

COMPARATIVE EVALUATION OF THE MATRIX ARCHITECTURE OF A BIORESORBABLE SYNTHETIC GRAFT MATRIX AND DEMINERALIZED FREEZE-DRIED BONE ALLOGRAFT: A SCANNING ELECTRON MICROSCOPIC AND ENERGY-DISPERSIVE SPECTROSCOPIC STUDY

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ABSTRACT

Introduction: The regenerative performance of a bone graft substitute is governed by its matrix architecture and elemental composition, yet direct ultrastructural comparisons between synthetic and allogenic grafts used in periodontal therapy remain limited.

Aim: The surface micro-architecture and elemental composition of a bioresorbable synthetic graft matrix and demineralized freeze-dried bone allograft (DFDBA) were comparatively evaluated using scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS).

Materials and Methods: In this in vitro comparative analytical study, six specimens each of a bioresorbable synthetic graft matrix (Group 1) and DFDBA (Group 2) were examined by field-emission SEM at 500×, 1000× and 1500× magnification, followed by EDS elemental mapping of representative areas.

Results: The synthetic matrix exhibited a finely porous, uniform and interconnected architecture at all magnifications, whereas DFDBA showed an irregular, comparatively denser and less interconnected porosity that became more compact at higher magnification. EDS revealed inverse elemental profiles: the synthetic matrix was mineral-dominant while DFDBA was organic-dominant.

Conclusion: The bioresorbable synthetic graft matrix demonstrates a more uniform, interconnected and mineral-rich scaffold favourable for osteoconduction and mechanical space maintenance, while DFDBA offers a collagen-dominant, osteoinductive matrix of comparatively lower porosity. The two materials appear structurally and compositionally complementary, and graft selection should be individualised to the regenerative requirements of the defect.

KEYWORDS: Bone graft substitutes; demineralized freeze-dried bone allograft; scanning electron microscopy; EDS-mapping; periodontal regeneration; β -tricalcium phosphate

INTRODUCTION

Periodontitis is a multifactorial, chronic inflammatory disease that progressively destroys the tooth-supporting apparatus, producing horizontal and angular bone defects of varying morphology and severity.¹ Reconstruction of lost alveolar bone, whether secondary to periodontal disease, trauma, tumour ablation or congenital deficiency, remains a central challenge of periodontal and implant therapy. Bone grafting continues to be the most widely used adjunct to guided bone regeneration (GBR), periodontal reconstructive surgery and implant site development, and the clinical performance of any graft material is governed by four interdependent properties: biocompatibility, biodegradability, mechanical stability and internal architecture.¹²

Autogenous bone has traditionally been regarded as the gold standard graft material because it alone combines osteogenic, osteoinductive and osteoconductive potential. Its clinical use, however, is constrained by donor-site morbidity, a finite harvestable volume and the requirement for a second surgical field, limitations that have sustained the continuing search for viable alternatives.² Allogeneic, xenogeneic and alloplastic substitutes have consequently evolved to bridge this gap,

each characterised by a distinct physicochemical and micro-architectural profile that governs its biological behaviour once placed within a defect.²³

Demineralized freeze-dried bone allograft (DFDBA), prepared from human cortical bone by dilute-acid demineralization and lyophilization, exposes non-collagenous matrix proteins, including bone morphogenetic proteins, that confer a degree of osteoinductive activity in addition to its osteoconductive scaffold function. It has been used extensively for the reconstruction of intraosseous and furcation defects and, in combination with barrier membranes, for ridge augmentation and peri-implant bone regeneration.³ Its regenerative behavior nevertheless remains inherently variable, being influenced by donor age, processing protocol and residual particle morphology, all of which alter the collagen architecture retained after processing and the subsequent kinetics of graft resorption.³

Synthetic (alloplastic) bone substitutes, most commonly calcium-phosphate ceramics such as β -tricalcium phosphate (β -TCP) and hydroxyapatite (HA), bioactive glasses and resorbable polymer composites, were developed to reproduce the structural and biological features of native bone while eliminating the risk of disease transmission and immunogenicity associated with biologically derived grafts.⁴ Contemporary fabrication techniques allow the porosity, pore interconnectivity, crystallinity and degradation kinetics of bioresorbable synthetic matrices to be engineered with a reproducibility that biological grafts cannot match, and formulations combining a polylactic-acid-based polymer scaffold with a silicate-modified β -TCP phase have been reported to display favorable handling characteristics and enhanced osteoinductive behavior once hydrated.⁵

The internal architecture of a graft material — its surface porosity, pore-size distribution, interconnectivity and surface topography — is now recognized as a principal determinant of regenerative capacity, since these parameters govern osteoblast attachment and migration, capillary ingrowth, and diffusion of nutrients and signaling molecules through the scaffold.⁶ A pore diameter of 100–500 μm is generally considered optimal for osteoblast migration and neovascularization, and scanning electron microscopy (SEM) remains the most direct means of characterizing these features at a resolution adequate for meaningful inter-material comparison. Complementing SEM with energy-dispersive spectroscopy (EDS) allows structural and elemental data to be acquired from the same specimen and interpreted together rather than in isolation.

Despite the widespread clinical use of both DFDBA and synthetic bioresorbable graft matrices, direct, side-by-side ultrastructural and elemental comparisons between the two remain limited. Such comparative characterization is clinically relevant, since the choice of graft material for a given periodontal or peri-implant defect should ideally be informed by objective micro-architectural and compositional evidence rather than by material category alone. The present study was therefore undertaken to compare, under identical experimental conditions, the surface micro-architecture and elemental composition of a commercially available bioresorbable synthetic graft matrix and DFDBA using high-resolution field-emission SEM coupled with EDS.

Aim And Objectives:

Aim

A detailed comparative evaluation was done of the matrix architecture of a bioresorbable synthetic graft matrix and demineralized freeze-dried bone allograft (DFDBA) using scanning electron microscopy, and to correlate these structural findings with elemental composition determined by energy-dispersive spectroscopy.

Objectives

- The surface micro-architecture — porosity, pore distribution and surface morphology using SEM at 500 $\times$, 1000 $\times$ and 1500 $\times$ magnification was characterized and compared.
- The elemental composition of both graft materials using energy-dispersive spectroscopy (EDS) To determined and compared.
- It was analyzed how the observed differences in matrix architecture and elemental composition may influence the regenerative efficiency and healing potential of each material.
- The insights were derived that may guide the rational, defect-specific selection of graft material and inform the future design of synthetic grafts that more closely emulate the architecture of native bone.

MATERIALS AND METHODS

Study Design And Specimen Preparation

This in vitro, comparative, analytical study was conducted in the Department of Periodontology, Darshan Dental College and Hospital, Udaipur, Rajasthan, in collaboration with the Central Research Laboratory, Indian Institute of Technology (IIT), Indore, where scanning electron microscopy and energy-dispersive spectroscopy were performed. As the study did not involve human subjects, patient material or animal experimentation, patient-related inclusion and exclusion criteria were not applicable.

Graft Materials

- Group 1 — Bioresorbable synthetic graft matrix (Powerbone): procured directly from the manufacturer in sheet form, dimensions 25 $\times$ 25 $\times$ 2 mm.
- Group 2 — Demineralized freeze-dried bone allograft (DFDBA): procured from the Tata Memorial Tissue Bank, dimensions 20 $\times$ 15 $\times$ 5 mm.

Armamentarium

Digital caliper, BP blade with handle, fine tweezers, vacuum sputter coater, a field-emission scanning electron microscope (FE-SEM, Zeiss) fitted with an energy-dispersive spectroscopy detector, and a digital documentation camera. (figure 1)



Figure 1: Armamentarium



Figure 2: Bone Allograft (Dfdba)



Figure 3: Bioresorbable Synthetic Graft Matrix



Figure 4: Scanning Electron Microscope

Sample Preparation

Six specimens were prepared from each material ($n = 6$ per group). Each block was sectioned under aseptic conditions into $1 \times 1 \times 1$ mm fragments using a sterile BP blade, sized to seat securely on aluminum specimen stubs. Sections were mounted and sputter-coated with a thin gold layer (coating time 7–8 minutes) to render the surface conductive and optimize image resolution.

SEM and EDS Analysis

Coated specimens were examined by field-emission SEM at the Central Research Laboratory, IIT Indore. The electron beam was accelerated under high vacuum, and secondary-electron images were acquired at three magnifications — 500 $\times$, 1000 $\times$ and 1500 $\times$ — in both horizontal and vertical planes, with observation directed toward surface porosity, surface morphology and crystallinity. Elemental composition was subsequently determined on selected representative areas of specimens from each group using energy-dispersive spectroscopy, with weight-percentage (wt%) and atomic-percentage (at%) values generated for each detected element through eZAF quantification.

Qualitative and Quantitative Evaluation

Photomicrographs were assessed qualitatively for surface porosity, pore interconnectivity, morphology and crystallinity. Pore dimensions were measured quantitatively in micrometers using the calibrated SEM software scale bar. Comparison between the two groups was descriptive in nature; given the exploratory scope of the ultrastructural and elemental dataset, no inferential statistical testing was undertaken, and this is acknowledged as a limitation of the study.

Ethical Considerations:

No human tissue, patient intervention or animal experimentation was involved; both graft materials were commercially procured, obviating the requirement for institutional ethics committee clearance.

RESULTS

Field-emission SEM examination of the bioresorbable synthetic graft matrix (Group 1) and DFDBA (Group 2) at 500 $\times$, 1000 $\times$ and 1500 $\times$ magnification revealed consistent and reproducible differences in surface porosity, pore interconnectivity and morphology between the two materials; these findings were further corroborated by EDS elemental mapping.

Bioresorbable Synthetic Graft Matrix (Group 1)

At 500 $\times$ magnification, the surface exhibited a fine, relatively uniform and interconnected porous network favorable for fluid permeation (Figure 4). At 1000 $\times$, the pores appeared more distinct, evenly spaced and interconnected, features consistent with a scaffold suited to cellular infiltration (Figure 5). At 1500 $\times$, finer surface detail became apparent, with a mesh-like, micro-irregular texture that would be expected to favor cell attachment and further cellular infiltration (Figure 6).

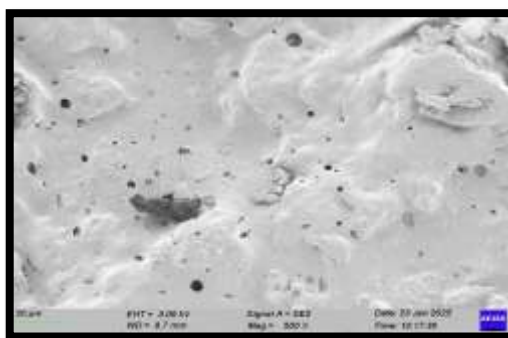


Figure 4. SEM photomicrograph of the bioresorbable synthetic graft matrix at 500 $\times$ magnification, showing a fine, interconnected porous surface.

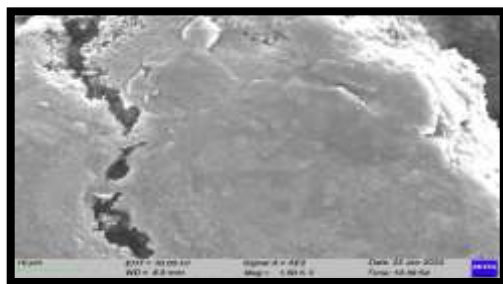


Figure 5. SEM photomicrograph of the bioresorbable synthetic graft matrix at 1000 $\times$ magnification, showing evenly spaced, interconnected pores.

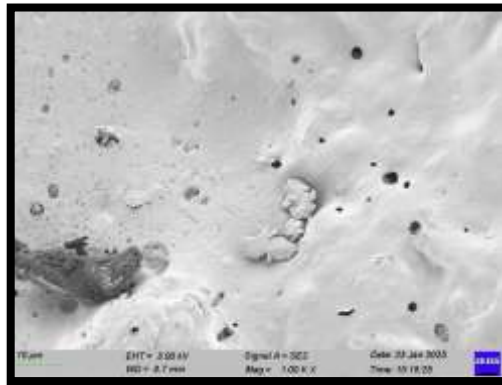


Figure 6. SEM photomicrograph of the bioresorbable synthetic graft matrix at 1500 $\times$ magnification, showing a mesh-like micro-textured surface.

Demineralized Freeze-Dried Bone Allograft (Group 2)

At 500 $\times$ magnification, DFDBA demonstrated an irregular, naturally porous trabecular architecture with an unevenly distributed, interconnected pore network (Figure 7). At 1000 $\times$, the surface appeared more compact, with smaller, less uniformly distributed pores and comparatively limited open pore space (Figure 8). At 1500 $\times$, the surface was denser still, with few visible pores and a rough, textured background consistent with a predominantly collagenous, minimally mineralized matrix (Figure 9).

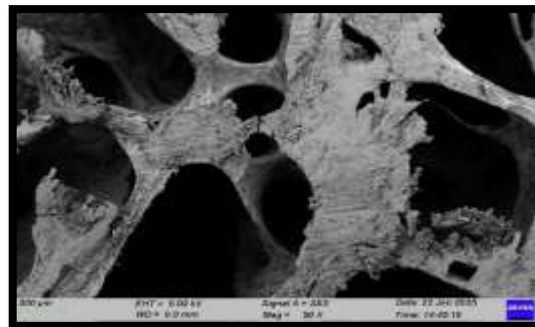


Figure 7. SEM photomicrograph of DFDBA at 500 $\times$ magnification, showing an irregular trabecular porous architecture.

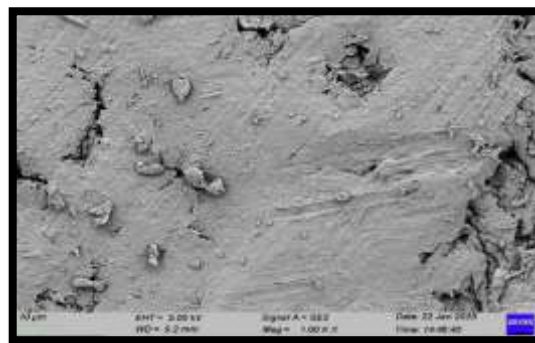


Figure 8. SEM photomicrograph of DFDBA at 1000 $\times$ magnification, showing a more compact surface with limited open pore space.

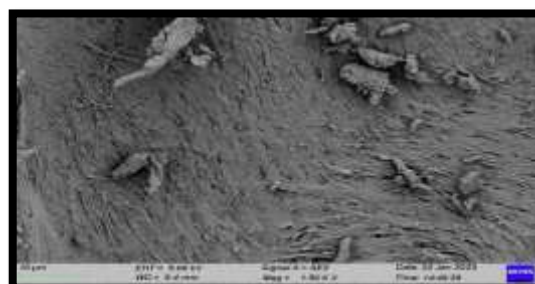


Figure 9. SEM photomicrograph of DFDBA at 1500 $\times$ magnification, showing a dense surface with minimal residual porosity.

Comparative Micro-architectural Features

Table 1 summarizes the principal micro-architectural differences observed between the two materials. The synthetic matrix displayed higher, more uniform and better-interconnected porosity than DFDBA, whose porosity, while biologically derived, was comparatively more restricted and less interconnected, particularly at higher magnification.

Feature	Bioresorbable Synthetic Graft Matrix	Bone Allograft (DFDBA)
Porosity	High, uniform, interconnected (superior scaffold)	Moderate, irregular, trabecular
Surface roughness	Engineered for cell attachment	Natural, variable
Crystallinity	High (HA/ β -TCP composite)	Low (collagen-influenced)
Resorption rate	Predictable, faster initial phase	Slower, biological remodeling
Regeneration role	Osteoconductive scaffold	Osteoinductive and osteoconductive

Table 1. Comparative micro-architectural features of the bioresorbable synthetic graft matrix and DFDBA.

Elemental Composition — Bioresorbable Synthetic Graft Matrix

EDS mapping of a representative area of the synthetic matrix (Graph 1) produced the spectrum shown in Figure 10, dominated by phosphorus (P K α) and calcium (Ca K α) peaks with minor magnesium and silicon contributions. Quantitative eZAF analysis (Table 2) demonstrated that calcium (63.5 wt%) and phosphorus (34.5 wt%) together accounted for the great majority of the detected mass, with silicon (1.8 wt%) and magnesium (0.2 wt%) present as minor constituents. Both phosphorus and calcium were detected with low relative error (4.0% and 4.9% respectively) and high net peak intensity, confirming a reliable, mineral-dominant, ion-substituted calcium-phosphate composition consistent with a β -TCP/hydroxyapatite-type matrix.

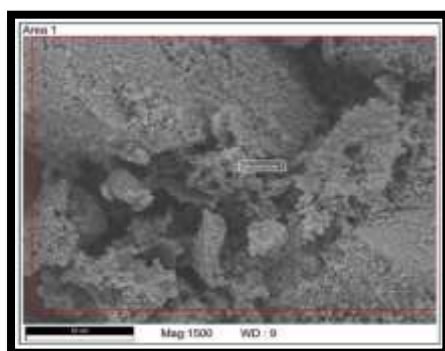
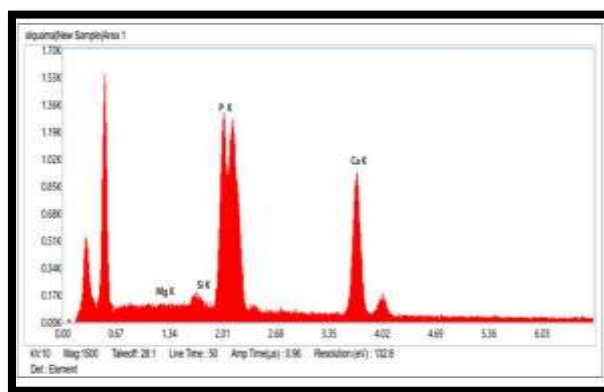


Figure 10. Selected area used for EDS mapping of the bioresorbable synthetic graft matrix.

Element	Wt %	MDL	At %	Error %	Net Int.	R	A	F
Mg K	0.2	3.10	0.3	63.7	2.4	0.8933	0.7029	1.0113
Si K	1.8	1.42	2.3	12.7	21.4	0.9050	0.8602	1.0333
P K	34.5	1.14	40.2	4.0	309.4	0.9107	0.8980	1.0168
Ca K	63.5	0.57	57.2	4.9	206.8	0.9386	0.9538	1.0086

Table 2. Quantitative EDS (eZAF) analysis of the bioresorbable synthetic graft matrix.



Graph 1 . EDS spectrum of the bioresorbable synthetic graft matrix, showing dominant phosphorus and calcium peaks with minor magnesium and silicon peaks.

Elemental Composition — DFDBA

EDS mapping of a representative area of DFDBA (Graph 2) produced the spectrum shown in Figure 11, in which carbon (C K α) and oxygen (O K α) peaks were markedly dominant, with only minor peaks for sodium, silicon, phosphorus, chlorine, potassium and calcium. Quantitative analysis (Table 3) showed carbon (74.2 wt%) and oxygen (19.8 wt%) to be the principal constituents, while phosphorus (5.1 wt%) and calcium (0.1 wt%) were present only in trace quantity. This composition is consistent with a predominantly organic, collagen-rich matrix that has undergone substantial loss of mineral phase during acid demineralization.

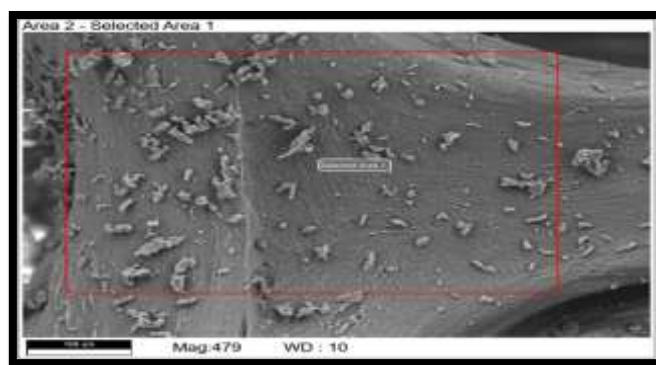
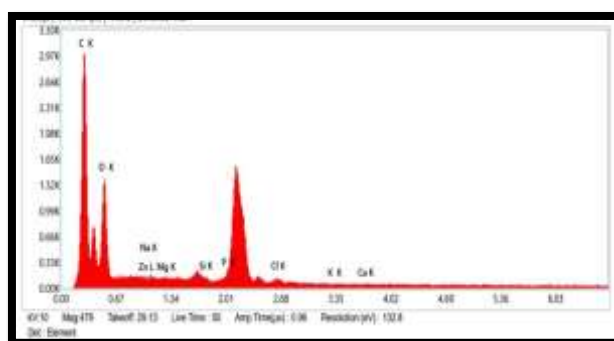


Figure 11. Selected area used for EDS mapping of DFDBA



Graph 2. EDS spectrum of DFDBA, showing markedly dominant carbon and oxygen peaks with only trace mineral peaks.

Element	Wt %	MDL	At %	Error %	Net Int.	R	A	F
C K	74.2	0.86	81.2	8.6	423.6	0.9412	0.3916	1.0000
O K	19.8	0.54	16.3	10.8	170.8	0.9500	0.2475	1.0000

Element	Wt %	MDL	At %	Error %	Net Int.	R	A	F
Na K	0.0	0.00	0.0	100.0	0.0	0.9600	0.6667	1.0025
Mg K	0.0	0.00	0.0	100.0	0.0	0.9630	0.7839	1.0045
Si K	0.3	0.07	0.1	52.9	6.9	0.9685	0.9111	1.0130
P K	5.1	0.37	2.2	3.7	104.3	0.9711	0.9418	1.0068
Cl K	0.5	0.12	0.2	35.6	7.2	0.9758	0.9645	1.0124
K K	0.0	0.00	0.0	91.8	0.1	0.9803	0.9824	1.0231
Ca K	0.1	0.16	0.0	62.8	0.6	0.9825	0.9878	1.0293
Zn L	0.0	0.00	0.0	100.0	0.0	0.9595	0.6477	1.0008

Table 3. Quantitative EDS (eZAF) analysis of DFDBA.

Comparative EDS Findings

The two materials exhibited essentially inverse elemental profiles. The synthetic graft matrix was mineral-dominant (Ca + P $\approx$ 98 wt%), consistent with a ceramic calcium-phosphate scaffold engineered principally for osteoconduction and mechanical space maintenance. DFDBA was organic-dominant (C + O $\approx$ 94 wt%), consistent with a demineralized, collagen-based scaffold retaining minimal residual mineral, in keeping with its role as a matrix for cell attachment and osteoinduction rather than as a load-bearing mineral scaffold.

Discussion:

SEM and EDS characterization of DFDBA and the bioresorbable synthetic graft matrix in the present study reveals structural and compositional differences that carry direct implications for mechanical stability, osteoconductive behavior and regenerative predictability. Interpreting these findings alongside the wider pre-clinical and clinical literature is essential to translate ultrastructural observation into rational, evidence-based graft selection.

Matrix Architecture and Porosity: Synthetic Graft versus DFDBA

The present SEM observations demonstrate that the bioresorbable synthetic graft matrix possesses a more uniform, more extensively interconnected porous network than DFDBA at every magnification examined. This finding is consistent with the physicochemical characterization reported by Anil et al,⁷ who compared five commercially available bone graft substitutes and similarly found that alloplastic, ceramic-based materials display more regular and reproducible porosity than allogeneic grafts, whose architecture is inherently subject to donor variability and processing artefact. Böhner et al⁸ have likewise documented that the sintering and fabrication parameters used in β -TCP manufacture allow pore size and interconnectivity to be controlled within narrow tolerances, a level of reproducibility that cannot be achieved with a biologically derived material such as DFDBA.

The comparatively denser, less interconnected architecture of DFDBA observed in this study corroborates earlier work indicating that, although demineralization preserves a loosely arranged collagen network capable of supporting osteoblast adhesion, variability in fibril orientation and partial matrix collapse during lyophilization can compromise both porosity and mechanical integrity. Since pore diameters of 100–500 μ m are widely regarded as optimal for osteoblast migration and neovascularization, the superior interconnected porosity demonstrated by the synthetic matrix in this study would be expected, on structural grounds, to favor more rapid vascular ingrowth and cellular infiltration than DFDBA.

Elemental Composition and its Biological Correlate

EDS quantification reinforced these structural observations. The synthetic matrix demonstrated a mineral-dominant calcium-phosphate composition together with trace magnesium and silicon substitution, a profile characteristic of an ion-modified β -TCP/hydroxyapatite ceramic. Ionic substitution of this kind has been shown by Zhao et al²⁶ to enhance osteogenic differentiation and to modulate the resorption kinetics of calcium-phosphate scaffolds, supporting the biological plausibility of the compositional profile identified in the present analysis. DFDBA, in contrast, displayed an organic-dominant profile with only trace phosphorus and calcium, confirming that the demineralization process largely removes the mineral phase, leaving a predominantly collagenous scaffold whose principal contribution to regeneration is expected to be osteoinductive rather than structural.

Comparative Clinical Evidence for β -TCP–Based Synthetic Grafts

These structural and compositional differences find support in the wider clinical and pre-clinical literature. Cheon et al¹⁰ demonstrated that a β -TCP/collagen composite produced significantly higher expression of osteogenic markers (alkaline phosphatase, osteocalcin) in vitro and achieved a greater proportion of new bone formation in vivo (69.5%) than a commercial collagenated xenograft (67.1%) at eight weeks, findings that parallel the favorable osteoconductive architecture identified for the synthetic matrix in the present study. Kumar et al¹¹ reported that β -TCP–based synthetic grafts achieve clinical attachment level gains comparable with autografts and superior vital bone formation relative to

xenografts (65–88% versus 45–55%), with more predictable resorption; a systematic review and meta-analysis by Jasser et al¹² similarly concluded that β -TCP produces probing depth and clinical attachment gains in infrabony defects broadly equivalent to those achieved with DFDBA, while avoiding the donor-dependent variability inherent to allogeneic grafts. Not all comparative clinical data, however, favor graft-augmented regeneration unequivocally. Gojkov-Vukelic et al¹³ found no statistically significant difference in clinical attachment gain or pocket depth reduction between open flap debridement performed with and without bone graft placement, and Zhang and Liu,¹⁴ in a meta-analysis comparing bone grafting alone with bone grafting combined with guided tissue regeneration, likewise reported no significant difference in six-month clinical attachment level, probing depth or radiographic bone gain between the two approaches, although the non-resorbable-membrane GTR combination produced greater gingival recession. Taken together, these observations suggest that while the ultrastructural and compositional advantages of a well-engineered synthetic matrix are biologically plausible determinants of regenerative performance, their translation into a measurable clinical advantage over DFDBA or unaugmented flap surgery remains material- and technique-dependent.

Comparative Clinical Evidence for DFDBA and Combination Grafts

DFDBA nonetheless continues to demonstrate meaningful clinical utility. Jaiswal et al²⁰ reported that DFDBA produced significant improvement in clinical attachment level and pocket depth reduction in the management of small osseous defects, an effect further enhanced by the addition of a collagen membrane, underscoring the value of combining DFDBA with an adjunctive barrier to compensate for its comparatively limited porosity and mechanical stability. Bansal et al¹⁹ showed that a hydroxyapatite/ β -TCP composite graft achieved substantial probing depth reduction (2.94 mm, 47.0%), clinical attachment gain (3.19 mm, 29.1%) and radiographic bone fill (3.20 mm, 63.2%) in intrabony defects, illustrating that combination ceramic grafts can further improve on the performance of either mineral phase alone. O'Hooley et al²¹, using

a β -TCP/calcium-sulfate composite, similarly documented enhanced vascularization, accelerated graft resorption and histological evidence of new bone formation with minimal residual graft material, reinforcing the concept that a predictable, engineered resorption profile — of the kind suggested compositionally for the synthetic matrix in this study — is advantageous for the timely replacement of graft material with vital bone.

Biological Augmentation of Graft Performance

Several groups have explored strategies to augment the inherent regenerative capacity of both graft categories. Bernhardt et al¹⁶, evaluating osteoblast-like cell attachment on a range of calcium-phosphate granules, found that bioglass-reinforced β -TCP supported markedly better cell attachment, proliferation and osteogenic marker expression than certain nanostructured calcium-phosphate materials, indicating that surface characteristics and composition, rather than mineral content alone, govern cellular response. Miletić et al¹⁵ demonstrated that pre-treating β -TCP with cold atmospheric plasma prior to seeding with periodontal ligament stem cells produced significantly greater bone regeneration, reduced inflammation and higher expression of bone-healing markers than β -TCP alone, indicating a potential adjunctive role for surface bio-functionalization and cell-based therapy in enhancing the performance of an already favorable scaffold architecture. Abdelaziz et al²² similarly reported that hydroxyapatite- and silver-nanoparticle-functionalized electrospun membranes improved cell adhesion, mechanical strength and antibacterial activity when used as GTR barriers, suggesting that combining an osteoconductive graft, such as the synthetic matrix characterized here, with a bio-functionalized membrane could offer a synergistic approach to periodontal bone regeneration.

Immunomodulatory and Ion-Mediated Considerations

The role of the host response in graft integration is increasingly recognized. Miron²³ and Miron et al²⁴ have emphasized that macrophage-mediated osteoimmunological responses to an implanted biomaterial critically influence its osteogenic integration, and that graft selection should account for immunomodulatory as well as structural properties. Ion incorporation — magnesium, strontium and silicon among others — has similarly been shown by Zhao et al²⁶ to modulate osteogenic differentiation, a mechanism plausibly relevant to the trace magnesium and silicon substitution identified within the synthetic matrix examined in the present study. Growth-factor-based biological adjuncts, such as injectable platelet-rich fibrin reviewed by Miron et al,²⁵ represent a complementary, biologically mediated strategy for enhancing angiogenesis and tissue healing that could, in principle, be combined with either graft material evaluated here to further improve regenerative outcomes.

Clinical Implications and Material Selection:

Collectively, the present ultrastructural and elemental findings, together with the supporting clinical and pre-clinical literature (references 7–9, 17–18, 27–30), indicate that the bioresorbable synthetic graft matrix offers a more uniform, predictably interconnected, mineral-dominant scaffold suited to defects in which rapid vascular ingrowth and mechanical space maintenance are priorities, whereas DFDBA provides a less uniformly porous but biologically active, collagen-dominant matrix whose principal contribution lies in osteoinduction. Bartold and Ivanovski²⁷ and Gallo et al¹⁷ both emphasize that successful periodontal regeneration depends on the interaction between scaffold architecture, growth factors and the host immune response rather than on any single material property in isolation, a view consistent with the structural and compositional complementarity identified between the two graft materials in this study. Balaji et al¹⁸ and Cheah et al²⁹ likewise conclude, in their respective reviews of periodontal graft materials, that no single alloplast, allograft or xenograft is universally superior, and that material selection should instead be individualized to defect morphology, the need for immediate mechanical support, and the desired balance between osteoconduction and osteoinduction — a

principle directly supported by the complementary architectural and compositional profiles demonstrated for the two materials examined here. Dayakar et al²⁸ and Barba-Rosado et al³⁰ provide further comparative ultrastructural data on alternative graft categories. Similarly, Khairnar SM et al³¹ underline the importance of detailed micro-architectural characterization through X-Ray diffraction study, relating to the present study, in guiding rational, defect-specific graft selection.

Limitations:

This study is not without limitations. The in vitro, descriptive nature of the SEM-EDS analysis, performed on a limited number of specimens without inferential statistical comparison, precludes definitive conclusions regarding the clinical superiority of either material, and elemental mapping was performed on selected representative areas rather than across the entire specimen surface. In vivo validation, incorporating standardized histomorphometry and clinical outcome measures, is required before the structural advantages identified for the synthetic matrix in this study can be translated into evidence-based clinical recommendations.

SUMMARY AND CONCLUSION

The evolution of periodontal regenerative therapy toward biologically informed, patient-specific strategies has placed increasing emphasis on the three-dimensional architecture of the biomaterials used to reconstruct lost alveolar bone. This study compared the surface micro-architecture and elemental composition of a bioresorbable synthetic graft matrix and DFDBA using field-emission SEM and EDS.

SEM demonstrated that the synthetic matrix possessed a finely porous, uniformly interconnected surface at all three magnifications examined, a configuration favorable for fluid permeation, cellular infiltration and vascular ingrowth. DFDBA, in contrast, exhibited an irregular but biologically derived porosity that became progressively denser and less interconnected at higher magnification, indicative of a more compact, collagen-dominant matrix. EDS confirmed that these structural differences were accompanied by essentially inverse elemental profiles: the synthetic matrix was mineral-dominant (calcium 63.5 wt%, phosphorus 34.5 wt%), consistent with an ion-substituted β -TCP/hydroxyapatite ceramic, whereas DFDBA was organic-dominant (carbon 74.2 wt%, oxygen 19.8 wt%), consistent with a demineralized collagenous scaffold.

These findings support the conclusion that the two graft materials occupy complementary rather than interchangeable positions within the regenerative armamentarium. The synthetic matrix's engineered porosity and mineral-dominant composition make it well suited to defects requiring predictable mechanical space maintenance and osteoconductive scaffolding, while DFDBA's collagen-rich, osteoinductive matrix retains clinical value, particularly when combined with a barrier membrane or biologically active adjunct, in defects where biological induction of bone formation is prioritized. Neither material should be regarded as universally superior; rather, the present micro-architectural and compositional data reinforce the principle that graft selection should be individualized according to defect morphology, the need for mechanical support, and the desired balance between osteoconduction and osteoinduction.

Future research should extend these in vitro observations to in vivo and clinical settings, incorporating histomorphometric and long-term radiographic and clinical outcome data, to confirm whether the structural advantages demonstrated for the synthetic matrix in this study translate into superior regenerative outcomes. Continued characterization of graft micro-architecture and composition, of the kind undertaken here, will remain central to the rational design of next-generation biomaterials for periodontal and peri-implant bone regeneration.

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Conflict of Interest

None declared.

Source of Support

Nil.

REFERENCES

1. Aslan S, Rasperini G. Fundamental concepts and new perspectives for periodontal regeneration. *Curr Oral Health Rep.* 2025;12(1):15.
2. AlGhamdi AS, Shibly O, Ciano SG. Osseous grafting part I: autografts and allografts for periodontal regeneration – a literature review. *J Int Acad Periodontol.* 2010;12(2):34-8.
3. Liu J, Kerns DG. Mechanisms of guided bone regeneration: a review. *Open Dent J.* 2014;8:56-65.
4. Winkler T, Sass FA, Duda GN, Schmidt-Bleek K. A review of biomaterials in bone defect healing, remaining shortcomings and future opportunities for bone tissue engineering: the unsolved challenge. *Bone Joint Res.* 2018;7(3):232-43.
5. Miron RJ, Fujioka-Kobayashi M, Pikos MA, Nakamura T, Imafuji T, Zhang Y, et al. The development of non-resorbable bone allografts: biological background and clinical perspectives. *Periodontol 2000.* 2024;94(1):161-79.
6. Fraser D, Caton J, Benoit DS. Periodontal wound healing and regeneration: insights for engineering new therapeutic approaches. *Front Dent Med.* 2022;3:815810.

7. Anil A, Sadasivan A, Koshi E. Physicochemical characterization of five different bone graft substitutes used in periodontal regeneration: an in vitro study. *J Int Soc Prev Community Dent.* 2020;10(5):634-42.
8. Bohner M, Santoni BLG, Döbelin N. β -tricalcium phosphate for bone substitution: synthesis and properties. *Acta Biomater.* 2020;113:23-41.
9. Miron RJ, Sculean A, Shuang Y, Bosshardt DD, Gruber R, Buser D, et al. Osteoinductive potential of a novel biphasic calcium phosphate bone graft in comparison with autografts, xenografts, and DFDBA. *Clin Oral Implants Res.* 2016;27(6):668-75.
10. Cheon EJ, Kim SH, Lee DK, Jo YK, Ki MR, Park CJ, et al. Osteostimulating ability of β -tricalcium phosphate/collagen composite as a practical bone-grafting substitute: in vitro and in vivo comparison study with a commercial product. *Biotechnol Bioprocess Eng.* 2021;26(6):923-32.
11. Kumar V, Naik GN, Tuteja S, Bhasin MT, Chattopadhyay D, Chowdhury S, et al. Evaluation of radiographic and clinical outcomes in the use of bone substitutes in periapical surgery and periodontal regeneration. *J Pharm Bioallied Sci.* 2025;17(Suppl 3):S2218-20.
12. Jasser RA, AlSubaie A, AlShehri F. Effectiveness of beta-tricalcium phosphate in comparison with other materials in treating periodontal infra-bony defects around natural teeth: a systematic review and meta-analysis. *BMC Oral Health.* 2021;21(1):219.
13. Gojkov-Vukelic M, Hadzic S, Pasic E. Evaluation of efficacy of surgical periodontal therapy with the use of bone graft in the treatment of periodontal intrabony defects. *Med Arch.* 2017;71(3):208-11.
14. Zhang F, Liu G. Comparison of the clinical efficacy of bone grafting and bone grafting combined with guided tissue regeneration in periodontal regenerative therapy: a meta-analysis. *Acta Odontol Scand.* 2024;83:40255.
15. Miletić M, Puač N, Škoro N, Brković B, Andrić M, Prokić BB, et al. Bone regeneration potential of periodontal ligament stem cells in combination with cold atmospheric plasma-pretreated beta-tricalcium phosphate: an in vivo assessment. *Appl Sci.* 2023;14(1):16.
16. Bernhardt A, Lode A, Peters F, Gelinsky M. Comparative evaluation of different calcium phosphate-based bone graft granules – an in vitro study with osteoblast-like cells. *Clin Oral Implants Res.* 2013;24(4):441-9.
17. Gallo S, Pascadopoli M, Pellegrini M, Pulicari F, Manfredini M, Zampetti P, et al. Latest findings of the regenerative materials application in periodontal and peri-implant surgery: a scoping review. *Bioengineering.* 2022;9(10):594.
18. Balaji VR, Manikandan D, Ramsundar A. Bone grafts in periodontics. *Matrix Sci Med.* 2020;4(3):57-63.
19. Bansal R, Patil S, Chaubey KK, Thakur RK, Goyal P. Clinical evaluation of hydroxyapatite and β -tricalcium phosphate composite graft in the treatment of intrabony periodontal defect: a clinico-radiographic study. *J Indian Soc Periodontol.* 2014;18(5):610-7.
20. Jaiswal Y, Kumar S, Mishra V, Bansal P, Anand KR, Singh S. Efficacy of decalcified freeze-dried bone allograft in the regeneration of small osseous defect: a comparative study. *Natl J Maxillofac Surg.* 2017;8(2):143-8.
21. O'Hooley D, Fairbairn P, Kilner S, Horowitz RA, Kurtzman GM. Bone regeneration using an alloplastic graft material that combines β -tricalcium phosphate and calcium sulphate: a case series report with histology. *Med Res Arch.* 2024;12(8).
22. Abdelaziz D, Hefnawy A, Al-Wakeel E, El-Fallal A, El-Sherbiny IM. New biodegradable nanoparticles-in-nanofibers based membranes for guided periodontal tissue and bone regeneration with enhanced antibacterial activity. *J Adv Res.* 2021;28:51-62.
23. Miron RJ. Optimized bone grafting. *Periodontol 2000.* 2024;94(1):143-60.
24. Miron RJ, Bohner M, Zhang Y, Bosshardt DD. Osteoinduction and osteoimmunology: emerging concepts. *Periodontol 2000.* 2024;94(1):9-26.
25. Miron RJ, Gruber R, Farshidfar N, Sculean A, Zhang Y. Ten years of injectable platelet-rich fibrin. *Periodontol 2000.* 2024;94(1):92-113.
26. Zhao Q, Ni Y, Wei H, Duan Y, Chen J, Xiao Q, et al. Ion incorporation into bone grafting materials. *Periodontol 2000.* 2024;94(1):213-30.
27. Bartold PM, Ivanovski S. Biological processes and factors involved in soft and hard tissue healing. *Periodontol 2000.* 2025;97(1):16-42.
28. Dayakar MM, Pooja H, Prakash Pai G, Shivananda H. Bone grafts in periodontal regeneration – a contemporary overview. *Dent J Indira Gandhi Inst Med Sci.* 2024;9(2):425-78.
29. Cheah CW, Al-Namnam NM, Lau MN, Lim GS, Raman R, Fairbairn P, et al. Synthetic material for bone, periodontal, and dental tissue regeneration: where are we now, and where are we heading next? *Materials.* 2021;14(20):6123.
30. Barba-Rosado LV, Realpe MF, Valencia-Llano CH, López-Tenorio D, Piñeres-Ariza IE, Grande-Tovar CD. Tomographic and electron microscopy description of two bone-substitute xenografts for the preservation of dental alveoli. *Int J Mol Sci.* 2024;25(20):10942.
31. Khairnar SM, Ravikiran N, Porwal KB, Vyas V, Rizvi A, Kamble A. Comparative evaluation of mineral & crystalline pattern of DFDBA & different CBR scaffolds. *J Neonatal Surg.* 2025;14(27 Suppl):207-215.