

Synergistic Integration of Collagen-Gel Bioactive Glass (Col-gel-BG) Scaffolds with Autologous Platelet-Rich Fibrin (PRF) for Enhanced Osteoconduction and Accelerated Bone Tissue Regeneration.

Prateek Veerendrakumar Siddhapur^{1*}, Dr. Dinesh kumar²

¹B.D.S-Undergraduate Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, India.

²Assistant professor, Department of Pedodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical sciences (SIMATS), Chennai – 600077 India

Abstract

Background: The regeneration of critical-sized bone defects remains a significant clinical challenge. While bioactive glass and collagen scaffolds offer osteoconductive properties, they often lack the potent biological cues required for accelerated healing. This study investigates the synergistic effect of integrating Collagen-Gel Bioactive Glass (Col-gel-BG) scaffolds with autologous Platelet-Rich Fibrin (PRF).

Methods: Col-gel-BG scaffolds (70:30 ratio) were synthesized via lyophilization. Thirty New Zealand White rabbits were divided into three groups (n=10): Control (empty defect), Scaffold Only (Col-gel-BG), and Composite (Col-gel-BG + PRF). A 5 mm segmental radial defect was created in each animal. Bone regeneration was evaluated at 4, 8, and 12 weeks using radiography, Micro-Computed Tomography (Micro-CT), and histomorphometry.

Results: At 12 weeks, the Composite group exhibited significantly higher Bone Volume/Total Volume ($62.18\% \pm 5.82\%$) compared to the Scaffold Only ($38.62\% \pm 4.55\%$) and Control ($12.45\% \pm 2.12\%$) groups ($p < 0.05$). Radiographic scoring indicated significantly earlier mineralization in the Composite group as early as 4 weeks. Histological analysis revealed mature lamellar bone formation and accelerated scaffold turnover in the presence of PRF, with significantly less residual material (7.12%) compared to the scaffold-only group (18.44%).

Conclusion: The integration of PRF into Col-gel-BG scaffolds creates a bioactive microenvironment that transitions the healing process from passive osteoconduction to accelerated osteogenesis. This autologous composite approach represents a promising advancement in bone tissue engineering for orthopedic and maxillofacial applications.

Keywords: Bioactive Glass; Collagen; Platelet-Rich Fibrin; Bone Tissue Engineering; Synergistic Regeneration.

Introduction

The reconstruction of critical-sized bone defects resulting from trauma, tumor resection, or congenital anomalies remains a formidable challenge in regenerative medicine [1]. Critical-sized defects are defined as those that do not heal spontaneously within the lifetime of the organism, requiring some form of intervention to achieve bony union. Such defects present significant clinical management difficulties due to the complex interplay of mechanical, biological, and spatial factors that must be addressed to achieve successful osseous regeneration. The consequences of inadequate bone reconstruction include persistent non-union, functional impairment, aesthetic deformity, and reduced quality of life for affected patients.

While autologous bone grafting continues to be regarded as the gold standard for bone reconstruction due to its inherent osteogenic, osteoinductive, and osteoconductive properties, its clinical utility is frequently hampered by significant limitations [2, 3]. The osteogenic capacity of autografts arises from the presence of viable osteoprogenitor cells within the graft, while osteoinductive factors such as bone morphogenetic proteins drive the differentiation of host mesenchymal stem cells along the osteoblastic lineage. The osteoconductive scaffold provided by the graft matrix supports the ingrowth of new blood vessels and bone-forming cells. Despite these advantages, autograft harvesting requires a second surgical site, which increases operative time, prolongs recovery, and is associated with donor-site morbidity including pain, infection, hematoma, and sensory disturbances. The quantity of available autograft is also limited by the donor site anatomy, making it unsuitable for large-volume defects. Consequently, there is an urgent need for synthetic bone graft substitutes that can effectively mimic the complex hierarchical structure and biological functionality of the natural bone extracellular matrix [4].

Among the various biomaterials explored for bone regeneration, bioactive glass has garnered significant attention for its ability to form a chemically bonded interface with host bone through the development of a surface hydroxyapatite layer [5]. Bioactive glass is a silica-based material that, upon exposure to physiological fluids, undergoes a series of surface reactions culminating in the precipitation of a carbonated hydroxyapatite layer that closely resembles the mineral phase of bone. This surface layer provides binding sites for extracellular matrix proteins and facilitates direct bonding between the material and surrounding bone tissue. However, the inherent brittleness and low fracture toughness of monolithic

bioactive glass limit its application in load-bearing sites where mechanical demands are substantial. To address these mechanical shortcomings and improve biological performance, the integration of bioactive glass into organic polymers, such as collagen, has been proposed [6]. Collagen, the most abundant protein in the bone extracellular matrix, provides a natural scaffold for cell attachment and proliferation, with its RGD integrin-binding motifs supporting cell adhesion and migration. The bioactive glass particles offer structural reinforcement to the collagen matrix while simultaneously releasing ions—such as calcium, silicon, and phosphate—that stimulate osteoblast differentiation and promote extracellular matrix mineralization [7, 8]. This composite approach combines the biological advantages of a native extracellular matrix component with the osteoinductive ion release properties of a synthetic ceramic.

Despite the osteoconductive potential of collagen-bioactive glass composites, achieving accelerated bone regeneration often requires the inclusion of bioactive signaling molecules that can actively direct cell behavior [9]. Platelet-rich fibrin, a second-generation platelet concentrate, has emerged as a potent autologous source of growth factors, including transforming growth factor-beta, platelet-derived growth factor, and vascular endothelial growth factor [10, 11]. Unlike earlier platelet concentrates that relied on anticoagulants and required the addition of bovine thrombin for activation, platelet-rich fibrin is prepared through a simple, cost-effective centrifugation process without any chemical additives. This results in a dense fibrin matrix that captures a high concentration of platelets and leukocytes within a natural, slowly degrading fibrin scaffold. The three-dimensional fibrin network allows for the sustained release of growth factors over an extended period of up to several weeks, thereby enhancing angiogenesis through VEGF stimulation, promoting mesenchymal stem cell recruitment and proliferation through PDGF, and driving osteogenic differentiation through TGF- β [12, 13]. The autologous nature of platelet-rich fibrin eliminates concerns regarding immunogenicity or disease transmission, making it an attractive biological adjunct for bone regeneration.

While the individual benefits of collagen-bioactive glass scaffolds and platelet-rich fibrin are well-documented in the literature, the potential synergistic effects of their combined application remain insufficiently explored in the context of accelerated bone tissue engineering [14, 15]. It is hypothesized that the collagen-bioactive glass scaffold can provide a stable three-dimensional architecture for the platelet-rich fibrin network to infiltrate and integrate, while the platelet-rich fibrin enriches the scaffold with essential biological cues to bridge the gap between simple osteoconduction and active osteoinduction [16, 17]. The combination may address key limitations of each component used in isolation: the scaffold provides structural support and osteoconductive guidance, while the platelet-rich fibrin supplies the soluble signals necessary to recruit host progenitor cells and direct their differentiation toward the osteogenic lineage. The fibrin matrix of platelet-rich fibrin may also serve to retain the bioactive glass particles within the defect site, preventing their dispersion and maintaining a consistent ion release profile. Conversely, the scaffold may provide a stabilizing framework for the fibrin network, preventing its premature collapse and extending its functional lifespan.

The present study aims to evaluate the efficacy of a novel synergistic integration of collagen-bioactive glass scaffolds with autologous platelet-rich fibrin. Using a standardized animal model, we characterize the morphological properties of the composite scaffold and assess its capacity for enhanced osteoconduction and accelerated bone tissue regeneration compared to scaffold-only and empty control groups. The study seeks to quantify the degree of new bone formation, assess the quality of regenerated tissue, and evaluate the temporal progression of healing in response to the combined treatment. By establishing a robust biological basis for this combined therapeutic approach, this research aims to provide evidence to support the translation of this strategy into clinical maxillofacial and orthopedic applications, where the need for effective, reliable, and biologically informed bone reconstruction strategies remains pressing [18, 19]. The findings of this investigation may ultimately contribute to the development of off-the-shelf, biologically active bone graft substitutes that combine the structural benefits of synthetic scaffolds with the biological potency of autologous growth factor delivery systems.

Materials and Methods

Scaffold Preparation and Characterization

The Collagen-Gel Bioactive Glass scaffolds were synthesized using a lyophilization technique, a method that allows for the creation of highly porous structures that mimic the natural extracellular matrix architecture of bone. High-purity Type I bovine collagen was dissolved in 0.05 M acetic acid to achieve a 1% concentration by weight. Acetic acid was selected as the solvent because it facilitates the dissolution of collagen fibers while preserving their native triple-helical structure, which is essential for maintaining biological activity.

Bioactive glass with the 45S5 composition, consisting of 45% silicon dioxide, 24.5% calcium oxide, 24.5% sodium oxide, and 6% phosphorus pentoxide, was selected for this study. This specific composition is well-established in the literature for its osteoconductive properties and ability to form a stable hydroxyapatite layer upon exposure to physiological fluids. The bioactive glass particles were sieved to achieve a particle size range of 40 to 60 micrometers, a dimension selected to balance surface area availability for ion release with the mechanical integrity of the composite. The bioactive glass

particles were added to the collagen slurry at a weight ratio of 70 parts bioactive glass to 30 parts collagen, a ratio chosen to provide adequate structural reinforcement while maintaining sufficient collagen content for cell adhesion.

The mixture was homogenized at 12,000 revolutions per minute for 15 minutes to achieve uniform dispersion of the bioactive glass particles within the collagen matrix. The homogenized mixture was poured into cylindrical molds measuring 8 millimeters in diameter and 5 millimeters in height, dimensions selected to match the critical-sized defect created in the animal model. The molds were frozen at -80°C to induce ice crystal formation, which serves as a porogen to create the interconnected pore network essential for cell infiltration and vascularization.

Following freezing, the samples underwent primary drying at -55°C for 24 hours under vacuum to remove ice crystals through sublimation, leaving behind a porous scaffold structure. Secondary drying was performed at 20°C for 6 hours under vacuum to remove any residual bound water that may have remained after primary drying. The resulting porous scaffolds were cross-linked using 0.1% glutaraldehyde to improve mechanical stability and resistance to enzymatic degradation. Following cross-linking, the scaffolds were extensively washed with deionized water to remove any residual cross-linking agents that could be cytotoxic to cells.

PRF Preparation and Integration

Autologous Platelet-Rich Fibrin was prepared according to the protocol established by Choukroun, which has become the standard method for producing this autologous growth factor concentrate. Briefly, 10 milliliters of whole blood was collected from each animal via marginal ear vein puncture into sterile 10 milliliter glass-coated plastic tubes without anticoagulants. The use of glass-coated tubes is critical to the process, as the glass surface initiates the coagulation cascade without the addition of exogenous activators.

The tubes were immediately centrifuged at 3000 revolutions per minute, equivalent to approximately $700 \times g$, for 12 minutes. This centrifugation speed and duration were selected based on established protocols to achieve optimal separation of blood components while preserving platelet integrity and viability. The centrifugation process resulted in three distinct layers: a base layer of red blood cells, a middle layer consisting of the PRF clot, and a top layer of acellular plasma.

The PRF clot was carefully harvested from the middle layer, separated from the red blood cell base, and compressed using a sterile PRF box to form a thin, high-density membrane. The compression process expels excess serum while preserving the fibrin matrix structure and entrapped growth factors, resulting in a mechanically stable membrane suitable for surgical handling. Integration of the PRF with the Col-gel-BG scaffold was achieved through a dual approach: first, the Col-gel-BG scaffolds were infiltrated with the expressed PRF exudate to impregnate the scaffold pores with soluble growth factors; second, the compressed PRF membrane was wrapped around the scaffold to provide an external source of growth factors and to retain the scaffold within the defect site.

Experimental Animal Model

Thirty adult male New Zealand White rabbits weighing between 3.0 and 3.5 kilograms were utilized for this study. The rabbit radius model was selected because it provides a well-characterized, critical-sized segmental defect that does not heal spontaneously, allowing for reliable assessment of bone regeneration capacity. The use of male animals of similar weight minimized variability due to sex and size differences.

The animals were housed in a temperature-controlled environment maintained at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with a 12-hour light and dark cycle. They were provided with a standard pellet diet and water ad libitum throughout the study period. All surgical procedures were performed under general anesthesia induced by intramuscular injection of ketamine at 35 milligrams per kilogram and xylazine at 5 milligrams per kilogram. This anesthetic combination provides adequate surgical anesthesia with minimal cardiovascular depression.

A 10 millimeter longitudinal incision was made over the mid-diaphysis of the radius, and the underlying soft tissues were carefully dissected to expose the bone. A 5 millimeter critical-sized segmental defect was created using a trephine burr under constant saline irrigation to prevent thermal necrosis of the bone margins. This defect size has been validated in previous studies as being critical, meaning that it does not heal spontaneously without intervention, allowing for clear differentiation between treatment effects.

Study Groups and Surgical Procedure

The animals were randomly assigned to three experimental groups, with 10 animals per group to provide adequate statistical power for detecting differences in bone regeneration outcomes. Group A served as the negative control, with the defect left empty to establish baseline healing capacity. Group B received the Col-gel-BG scaffold only, allowing assessment of the osteoconductive potential of the scaffold alone. Group C received the Col-gel-BG scaffold integrated with autologous PRF, representing the experimental composite group.

Following implantation of the respective materials, the periosteum and muscle layers were sutured with 4-0 Vicryl absorbable sutures to maintain soft tissue integrity and to retain the implant within the defect site. The skin was closed

with 3-0 silk non-absorbable sutures. Postoperative analgesia was administered using buprenorphine at 0.05 milligrams per kilogram for three days to ensure animal comfort during the early healing period.

Radiographic and Micro-Computed Tomography Analysis

Standardized lateral radiographs were taken at 4, 8, and 12 weeks post-surgery to monitor callus formation and bone healing progression over time. Radiographs were performed under sedation using standardized exposure parameters to allow for consistent comparison between time points and groups.

At 12 weeks post-surgery, the animals were euthanized via anesthetic overdose, and the radii were harvested en bloc. The specimens were fixed in 10% buffered formalin for 48 hours to preserve tissue architecture and to inactivate any residual biological activity. The fixed specimens were scanned using a Micro-CT system at a resolution of 18 micrometers to provide high-resolution three-dimensional reconstruction of the defect site.

Quantitative parameters were calculated within the defect region of interest. Bone Volume relative to Total Volume, expressed as a percentage, was calculated to assess the extent of new bone formation within the defect. Trabecular Thickness was measured to evaluate the structural quality of the newly formed bone. Bone Mineral Density was calculated to assess the mineralization status of the regenerated tissue.

Histomorphometric Analysis

Following Micro-CT scanning, the samples were decalcified in 10% ethylenediaminetetraacetic acid for 4 weeks to soften the mineralized tissue sufficiently for sectioning. The decalcification solution was changed every 3 days to maintain chelating activity. The decalcified specimens were dehydrated in graded ethanol solutions and embedded in paraffin wax. Sections of 5 micrometers thickness were cut along the longitudinal axis of the radius to provide consistent orientation for histomorphometric analysis. The sections were stained with Hematoxylin and Eosin for general histological evaluation and with Masson Trichrome to specifically visualize collagen deposition and distinguish between mature bone and osteoid tissue.

Histomorphometric evaluation was performed by two blinded observers who were unaware of the group assignments to prevent observer bias. The percentage of newly formed bone and the percentage of residual scaffold material within the defect area were quantified using ImageJ software. Five representative fields per section were analyzed, and the average values were calculated for each specimen.

Statistical Analysis

All quantitative data were expressed as Mean \pm Standard Deviation. Statistical significance was determined using one-way Analysis of Variance followed by Tukey post-hoc test for multiple comparisons. This approach was selected to control for Type I error when comparing three groups while maintaining statistical power. A p-value of less than 0.05 was considered statistically significant for all comparisons. All statistical analyses were performed using dedicated statistical software with the group assignments coded to maintain blinding until after the completion of the analysis.

Results

General Observations

All thirty animals survived the surgical procedure and the 12-week experimental period without signs of systemic infection, wound dehiscence, or significant weight loss, indicating that the surgical interventions and implant materials were well-tolerated. Throughout the study period, all animals maintained normal activity levels and feeding behavior. At the time of harvest, macroscopic examination of the surgical sites revealed distinct differences among the three experimental groups.

In Group C, which received the Collagen-Gel Bioactive Glass scaffold integrated with autologous Platelet-Rich Fibrin, the defect sites demonstrated complete bridging with firm, bone-like tissue that was contiguous with the native bone margins. The tissue consistency was hard and resistant to manual pressure, suggesting the formation of mineralized bone. The surface of the healed defect was smooth and well-integrated with the surrounding cortical bone.

In Group B, which received the Collagen-Gel Bioactive Glass scaffold only, the defect sites exhibited partial bridging with some visible residual scaffold material still present within the defect. The tissue consistency was firm but less dense than that observed in Group C, and the interface between the new tissue and native bone was less well-defined. Some specimens showed gaps between the regenerated tissue and the host bone margins.

In Group A, the empty control group, the defect sites showed persistent defects filled with fibrous connective tissue that was soft and pliable. There was minimal bone formation evident at the host-stump margins, and no bridging of the defect was observed in any specimen. The tissue within the defect was easily distinguished from the adjacent cortical bone by its texture and color.

Micro-Computed Tomography Analysis

Quantitative Micro-Computed Tomography analysis at 12 weeks post-implantation revealed significant differences in bone regeneration parameters among the three experimental groups. Group C demonstrated a dense, trabecular network extending throughout the original defect site, with well-mineralized bone bridging the defect margins. Group B showed moderate bone formation with a less organized trabecular architecture, while Group A exhibited minimal bone formation confined primarily to the bone margins.

Table 1: Quantitative Micro-CT Parameters at 12 Weeks Post-Implantation

Group	BV/TV (%)	Tb.Th (mm)	BMD (g/cm ³)
Group A (Control)	12.45 ± 2.12	0.042 ± 0.008	0.215 ± 0.034
Group B (Scaffold Only)	38.62 ± 4.55	0.088 ± 0.012	0.482 ± 0.051
Group C (Composite)	62.18 ± 5.82	0.134 ± 0.015	0.744 ± 0.068

Statistical significance ($p < 0.05$) was observed between Group C and all other groups for all parameters. The Bone Volume to Total Volume ratio, representing the percentage of the defect volume occupied by mineralized tissue, was highest in Group C at 62.18%, compared to 38.62% in Group B and 12.45% in Group A. This difference was statistically significant between Group C and both other groups, with p -values less than 0.05 for all comparisons. The Trabecular Thickness followed a similar pattern, with Group C showing the greatest trabecular thickness at 0.134 millimeters, compared to 0.088 millimeters in Group B and 0.042 millimeters in Group A. Bone Mineral Density was also highest in Group C at 0.744 grams per cubic centimeter, approximately 1.5 times greater than Group B and more than 3 times greater than Group A. These quantitative parameters collectively indicate that the composite scaffold integrated with Platelet-Rich Fibrin resulted in superior bone regeneration in terms of both quantity and quality of new bone formation.

Radiographic Progression

Serial radiographs taken at 4, 8, and 12 weeks post-surgery illustrated the temporal progression of bone healing across the three groups. The modified Lane and Sandhu scoring system was employed to provide a semi-quantitative assessment of healing, with higher scores indicating more advanced bone regeneration.

Table 2: Radiographic Bone Healing Scores (Modified Lane and Sandhu Scoring)

Group	4 Weeks	8 Weeks	12 Weeks
Group A (Control)	0.4 ± 0.2	1.1 ± 0.3	1.8 ± 0.5
Group B (Scaffold Only)	1.2 ± 0.4	4.5 ± 0.7	6.2 ± 0.9
Group C (Composite)	2.8 ± 0.5	6.8 ± 0.9	10.4 ± 1.2

Note: Maximum score is 12. Group C scores were significantly higher at all time points ($p < 0.01$).

At 4 weeks, Group C already showed cloudy radiopaque areas within the defect, suggesting early mineralization and callus formation. The radiographic score for Group C at this time point was 2.8, significantly higher than both Group B at 1.2 and Group A at 0.4. At 8 weeks, the radiopacity in Group C had increased substantially, with scores reaching 6.8 compared to 4.5 in Group B and 1.1 in Group A. By 12 weeks, the radiopacity in Group C was nearly indistinguishable from the adjacent cortical bone, achieving a score of 10.4 out of a maximum of 12, indicating near-complete radiographic union. In contrast, Group B showed moderate callus formation with a score of 6.2, while Group A showed minimal healing with a score of 1.8. The differences between Group C and both other groups were statistically significant at all time points, with p -values less than 0.01.

Histomorphometric Evaluation

Histological sections stained with Hematoxylin and Eosin and Masson Trichrome confirmed the Micro-Computed Tomography findings and provided detailed information about the cellular and tissue-level events occurring within the defect sites.

In Group C, the Col-gel-BG scaffold was largely degraded and replaced by mature lamellar bone containing osteocytes within lacunae. The newly formed bone exhibited organized collagen fibers oriented along the lines of mechanical stress, as visualized by Masson Trichrome staining. Active osteoblasts were observed lining the trabecular surfaces, and there was evidence of ongoing remodeling with osteoclasts present in Howship lacunae. Vascular channels were abundant throughout the regenerated tissue, indicating good revascularization of the defect site. The residual scaffold material was minimal, comprising only 7.12% of the defect area.

In Group B, active osteoconduction was observed with new bone forming along the scaffold struts. However, significant amounts of residual bioactive glass remained within the defect, accounting for 18.44% of the defect area. The newly formed bone was less organized than in Group C, with a mixture of woven and lamellar bone. The interface between the scaffold material and new bone appeared intact, suggesting good integration, but the extent of bone formation was less extensive. Inflammatory cells were minimal, indicating good biocompatibility of the scaffold material.

In Group A, the defect was primarily occupied by dense irregular connective tissue consistent with fibrosis. Limited bone formation was observed only at the host bone margins, with no bridging across the defect. There was no evidence of scaffold material as none was implanted.

Table 3: Histomorphometric Quantification of New Bone Formation (NFB) and Residual Material

Group	New Bone Formation (NFB%)	Residual Scaffold (%)
Group A (Control)	8.34 ± 2.65	0.00 ± 0.00
Group B (Scaffold Only)	32.15 ± 4.22	18.44 ± 3.12
Group C (Composite)	58.76 ± 5.34	7.12 ± 1.88

The NFB% in Group C was significantly higher than Group B ($p < 0.001$), while residual material was significantly lower in Group C, indicating accelerated scaffold turnover.

The percentage of new bone formation within the defect area was highest in Group C at 58.76%, significantly higher than Group B at 32.15% and Group A at 8.34%. The difference between Group C and Group B was highly significant, with a p-value less than 0.001, indicating that the addition of Platelet-Rich Fibrin to the scaffold resulted in a nearly twofold increase in new bone formation. The residual scaffold material was significantly lower in Group C at 7.12% compared to 18.44% in Group B, indicating accelerated scaffold degradation and turnover in the presence of Platelet-Rich Fibrin. This finding suggests that the biological factors released from the Platelet-Rich Fibrin not only enhanced new bone formation but also facilitated the resorption and replacement of the scaffold material with host tissue. The histomorphometric data corroborate the radiographic and Micro-Computed Tomography findings, providing consistent evidence that the integration of autologous Platelet-Rich Fibrin with Col-gel-BG scaffolds significantly enhances bone regeneration in critical-sized defects compared to scaffold alone.

Discussion

The results of this study confirm that the synergistic integration of autologous Platelet-Rich Fibrin with Collagen-Gel Bioactive Glass scaffolds significantly enhances osteoconduction and accelerates the rate of bone regeneration in critical-sized segmental defects. While the Col-gel-BG scaffold alone provided a suitable osteoconductive template for bone growth, the addition of Platelet-Rich Fibrin acted as a biological catalyst, shifting the healing process from passive osteoconduction, where new bone grows along the scaffold surface, to active, accelerated osteogenesis driven by the sustained release of growth factors and the recruitment of host progenitor cells [1, 5]. The findings from this study provide strong evidence that the combination of a synthetic osteoconductive scaffold with an autologous biological growth factor delivery system yields regenerative outcomes that are superior to either approach used in isolation.

The observed increase in Bone Volume to Total Volume ratio and Bone Mineral Density in the composite group can be attributed to the sustained release of growth factors inherent to the Platelet-Rich Fibrin matrix. Platelet-Rich Fibrin acts as a natural reservoir for transforming growth factor-beta, platelet-derived growth factor, and vascular endothelial growth factor, which are crucial during the early stages of the healing cascade [10, 11]. Transforming growth factor-beta plays a central role in osteoblast differentiation, promoting the commitment of mesenchymal stem cells toward the osteogenic lineage and stimulating the production of extracellular matrix proteins. Platelet-derived growth factor acts as a potent mitogen and chemoattractant for mesenchymal stem cells and osteoblasts, recruiting these progenitor cells to the defect site and promoting their proliferation. Vascular endothelial growth factor, in particular, promotes rapid angiogenesis, which is a prerequisite for osteogenesis in large defects. Without adequate vascularization, new bone formation is limited to the periphery of the defect where oxygen and nutrient diffusion can support cellular activity. The establishment of a robust vascular network ensures the delivery of oxygen, nutrients, and circulating osteoprogenitor cells to the center of the scaffold, enabling three-dimensional bone formation throughout the defect volume rather than only at the margins [12, 14]. Our radiographic data at 4 weeks support this interpretation, showing significantly earlier mineralization in the composite group compared to the scaffold-only group, indicating that the biological factors released from the Platelet-Rich Fibrin accelerated the initiation of the mineralization process.

The interaction between the bioactive glass component and the fibrin network also appears to be critical to the enhanced performance of the composite scaffold. Bioactive glass with the 45S5 composition is known to release calcium and phosphate ions upon exposure to physiological fluids, which elevate the local pH and osmotic pressure, thereby stimulating osteoblast activity and promoting the precipitation of a carbonated hydroxyapatite layer on the scaffold surface [7, 8]. This surface layer serves as a favorable substrate for osteoblast attachment and provides a chemical signal that directs osteogenic differentiation. When integrated with Platelet-Rich Fibrin, the fibrin mesh likely serves to trap these ions and the growth factors in close proximity to the scaffold surface, creating a highly localized bioactive niche where the concentrations of signaling molecules and mineral ions are optimized for osteogenesis [15, 16]. This spatial concentration of bioactive factors may explain the significantly lower percentage of residual scaffold material observed in Group C, where only 7.12% of the scaffold remained at 12 weeks, compared to 18.44% in the scaffold-only group. The

presence of Platelet-Rich Fibrin-derived leukocytes and cytokines may have accelerated the cell-mediated degradation of the collagen matrix through the action of matrix metalloproteinases and facilitated the subsequent turnover of the bioactive glass particles into native bone through osteoclast-mediated resorption and osteoblast-mediated deposition [17]. The accelerated scaffold degradation in the composite group suggests that the biological factors released from the Platelet-Rich Fibrin not only enhanced new bone formation but also promoted the timely resorption and replacement of the scaffold material, which is desirable for achieving complete defect reconstruction with host bone.

Furthermore, the mechanical stability provided by the Col-gel-BG scaffold prevents the premature collapse of the Platelet-Rich Fibrin fibrin clot, which is a critical factor in the success of this composite approach. One of the primary limitations of using Platelet-Rich Fibrin alone in large bone defects is its rapid contraction and lack of structural integrity under load. The fibrin clot that forms from Platelet-Rich Fibrin is mechanically weak and tends to shrink over time, which can reduce the volume of the defect that is exposed to its biological factors and can compromise the spatial orientation of new bone formation [13]. By using the Col-gel-BG scaffold as a carrier, the Platelet-Rich Fibrin is stabilized across the entire defect volume, allowing its biological cues to operate over a three-dimensional space rather than being confined to a localized surface where the clot maintains contact with the host tissue [18]. This spatial stabilization is reflected in the histomorphometric findings, where Group C displayed mature lamellar bone with organized collagen fibers and well-developed trabecular architecture throughout the defect volume, whereas Group B showed more immature, woven bone formation concentrated primarily around the scaffold periphery, with less complete filling of the central defect region [19]. The organization of newly formed bone into lamellar structures with osteocytes embedded within lacunae indicates that the composite scaffold supported not only the quantity of bone formation but also the quality, with the regenerated tissue achieving a mature, functional architecture.

The clinical implications of these findings are substantial. The use of autologous Platelet-Rich Fibrin eliminates concerns regarding immunogenicity, disease transmission, or the ethical issues associated with the use of xenogeneic or allogeneic growth factor products. The preparation of Platelet-Rich Fibrin requires only a simple centrifugation step that can be performed at the point of care, making it accessible even in resource-limited settings. The combination of this autologous biological component with a synthetic Col-gel-BG scaffold creates an off-the-shelf osteoconductive carrier that can be stored and prepared in advance, with the biological activation occurring at the time of surgery through the addition of the patient's own Platelet-Rich Fibrin. This approach aligns with the principles of personalized medicine while maintaining practicality for routine clinical application.

However, it is important to note that while the autologous nature of Platelet-Rich Fibrin eliminates concerns regarding immunogenicity or cross-infection, its composition can vary between individuals based on hematological profiles, including platelet count, white blood cell count, and the concentration of circulating growth factors [10]. Despite this inherent variability, the results across our experimental cohort remained consistent, with all animals in the composite group demonstrating superior healing compared to the scaffold-only group. This consistency suggests that the composite approach is robust and that even variations in Platelet-Rich Fibrin quality do not negate its beneficial effects when combined with an osteoconductive scaffold. The standardization of the centrifugation protocol and the use of a consistent scaffold material likely contributed to minimizing the impact of inter-individual variability.

Several limitations of this study should be acknowledged. The use of a rabbit radius model, while well-established for critical-sized defect research, may not fully recapitulate the mechanical loading conditions or the healing kinetics of human bone, particularly in load-bearing sites. The 12-week study duration, while adequate to assess early to mid-stage bone regeneration, does not provide information on the long-term stability and remodeling of the newly formed bone. The evaluation of bone regeneration was limited to radiographic, micro-computed tomographic, and histological analyses; mechanical testing to assess the functional properties of the regenerated bone was not performed. Additionally, the mechanisms underlying the observed synergy between the scaffold and Platelet-Rich Fibrin were inferred from the literature but not directly investigated in this study through molecular or cellular analyses.

These findings suggest that the combination of a mineral-rich synthetic scaffold with autologous blood concentrates represents a highly effective and clinically viable strategy for bone tissue engineering [2, 4]. The results support the concept that successful bone regeneration in critical-sized defects requires not only an osteoconductive scaffold to guide new bone formation but also a source of osteoinductive signals to actively recruit and direct progenitor cells. The Col-gel-BG scaffold provides the former, while the Platelet-Rich Fibrin provides the latter, and the combination of the two yields outcomes that are greater than the sum of their individual contributions. Future studies should explore the long-term remodeling of the regenerated bone, investigate the cellular and molecular mechanisms underlying the synergistic effects observed, and translate this approach to larger animal models that more closely approximate human bone healing. The translation of this composite strategy to clinical practice has the potential to improve outcomes for patients requiring reconstruction of critical-sized bone defects in maxillofacial, orthopedic, and trauma surgery.

Conclusion

This study demonstrates that the integration of autologous Platelet-Rich Fibrin (PRF) with Collagen-Gel Bioactive Glass (Col-gel-BG) scaffolds results in a synergistic effect that significantly outperforms the use of the scaffold alone. The composite system not only provides the necessary mechanical and osteoconductive framework via the Col-gel-BG matrix but also introduces a sustained release of essential growth factors through the PRF fibrin network. Our quantitative Micro-CT and histomorphometric analyses confirmed that this combined approach leads to higher bone mineral density, increased bone volume, and accelerated scaffold degradation and replacement by mature lamellar bone. These findings suggest that the Col-gel-BG/PRF composite is a highly effective, biocompatible, and clinically accessible strategy for the treatment of critical-sized bone defects, offering a viable alternative to traditional autologous bone grafting.

References

1. Giannoudis PV, Dinopoulos H, Tsiridis E. Bone substitutes: an update. *Injury*. 2005;36 Suppl 3:S20-7.
2. Oryan A, Alidadi S, Moshiri A, Maffulli N. Bone regenerative medicine: classic options, novel strategies, and future directions. *J Orthop Surg Res*. 2014;9(1):18.
3. Dimitriou R, Jones E, McGonagle D, Giannoudis PV. Bone regeneration: current concepts and future directions. *BMC Med*. 2011;9:66.
4. Hench LL. The story of Bioglass. *J Mater Sci Mater Med*. 2006;17(11):967-78.
5. Jones JR. Review of bioactive glass: from Hench to hybrids. *Acta Biomater*. 2013;9(1):4457-86.
6. Wahl DA, Czernuszka JT. Collagen-hydroxyapatite composites for hard tissue repair. *Eur Cell Mater*. 2006;11:43-56.
7. El-Ghannam A. Bone reconstruction: from bioceramics to tissue engineering. *Expert Rev Med Devices*. 2005;2(1):87-101.
8. Xynos ID, Edgar AJ, Buttery LD, Hench LL, Polak JM. Gene-expression profiling of human osteoblasts following treatment with the ionic products of 45S5 bioactive glass dissolution. *J Biomed Mater Res*. 2001;55(2):151-7.
9. Bessa PC, Casal M, Reis RL. Bone morphogenetic proteins in tissue engineering: the road from the laboratory to the clinic, part I (basic concepts). *J Tissue Eng Regen Med*. 2008;2(1):1-13.
10. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101(3):e37-44.
11. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on graft stability. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101(3):e56-60.
12. He L, Lin Y, Hu X, Zhang Y, Wu H. A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009;108(5):707-13.
13. Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol*. 2009;27(3):158-67.
14. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. *Biomaterials*. 2005;26(27):5474-91.
15. Simonpieri A, Del Corso M, Sammartino G, Dohan Ehrenfest DM. The relevance of Choukroun's platelet-rich fibrin and autologous fibrin membranes in implantology, bone graft and soft tissue regeneration. *Implant Dent*. 2009;18(3):220-9.
16. Bose S, Roy M, Bandyopadhyay A. Recent advances in bone tissue engineering scaffolds. *Trends Biotechnol*. 2012;30(10):546-54.
17. Wu G, Pan M, Wang X, Wen J, Cao S, Li Z, et al. Osteogenesis of peripheral blood mesenchymal stem cells in self-assembling peptide nanofiber scaffold. *Biomaterials*. 2010;31(1):79-87.
18. Gerhardt LC, Boccaccini AR. Bioactive glass and glass-ceramic scaffolds for bone tissue engineering. *Materials*. 2010;3(7):3867-910.
19. Dohan Ehrenfest DM, Doglioli P, de Peppo GM, Del Corso M, Charrier JB. Choukroun's platelet-rich fibrin (PRF) stimulates proliferation and differentiation of human oral osteoblasts and periodontal ligament cells. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2007;104(3):336-42.