration also address many treatment modalities such as edentulous ridges, residual defects, post extractions sockets, dehiscence, and fenestrations following immediate implant placements[2-4]. Effective vascularization and the supply of osteogenic cells are crucial for new bone formation. To ensure that the membrane does not collapse in the space occupied by a blood clot, which serves as a breeding ground for osteogenic cells, the injury place maintain mechanical stability along the process of regeneration.

Expanded polytetrafluoroethylene (e-PTFE) is one of the extensively studied and commonly used membrane materials in GBR procedures. Under physiological conditions, e-PTFE does not degenerate chemically. Despite its high success rate in bone regeneration, e-PTFE membranes have the drawback of potential bacterial contamination and inflammatory reactions, which may necessitate their early removal[5-6]. Thus, platelet concentrates as concentrated growth factor (CGF) membranes, which are derived entirely from autologous blood without additives, are a promising alternative for use as barrier membranes in GBR treatments as they minimize the risk of foreign body or inflammatory reactions. Since no anticoagulants are used during blood collection, a variety of cell types are trapped in the fibrin clot, serving as a defense mechanism against invading viruses. Unlike many collagen barrier membranes from animal sources, autologous platelet concentrates do not provoke foreign-body giant cell formation. However, the resorption period for (CGF) membranes is typically short, ranging from 10 to 28 days, with a gradual deliverance of growth factors (GF) from the platelet-rich fibrin (PRF) matrix during resorption[7-8].

CGF is a favorable material for osseous regeneration, potentially enhancing its recovery and remodeling in jaw defects [9]. CGF has proven useful for bone regeneration, particularly in jaw deformities. It has been associated with (PRF) and is noted for its superior viscosity, adhesive strength, and traction resistance[10]. Previous research studies indicate that using a centrifugal force technique with varying speeds and constant temperature can stimulate platelet α-granules, resulting in autologous blood products with increased percent of GF than PRF [11]. CGF retains higher GF compared to platelet-rich proteins (PRP) and PRF and is not disband immediately after implementation, with GF being released for up to 13 days[12]. In this study, the effectiveness of CGF membranes as guided tissue membranes for treating one-wall defects has been evaluated through histochemical and histomorphometric analysis.

MATERIALS AND METHODS

Study Design: This study involved thirty six White New Zealand rabbits, the weight of each of them range from two to three kilograms. The rabbits were kept in specially designed cages within a room maintained at 24-28°C & 45-64% humidity, along 12-hour cycle of light-dark[13]. They were fed a semipurified diet and had unrestricted access to water for 10 days before the experiment began.

Sample Size Calculation: To evaluate the effects of CGF as a guided bone regenerative material between Groups I and II across three time intervals (7, 30, and 45 days), one-way ANOVA analysis was planned. Based on Cohen's recommendation (1988), a total sample size of thirty six rabbits is deemed appropriate to recognize the size effect of 0.25 with a 95% power (1-β=0.95) at a significance level of $p < 0.05$. Each group required at least 18 samples, as per calculation by the software (G*Power, version 3.1.9.3).

Grouping and Randomization: A one-wall defect was created on the left buccal bone near the lower first molar of each rabbit. The defects were either left untreated as a control group (Group I, n=18) or filled with CGF (Group II, n=18). Each group was further divided into three subgroups (a, b, and c) based on time intervals of 7, 30, and 45 days.

Surgical Procedures: Anesthesia was administered via an intramuscular injection of 10 mg/kg xylazine hydrochloride and 100 mg/kg ketamine hydrochloride. The surgical site was cleaned, shaved, and disinfected with iodine. The procedure involved a superficial incision followed by a deeper incision to expose the root surface. A gauge was used to determine the surface area of 5 mm square defect, a round bur number 4 was uses to make 4 pinholes each at the point angle of the squared defect, then the bone was removed in between the holes of an external surface area of 5x5 mm^2 leaving a resulting defect . A curette was used to remove any remaining periodontal ligaments and ensure complete removal of bone. The muscles and skin were closed with resorbable sutures. The rabbits were then returned to their cages and maintained in an incubator at 24-28°C with 45-64% humidity.

CGF Preparation: The samples obtained from rabbit's blood at the area of the ear lobes after shaving, disinfecting, and applying local anesthesia. Samples of five millimeters were obtained from the auricular artery in a sterilized test tube with no anticoagulants then prepared for centrifugation in a specialized machine (Medifuge TM; Silfradent srl, Sofia, Italy) for thirteen Min. with the following centrifugation steps: two Min. (2700 rpm) , four Mins. (2400 rpm), 4 four Mins. (2700 rpm), and three Mins. (3000 rpm). The fibrin buffy coat of the centrifuged blood was separated and pressed between two glass slides to remove the liquid components and to get the membrane rich in CGF, then the membrane between the glasses was carefully handled with a tweezer and applied to the bony defect area[16].

Specimen Collection: The timing of rabbit's euthanization planned at 7, 30, and 45 days using an overdose of ketamine (250 mg/kg) administered intravenously. The mandibles were split into two halves; the left half was cut transversely from buccal to lingual directions using a diamond disc. The specimens were then prepared for histochemical analysis.

Specimen Preparation for Histochemical Analysis: They were fixed in ten percent buffered formalin then decalcified in ten percent formal formic acid. After washing, dehydration done through increased concentrations of alcohol then inserted in blocks of paraffin. Histological slides from each group stained with Masson's Trichrome stain to differentiate between immature and newly formed bone[15], with immature bone appearing green and mature bone reddish[17].

Histomorphometric Analysis: Newly formed bone matrix in the repair zone was assessed by image analyzer system (Leica Qwin 500), which includes an IBM personal computer, a color monitor,

and a video camera connected to a microscope. The system, calibrated to convert pixel measurements into units of μm units, analyzed the percent and detected areas of bony marrow trabeculation by a 10x lens. Color detection was used to analyze five fields per specimen, excluding areas obscured by binary blue color. The percentage of mature bone matrix areas was calculated[18]. (Table 1)

Statistical Analysis: Data were summarized using median and range values. The test of Kolmogorov-Smirnov was applied to determine normality of the data and eventually their normal distribution[14] . One-way ANOVA was the test that applied for the groups comparison with a significance level set at $p \leq 0.05$. This analysis was done using the statistical Package for the Social Sciences, SPSS 18.0, Inc., Chicago, IL, USA).

Histological Results (H&E stain)

No bone formation was observed in the control group at day 7; the defect appeared filled with fibrous tissue formed of relatively parallel collagen bundles, including multiple spaces of variable size (Fig.1.A). In the CGF group on day 7, the membrane appeared intact adjacent to the tooth root, and no new bone formation was obvious. (Fig.1.B).

On day 30, There was a formation of bone trabeculae of different sizes and stainability. Also, large areas appeared without new bone formation in the control group (Fig.2.A). In the CGF group, new bone trabeculae appeared distributed through the defect. There was an osteon pattern that appeared in the internal bone (Fig.2.B).

The defect in the control group on day 45 was filled with bone but contained a small defect on the surface (Fig.3.A). There were relatively wide spaces observed within the healed bone tissue. while in the CGF group, the defect was filled with bone. The outer surface is formed of parallel lamellae with a smooth surface, the adjacent bone exhibited a spongy nature simulating that of normal alveolar bone but the marrow spaces appeared wider. (Fig. 3.B)

Histochemical Results (Masson trichrome stain):

At the 7-day interval, no bony regeneration was detected in any of the studied groups. Only a relatively loose fibrous layer was present in control group. Conversely, the CGF group showed a thick, dense membrane covering the defect, with no signs of breakdown, as interpreted in Figures; 4A and 4B.

At the 30-day interval, the control group displayed relatively thin, newly formed bone trabeculae composed of immature collagen, which stained green (Figure 5A). In contrast, the CGF group demonstrated thicker bone trabeculae with scattered red areas indicating maturation of the bone matrix (Figure 5B).

By the 45-day interval, both groups exhibited increased measurements of new bone formation, showing red-colored staining presenting mature osseous matrix mixed with green immature bone matrix. However, the CGF group had a more extensive area of mature bone matrix covering larger regions (Figures 6A and 6B).

C. Statistical Results:

The Statistical Results confirmed the significance in differences between different groups at two time intervals, favoring CGF groups. The percentage of mature bone matrix recorded the highest mean in Group II (CGF), subgroup C at 45 days, with a mean value of 58.31 ± 6.76, that was higher than the control group, Group I, subgroup C at 45 days, with a significant difference with a mean value of 41.11 ± 9.90. This was followed by subgroup B at 30 days, where Group II had a mean value of 26.63 ± 6.33 and Group I had a mean value of 13.74 ± 3.21, exhibiting a significant advantage for the CGF group.

Lowest measurements were detected in both Group (I) and (II) at the 7-day mark (subgroup A), with mean values of 0.00 ± 0.00, indicating no significant difference between them (Table 1).

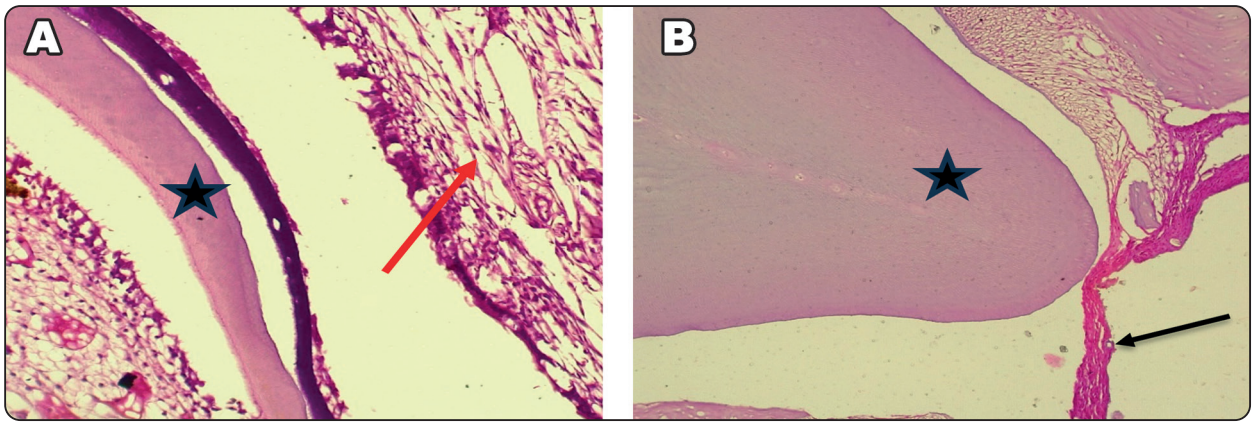


Fig. (1) Photomicrograph showing the defect area of the control group (A) and CGF group (B) at 7 days demonstrating the defect in group (A) appeared filled with fibrous tissue formed of relatively parallel collagen bundles (red arrow). The tooth root is indicated by (black stars), and the CGF membrane is indicated by (a black arrow). H&E, X100..

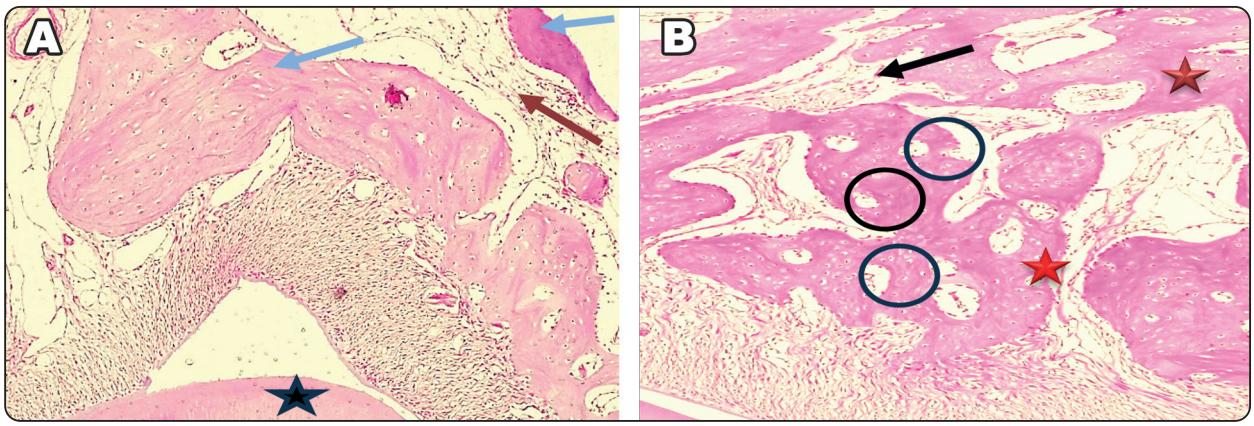
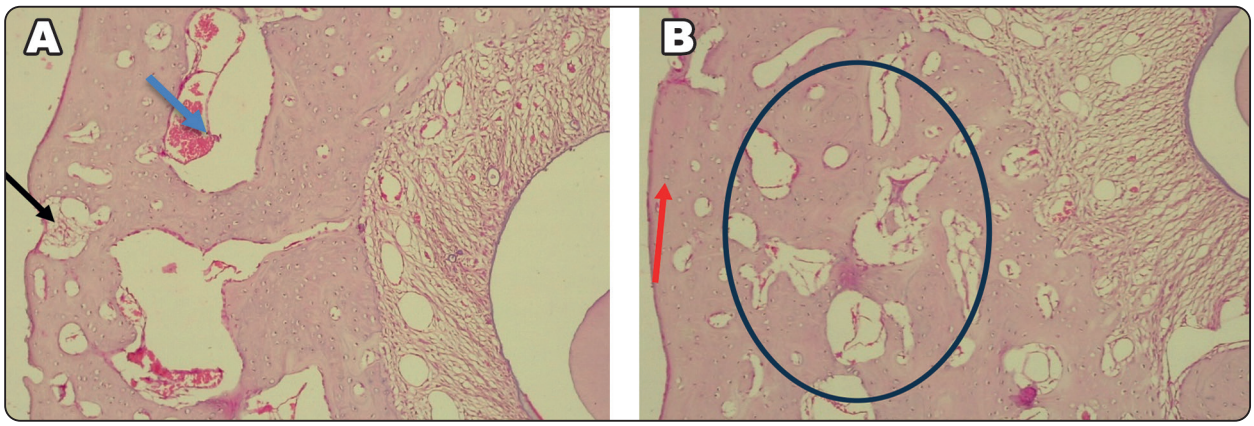


Fig. (2) Photomicrograph showing the defect area of the control group (A) and CGF group (B) at 30 days displaying in the control group tooth root (black stars), formation of bone trabeculae of different sizes and stainability (blue arrow), and loose connective tissue (brown arrows). In the CGF group, new bone trabeculae appeared distributed through the defect (red stars), bone marrow (black arrow), osteon pattern that appeared in the internal bone (black circles). H&E, X100.



Fig, (3) Photomicrograph displaying the defect area of the control group (A) and CGF group (B) at 45 days showing defect area (black arrow), wide bone marrow space (blue arrow). The CGF group (B) showing spongy bone (circle) and lamellated bone (red arrow). H&E, X100

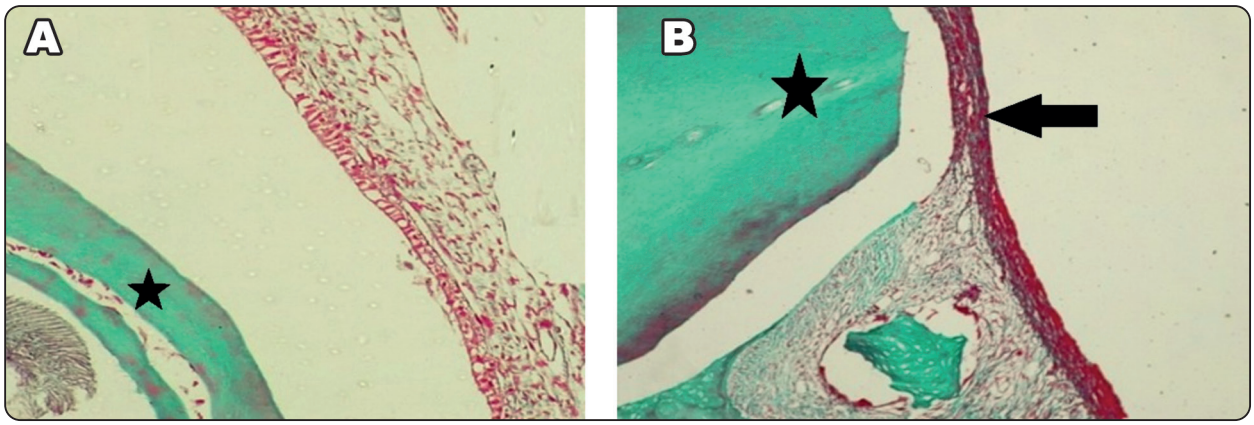


Fig. (4) Photomicrograph showing the defect area of the control group (A) and CGF group (B) at 7 days demonstrating no bone formation in the defect area. The tooth root is indicated by black stars, and the CGF membrane is indicated by a black arrow. Masson trichrome, X100

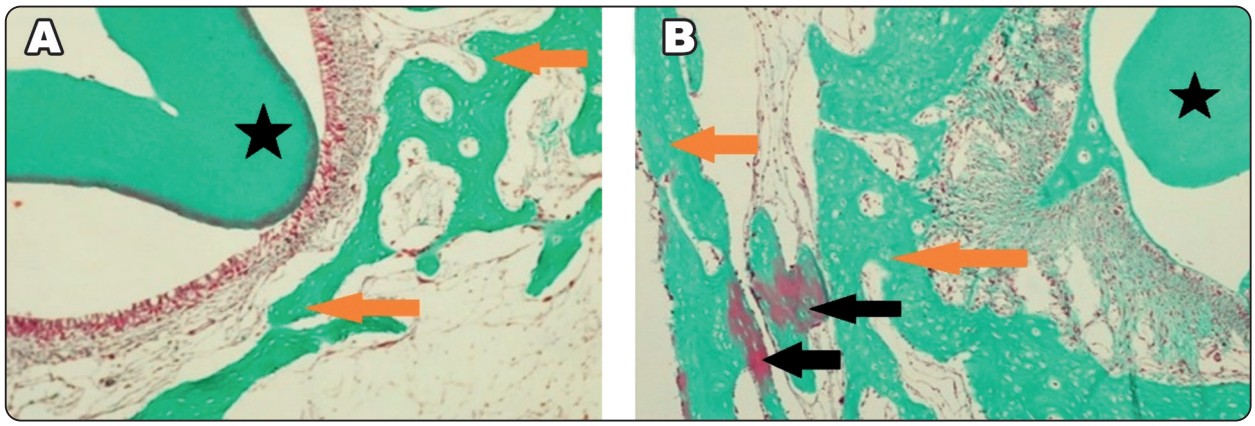


Fig. (5) Photomicrograph showing the defect area of the control group (A) and CGF group (B) at 30 days displaying tooth root (black stars), immature collagen in newly formed bone trabeculae (orange arrows), and islands of red maturing bone matrix in the CGF group (black arrows). Masson trichrome, X100.

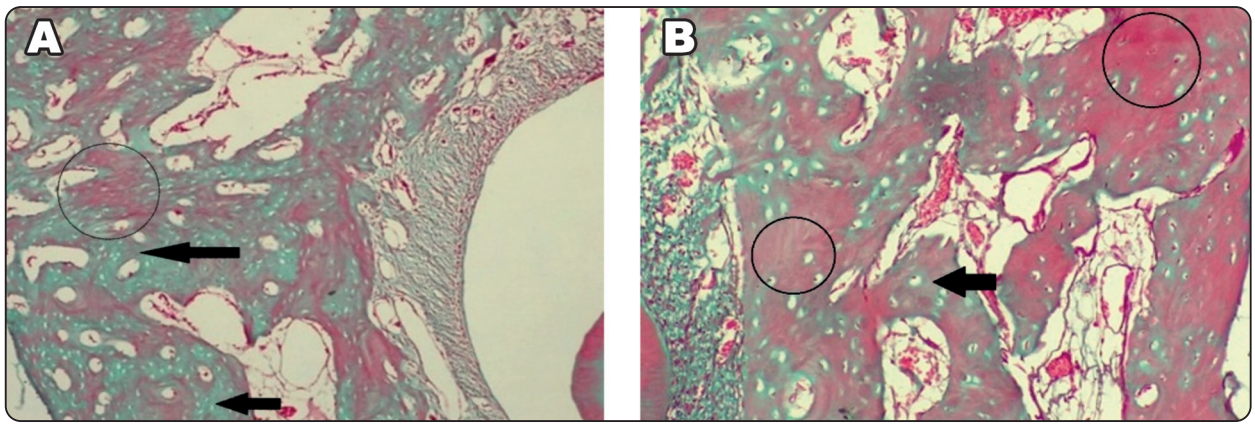


Fig. (6) Photomicrograph displaying the defect area of the control group (A) and CGF group (B) at 45 days showing immature newly formed bone trabeculae (black arrows) expressing red mature bone matrix interspersed with green immature bone matrix (staining green). The CGF group (B) showing islands of red maturing bone with a more pronounced area of mature bone matrix occupying larger regions (black circles). Masson trichrome, X100

Table (1): Showing the mean ± SD values, and post hoc tests for the comparison between different groups regarding Area% for mature bone matrix.

Area% for mature bone matrix	Group I (Control group)			Group II (CGF group)			p- value
Sub group	**Mean**	**±SD**	**Std. Error**	**Mean**	**±SD**	**Std. Error**	
(A)At day 7	0.00^{E}	0.00	0.00	0.00^{E}	0.00	0.00	1.000(NS)
(B)At day 30	13.74^{D}	3.21	2.33	26.63^{C}	6.33	2.83	0.008 (S)
(C)At day 45	41.11^{B}	9.90	4.43	58.71^{a}	6.76	3.023	0.013 (S)

Tukey's post hoc: Means sharing the same superscript letter are not significantly different

NS: Non significant; S: Significant

DISCUSSION

GBR is a technique that apply a biological membrane barrier to cover bony defects, preventing the invasion of soft tissues and facilitating effective bone healing. For optimal healing of bone defects, it is essential to achieve successful wound closure and ensure a proper blood supply [19].

CGF, a new regenerative material derived from platelets, has been identified by Migauskaitė et al. (2022) as a viable option for bone regeneration [20]. Regenerating one-wall intra-bony defects presents a significant challenge. This study utilized CGF as a treatment for these defects due to its promising efficacy.

Rabbits were selected for this study due to their skeletal similarity to humans and their manageable size for surgical procedures. A critical-sized bone defect was created to replicate a significant injury.

This model, which involves flap surgery and the removal of alveolar bone with a dental bur, is commonly used for studying bone reconstruction and regeneration due to its relevance to human onewall defects [21].

The CGF membrane made from the blood of the white rabbits was applicated to the bony defects to enhance healing process as it is rich in growth factors. Unlike other platelet concentrates used in tissue engineering, CGF can be utilized both as a serum and a membrane biomaterial, using donor derived platelet concentrates as a membrane for sustained growth factor release aids in activating and differentiating stem cells [22].

The application of the CGF membrane led to improved bone regeneration, with a better quality ,density and mineral contents of new bone at 45 days in comparison to controls. The prominent osteogenic effect of CGF is due to the thick fibrinogen fibers in the fibrin structure, which allows the gradual delivery of GF and stimulates osteogenesis by encouraging the migration of bone marrow-derived mesenchymal stem cells (MSC) and periodontal blood vessels, leading to osteoblast differentiation and maturation [23].

One week after inducing the Bone Defect in experimental animal model : Neither group showed signs of bone healing. Granulation tissue filled the defect in the control group, while a thick fibrin band was seen in the defect treated with CGF. There were no statistically significant differences between the groups under study in the area of mature bone matrix. At this early stage, these differences were

not statistically significant, despite the fact that the CGF group displayed bigger patches of red staining, indicating faster bone matrix development. GF release typically begins around 14 days, following fibrin degradation and GF production by the cells of CGF. At this early point, the defect site remained clearly visible with minimal bridging or new bone formation, mainly occupied by granulation tissue with early repair signs. There were no indications of disintegration in the largely complete graft of the CGF-treated defect, which was composed of a fibrous layer and a thick, dense membrane. Green patches seen by Masson's Trichrome staining were indicative of immature collagen, early collagen deposition, and tissue healing. According to these results, the CGF membrane is beginning to start the healing process at 7 days, causing granulation tissue production and early inflammation, but substantial bone formation has not yet seen.

The control group displayed a thin, delicate rim of osseous trabeculae with immature collagen at one month interval while the CGF group, on the other hand, had thicker bone trabeculae with fewer red spots strewn about, indicating more mature bone matrix. A less ordered structure and a greenish tint in Masson's Trichrome staining, which represents juvenile collagen, are characteristics of immature bone trabeculae. Early indications of bone production were seen, despite the fact that the bone matrix was not yet completely developed. Partial disintegration of the CGF membrane indicated that their GF was absorbed and used for bone repair. Increased bone production and early remodeling were seen in the CGF-treated areas, and some woven bone began to change into a more ordered lamellar structure.

Although it was not as noticeable as it was at the 45-day interval, histomorphometric examination at one month's interval showed an increase in bone volume when compared to baseline groups.

After 45 days, both groups demonstrated enhanced bone development, however the area of mature bone matrix was larger in the CGF group. Higher bone maturation and remodeling were indicated by the CGF group's notable areas of well-formed, reddish mature bone matrix. The CGF group's bone trabeculae were thicker and more extensive, indicating the development of adult bone structures from immature ones. Increased mineralization, which indicates continued bone maturation and deposition, replaced the original woven bone with more ordered lamellar bone as evidence of bone remodeling. The CGF membrane's function in preserving space was validated by its partial breakdown and gradual release of growth factors.

CONCLUSIONS

1. The current study aimed to assess the effectiveness of CGF membranes in enhancing bone regeneration. By combining light microscopy with histochemical and histomorphometric analyses, researchers evaluated both qualitative aspects of bone healing and quantitatively measured improvements in bone formation and quality.
2. The CGF membrane proved to be a more effective guided bone regenerative material, showing greater acceleration and maturation of bone in the one-wall defect model. It enhanced cellular activity and tissue repair compared to the control group, particularly at longer time intervals (45 days).
3. This research provides valuable insights into the clinical application of CGF membranes, bridging research findings with clinical practice and potentially leading to better outcomes for patients undergoing bone regeneration therapies.

Implications and Clinical Relevance:

1. CGF membranes significantly improved bone regeneration, with enhanced maturation of the bone matrix and increased bone volume compared to the control group.

2. Over time, defects treated with CGF showed superior bone formation and structural integrity, indicating effective bone healing and remodeling. The presence of mature bone matrix and increased mineralization suggests successful bone healing.
3. CGF membranes have potential as a promising biomaterial for guided bone regeneration, possibly translating to better clinical outcomes in human bone defect treatments.
4. This article provides foundational information on the use of bio-regenerative materials, valuable for dental professionals—including surgeons, implantologists, periodontists, and prosthodontists—by enhancing bone healing and regeneration in routine dental practice.

Recommendation:

Further clinical trials are recommended to investigate the effects of CGF application on bone defects in terms of bone quality, density, and mineralization in areas treated with CGF membranes.

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Data Availability: The row data of this research could be obtained from the authors upon request

Declaration: The authors disclose no potential conflicts of interest to declare.

Ethical Approval: The methodology approved by the Research Ethics Committee (REC) of the Faculty of Dental Medicine for Girls, Al-Azhar University and the study adhered to its guidelines, licensing demands, and their regulations. The research ethics committee (REC) approval reference number is REC-PD-23-20.

Conflicts of Interest: The authors have no conflicts of interest to disclose in relation to this study.

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