

Physicochemical Characterization of Five Different Bone Graft Substitutes Used in Periodontal Regeneration: An *In Vitro* Study

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ABSTRACT **Background:** Periodontal regeneration involves using a variety of bone graft substitutes (BGS) of varying origin and manufacturing processes. These include a wide range of biomaterials that are mainly of two types: the xenografts and alloplasts. The efficacy of these BGS depends upon the physical characteristics such as particle size, porous nature, surface morphology, as well as the chemical characteristics like composition, crystallinity and resorption properties. **Aims:** The present study is a descriptive study that focuses on describing the physicochemical characteristics of five selected commercially available BGS that are frequently used in periodontal regeneration procedures. The BGS studied here included two xenografts (colocast and osseograft) and three alloplasts (B-OstIN, biograft HABG active and biograft HT). **Materials and Methods:** The physical properties of the BGS, including particle size, morphology, and surface topography, were analyzed using SEM. The mineral phases and crystallinity of the BGS were analyzed using XRD. **Results:** The results showed that the xenografts (colocast and osseograft) had minimal mineral composition and crystalline structure. The physical properties such as surface roughness and porosity were less compared to alloplastic materials. The alloplasts (B-OstIN, biograft HABG and biograft HT) that had different chemical compositions showed varying physical and crystalline properties. Biograft HT showed a superior porous scaffold architecture among all BGS studied. **Conclusion:** It is important for a clinician to have a thorough understanding about the physicochemical characteristics of BGS they use in periodontal regeneration. The xenografts evaluated here had minimal physical and crystalline properties. Among the alloplasts studied, biograft HT showed superior physicochemical properties, while the presence of bioactive glass in biograft HABG enhanced regeneration.

KEYWORDS: Alloplasts, bone graft substitutes, characterization, physicochemical, xenografts

INTRODUCTION

Periodontal disease, a bacteria-induced immune-inflammatory disease, causes a progressive destruction of gingival tissues. Conventional periodontal treatment, including oral prophylaxis and open flap debridement, results in the arrest of disease progression with the repair of tissues but minimal periodontal regeneration. This has led to the evolution of periodontal tissue engineering (PTE) modalities including the use of

bone graft substitutes (BGS), guided tissue regeneration (GTR), delivery of growth factors, and root surface biomodifications.^[1] PTE strategies aim at the formation of new alveolar bone, cementum, and periodontal ligament.^[2]

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The role of BGS is to act as a scaffold or a provisional matrix for tissue-forming cells, growth factors, and vascular ingrowth. These BGS should get resorbed in symphony with new bone formation.^[3] Autografts have been considered the ‘gold standard’ in bone reconstructive surgery due to their osteogenic, osteoinductive, and osteoconductive properties; however, donor site morbidity and limitations on the quantity of graft that can be harvested have curtailed their use. This has resulted in searching for alternative graft substitutes for use in clinical applications.^[4] Allografts are harvested from a donor of the same species but with a different genotype. The advantages include eliminating a second surgical procedure and unlimited quantities being available. Based on the processing, allografts are divided into decalcified freeze-dried bone allograft (DFDBA) or freeze-dried bone allograft (FDBA).^[5] Xenografts are bone substitutes derived from animal sources (mainly bovine and porcine origin). They have the advantage of being available in large quantities, but face problems of antigenicity and patients disagreeing to their use because of ethical and religious concerns.^[2]

The disadvantages of autografts, allografts and xenografts led to an interest in manmade biocompatible materials (alloplasts). Alloplasts include ceramics, polymers, or their combination. Different types of bioceramics include calcium phosphates, calcium sulfates, and bioactive glasses.^[6] The ability of BGS to induce periodontal regeneration will depend on the mechanical properties, which include the macroscopic geometry, surface characteristics, presence of porosity, pore size distribution, and interconnectivity of pores, which in turn affects the rate of vascularization, diffusion of nutrients, and degradation rates.^[7] Samavedi *et al.* have shown that the chemical composition of different calcium phosphate-based BGS would influence tissue response when placed in bone defects.^[8] There is a wide variation in the physicochemical properties among different BGS due to their origin and different processing techniques, among others. Trajkovski *et al.* studied the hydrophilic, viscoelastic, and physicochemical variations in dental bone grafting substitutes. They opined that physicochemical properties have been poorly studied.^[9]

Clinicians need to be aware of the characteristics of BGS they are using as their responses might vary when placed in bone defects or when used along with implant placement. The aim of the present study was to describe the physical characteristics and chemical composition of five types of bone graft materials used in dental practice for periodontal regeneration by SEM to assess the particle size, morphology and surface topography, and by XRD to identify different phases and crystallinity of materials.

MATERIALS AND METHODS

BONE GRAFT SUBSTITUTES

The study was performed to describe the physicochemical characteristics of five commercially available BGS commonly used in periodontal regeneration. The study was approved by the research and ethical committee of Sree Mookambika Institute of Dental Sciences, Kulashekaram, Tamil Nadu. The BGS studied included two xenografts (colocast and osseograft) and three alloplasts (B-OstIN, biograft HABG active and biograft HT).

Sample A (colocast; xenograft from ColoGenesis Healthcare Pvt Ltd, India) was demineralized bone matrix of type 1 collagen prepared from bovine cortical bone. It was of particle size approximately 200–700 microns.

Sample B (osseograft; xenograft from Advanced Biotech Products Ltd, India) was a DMBM of type 1 collagen protein component. It was of bovine origin, non-immunogenic in nature, and of particle size approximately 250 microns.

Sample C (B-OstIN; alloplast from Basic Healthcare Products Pvt. Ltd.) was a synthetically produced hydroxyapatite (HA) with 100% phase purity, with a calcium-to-phosphate (Ca/P) ratio similar to that of natural bone. It has a porosity in range of 60–70% and particle size in the range of 100–300 microns.

Sample D (biograft HABG active; alloplast from IFGL Bioceramics Limited, India) was a combination of synthetic HA and calcium-phospho-silicate (bio-active glass), making it a bioactive composite. The manufacturer specifies a particle size ranging from 100 to 700 microns.

Sample E (biograft HT; alloplast from IFGL Bioceramics Limited, India) was a synthetic biphasic BGS consisting of 60% HA and 40% beta-tricalcium phosphate (β -TCP) in a porous granular form with a particle size of 350–500 microns.

PHYSICOCHEMICAL ANALYSIS

All samples were in granular form and obtained from the manufacturer in sealed vials. The BGS samples were sent for characterization without subjecting them to any further treatment. Physicochemical analyses of BGS were done at the Biomedical Technology Wing of Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, India. Characterization of BGS samples was completed within a period of 1 month (January 2020). Physicochemical studies performed on BGS included SEM to evaluate its physical properties, and XRD to analyze its mineral phases and crystallinity.

Scanning electron microscopy

SEM was used to assess the particle size, morphology, and surface topography. The samples were small enough

to ensure stable fitting on the specimen stage and to increase their electrical conductivity. The granules were washed with acetone and deionized with distilled water, air-dried, and critically point-dried to remove moisture. SEM images of the sample were obtained using a high-energy electron beam. This interaction produced secondary electrons, back-scattering of electrons, and characteristic X-rays. These signals were collected by multiple detectors to form images. Since the electron beam has a shorter wavelength, SEM micrographs will have a better resolution and a larger depth of field, giving a three-dimensional appearance of the sample. The topography of the samples was viewed under four magnifications (200X, 400X, 800X and 3000X). An FEI QUANTA 200 Environmental Scanning Electron Microscope was used in the study. Image J software

was used to determine average particle size from SEM images.

X-ray diffractometry

XRD was used to determine the mineral phases and crystallinity of the materials. X-rays were generated by a cathode ray tube, filtered to produce monochromatic radiation towards the sample, which in turn produces diffracted rays that are then detected, processed, and counted. Enough material from the sample was used to fill an empty sample holder. The wavelength of the X-ray was of the same order of magnitude as the bond distances between atoms in the crystal, producing a pattern unique to the atomic arrangement in the sample. The results obtained from each material were compared with standards to characterize its

Table 1: Comparison of particle sizes by SEM

Sample	Magnification	Scale of measurement, μm	Mean particle size, μm
Colocast	800 \times	100	131.1
Osseograft	800 \times	100	35.7
B-OstIN	800 \times	100	23.1
Biograft HABG	800 \times	100	18.9
Biograft HT	800 \times	100	29.5

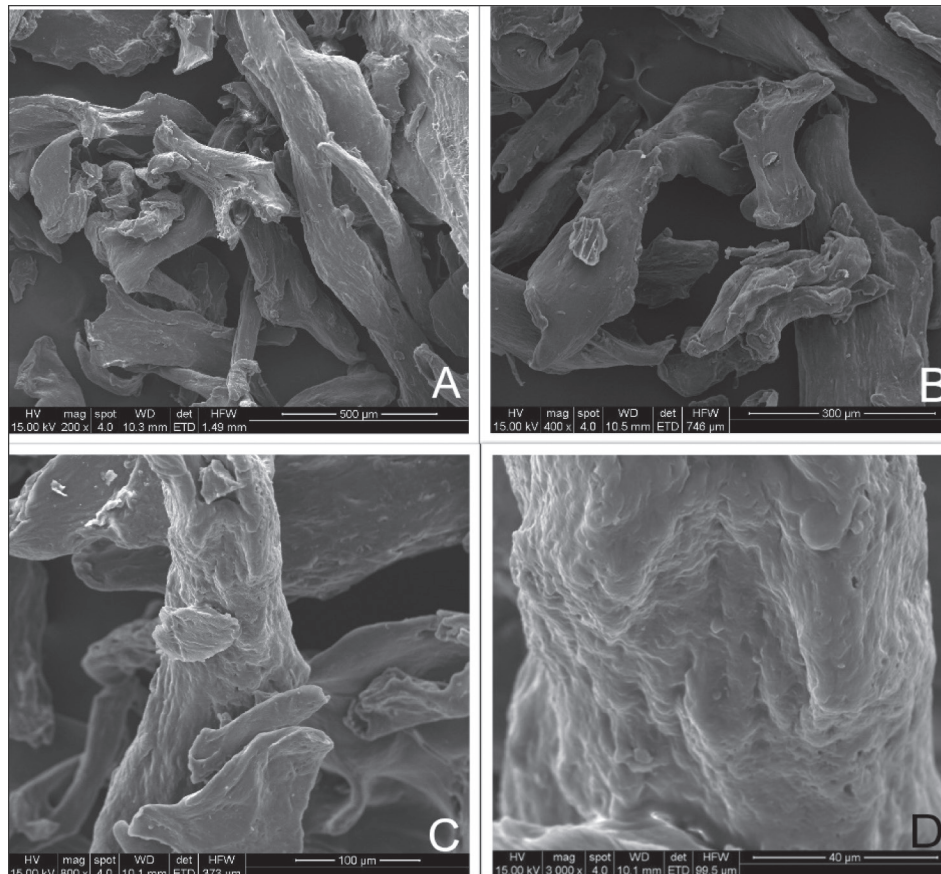


Figure 1: Colocast morphology and microstructure, SEM analysis: (A) 200 \times , (B) 400 \times , (C) 800 \times , and (D) 3000 \times . Fibrous architecture with minimal porosity seen.

phase purity and crystallinity. A graph obtained was compared to JCPDS (Joint Committee on Powder Diffraction and Standards). The analysis was done on a Bruker D8 XRD machine.

RESULTS

SEM ANALYSIS

The SEM analysis of BGS materials under magnification (800X) and scale of measurement of 100 μm showed the mean particle size of biograft HABG to be the smallest (18.9 μm) and that of colocast particles to be the largest (131.1 μm). Osseograft showed a mean particle size of 35.7 μm . Among the alloplastic BGS, the particle size of biograft HABG was the smallest at 18.9 μm followed by B-OstIN (23.1 μm) and biograft HT (29.5 μm) as shown in Table 1. The morphology and microstructure characterization of different bone-grafting materials are shown in Figures 1 to 5. Colocast samples showed a fibrous architecture with higher magnifications without showing a porous architecture [Figure 1]. Osseograft presented a roughened surface with not much porosity [Figure 2]. B-OstIN showed a minimal porous structure

[Figure 3]. Biograft HABG revealed a minimal surface roughness and porosity [Figure 4]. Biograft HT revealed the presence of macropores and micropores at SEM magnification [Figure 5].

XRD ANALYSIS

The XRD patterns of bone-grafting materials showed the mineral composition as well as orientation of grains especially in the polycrystalline sample. The colocast graft material was amorphous, and no calcium phosphate mineral was detected in the sample, whereas osseograft particles were not fully de-mineralized but contained a mineral phase β -TCP (whitlockite). All alloplastic materials showed a crystalline pattern with differences in the compositions of materials. B-OstIN (pure HA graft) showed a pure crystalline pattern, whereas biograft HABG active showed a composite consisting of crystalline HA, β -TCP and calcium phosphate silicate as major phases and calcium silicate (wollastonite) as minor phase. Biograft HT (HA-TCP) showed a composite of crystalline HA and β -TCP approximately in a 50:50 mass ratio. XRD analysis and patterns of all the BGS tested are presented in Table 2 and Figure 6.

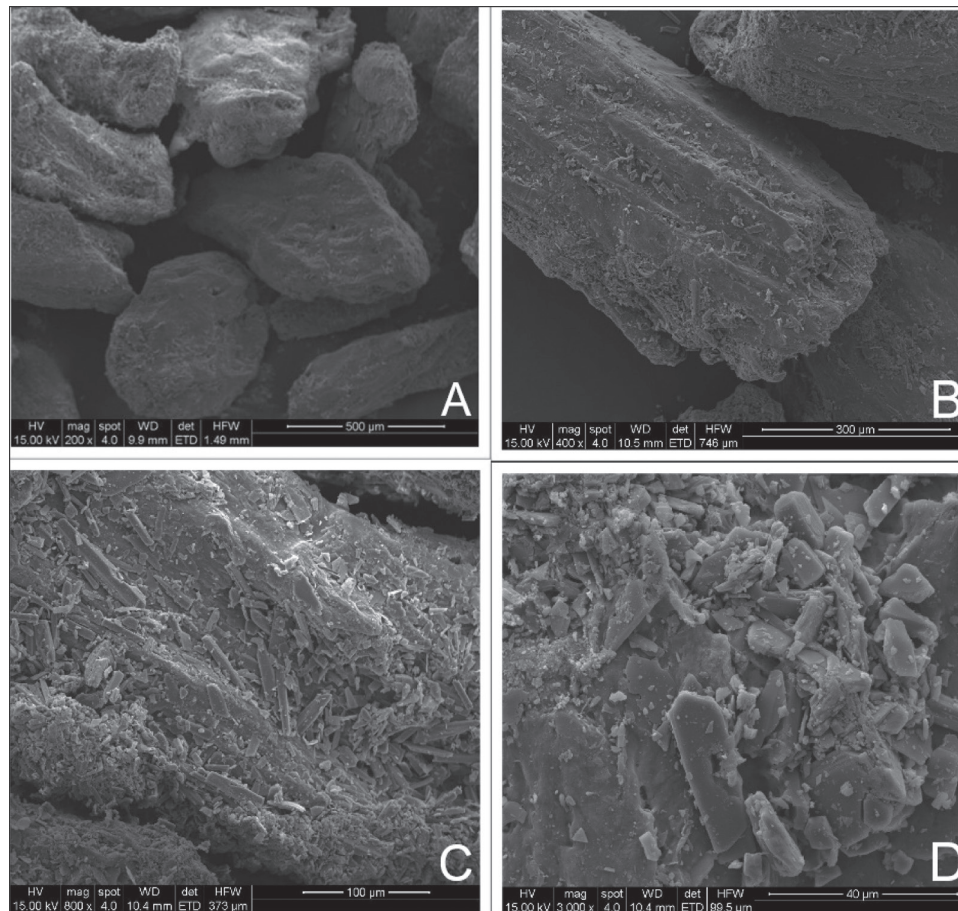


Figure 2: Osseograft morphology and microstructure, SEM analysis: (A) 200 \times , (B) 400 \times , (C) 800 \times , and (D) 3000 \times . Granular morphology with surface roughness seen with minimal porosity.

DISCUSSION

A wide range of BGS materials is available for periodontal regeneration, which include autografts, allografts, xenografts, and alloplastic materials. Porosity, interconnectivity, chemical composition, and mechanical properties affect the performance of a BGS *in vivo*. The graft should be highly hydratable to ensure an isosmotic environment that favors the diffusion of angiogenic factors. Another important property is that the BGS should not be immunogenic and should serve as a scaffold for new bone formation, getting itself resorbed during the process.^[8] The purpose of this study was to analyze and compare the physical and chemical characteristics of five different bone-grafting materials, which include two different xenografts of bovine origin and three alloplasts (HA, HA+BAG, and HA+ β TCP). The chemical composition and morphology influence the biological performance of an implanted graft material within the bone defect. Considering the selection of BGS materials, we employed SEM to assess their graft particle size, porosity, surface irregularity, and shape, and XRD to evaluate their chemical composition (presence of crystalline phases).

The particle size range of BGS determines the amount of surface area available for interaction with surrounding cells and biological fluids, as well as the packing characteristics of the material in defect. It has been postulated that if there is a wide size range, the smaller particles will obstruct the space between larger particles, thereby hindering vascularization. If the particles are very small in size, they may get resorbed at a faster rate.^[10] Al Ruhaimi suggested that the inflammatory response at the grafted site is directly proportional to the size, shape, and surface characteristics of BGS. A greater inflammatory response can be seen with sharp-edged small particles (1–30 μm), with an increased production of pro-inflammatory cytokines such as TNF- α and IL-6.^[11] SEM showed smaller mean particle sizes of biograft HABG and B-OstIN (18.9 and 23.1 μm , respectively), while biograft HT showed larger particles (29.5 μm). The largest particle size of osseograft was 35.7 μm , while that of Colocast was 131.1 μm . Beriberi *et al.* showed a median particle size range of 1.32 μm for BioOss and 663 μm for cerabone (both xenografts). For alloplastic BGS, they showed a median size of 262 μm for macrobone, 6.72 μm for

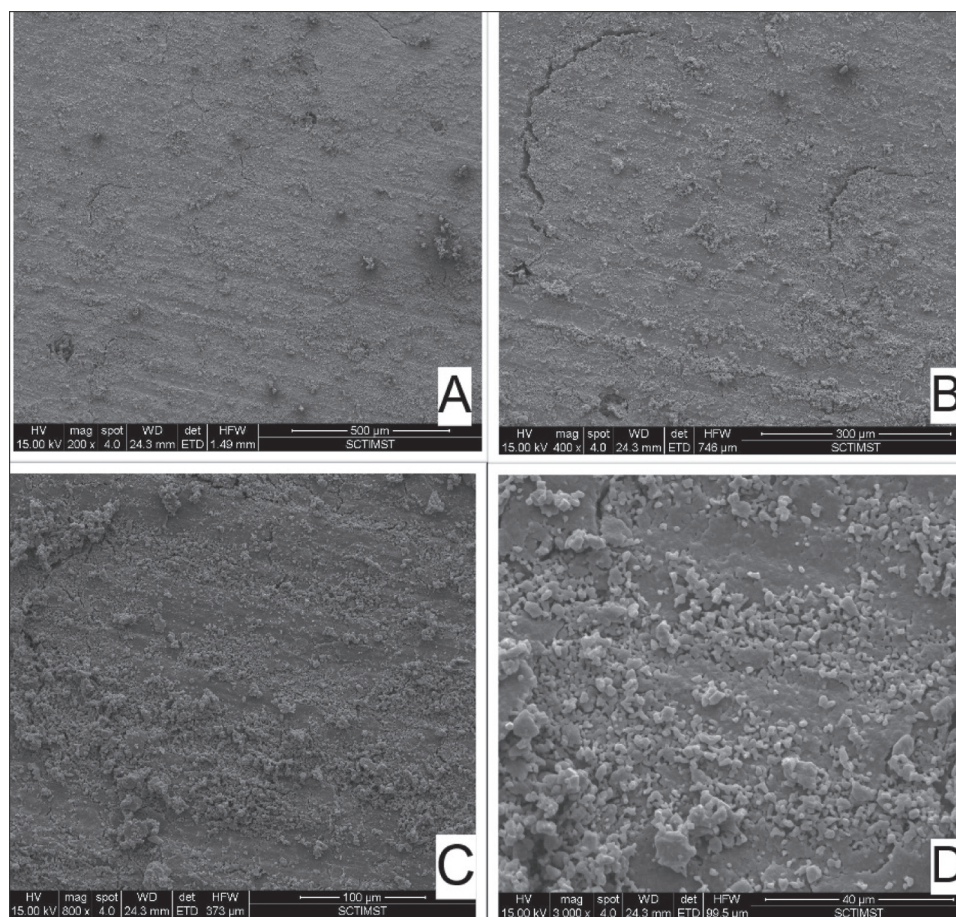


Figure 3: B-OstIN morphology and microstructure, SEM analysis: (A) 200 \times , (B) 400 \times , (C) 800 \times , and (D) 3000 \times . Dense particles with minimal porosity seen.

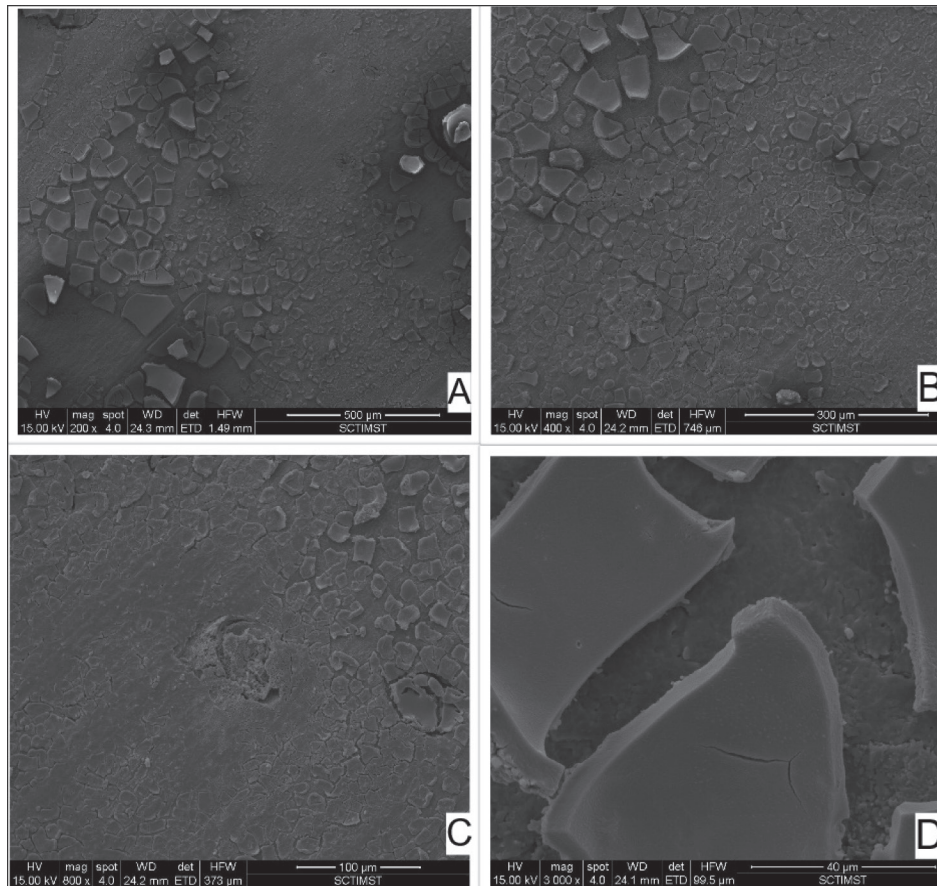


Figure 4: Biograft HABG morphology and microstructure, SEM analysis: (A) 200×, (B) 400×, (C) 800×, and (D) 3000×. Surface architecture with minimal surface roughness and porosity seen.

ingenios TCP, and 592 µm for ingenios HA.^[12] What we can conclude from the particle size data in the present study is that actual size ranges measured were different from what the manufacturers have reported. It might be due to the use of a different characterization technique by manufacturers.

Porosity is an important feature as all other parameters are strongly dependent on it. Therefore, many studies have been carried out to determine which pore size, pore interconnectivity, and pore distribution are ideal for enhancing osteoblast proliferation and bone ingrowth.^[13] Centripetal bone ingrowth by differentiated cells is responsible for the remodeling of bone to occur. Osteogenesis also depends on the degree of vascularization for which a high degree of porosity and a large pore size (>100 microns) is favorable for blood vessel access and development.^[14] The BGS presents macropores on the surface, while there are micropores within the internal architecture. Within the micropores (<10 microns), dissolution results in the release of calcium, phosphate, and carbonate ions leading to the formation of apatite crystals. The macropores (>100 microns) provide an environment for differentiated cells such as osteoblasts and osteoclasts to function in

addition to neo-vascularization. The interconnection of pores is an important parameter for the remodeling of bone to happen.^[15] Scaffold biomaterials should provide a microenvironment allowing the transmission of specific signals to stem cells that will be decoded into biochemical signals. The surface topography and chemical composition are said to influence cell adhesion, proliferation, migration, and differentiation.^[16] Cells are said to use the morphology of BGS for attachment, orientation, and migration by a process known as contact guidance. The focal adhesion of cells on graft surface is said to influence phenotypic expression in mesenchymal stem cells. Cellular response to a BGS is said to be affected by variations in surface texture or microtopography. Studies have shown that osteoblasts predominantly attach to a rougher surface.^[17] In our SEM analysis, colcast, osseograft, B-OstIN and biograft HABG showed an irregular surface with minimal porous architecture. Biograft HT showed a porous architecture with both macro- and micropores.

An advantage of XRD characterization is that it can be done with minimal sample preparation. A standard reference database is required to interpret data from new BGS. In our study, the graph obtained

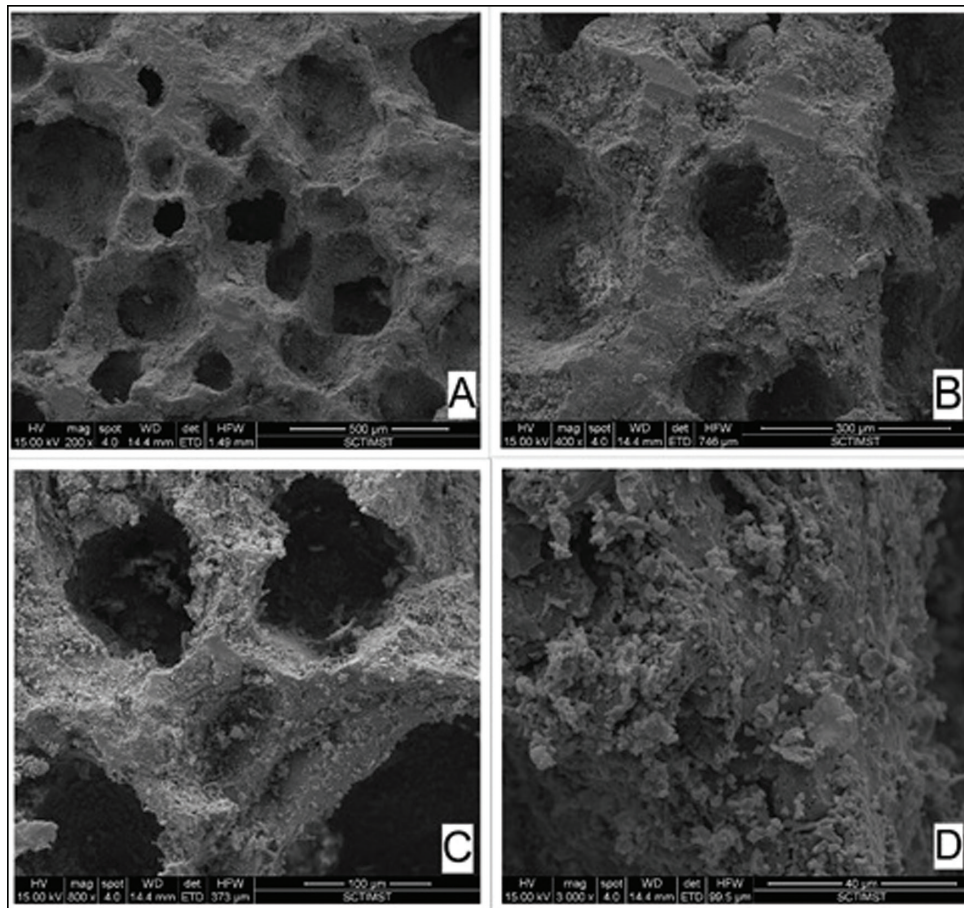


Figure 5: Biograft HT morphology and microstructure, SEM analysis: (A) 200×, (B) 400×, (C) 800×, and (D) 3000×. Granular particles seen with a porous architecture.

was compared to JCPDS, which is now known as ICDD (International Centre for Diffraction Data). The results depicted high peaks for materials with high crystallinity and small peaks for amorphous materials. In the present study, colocast (xenograft) was not fully de-mineralized but contained a mineral phase β -TCP (whitlockite). Osseograft (xenograft) was amorphous, and no calcium phosphate mineral was detected. This could be attributed to the presence of collagen remnants in the xenogenic BGS. The presence of collagen results in a lower degree of crystallinity, which makes the BGS material prone to faster degradation.^[18] Among the alloplastic materials, B-OstIN, biograft HABG and biograft HT showed typical crystalline structures. B-OstIN showed a pure crystalline pattern, whereas biograft HABG showed a composite consisting of crystalline HA, β -TCP and calcium phosphate silicate as major phases and calcium silicate (wollastonite) as minor phase. Biograft HT showed a composite of crystalline HA and β -TCP in 50:50 mass ratio.

The chemical composition of BGS will affect its rate of dissolution, crystallinity, mechanical properties, and cellular response. Calcium phosphate bioceramics are said to be ideal candidates for scaffold-based bone tissue engineering because they release calcium and phosphate ions during dissolution, which are said to play a role in the proliferation and differentiation of osteoblasts. Specific concentrations of these inorganic ions have been suggested to induce increased proliferation and osteogenic differentiation of mesenchymal stem cells.^[19] Ceramic materials show better volume stability at the grafted site compared to BGS having organic content.^[20]

Ceramics can either be crystalline or non-crystalline (amorphous). Crystallinity of HA affects its solubility; the higher the crystallinity, the lesser the solubility. The fact that pure HA ceramics resorb at a slower rate will result in a fibrous encapsulation of graft particles, preventing bone regeneration, which is a big disadvantage.^[2] TCP has shown good biocompatibility and regenerative potential; however, it has poor mechanical properties and a faster resorption rate.^[21] This has led to the development of biphasic ceramics

Table 2: XRD analysis of bone graft substitutes

Sample	Results
Colocast	Not fully de-mineralized but contains mineral phase β -TCP (whitlockite)
Osseograft	Amorphous and no calcium phosphate mineral detected
B-OstIN	Pure crystalline HA mineral
Biograft HABG	Composite consisting of crystalline HA, β -TCP, and calcium phosphate silicate as major phases and calcium silicate (wollastonite) as minor phase
Biograft HT	Composite of crystalline HA and β -TCP (50:50 mass ratio)

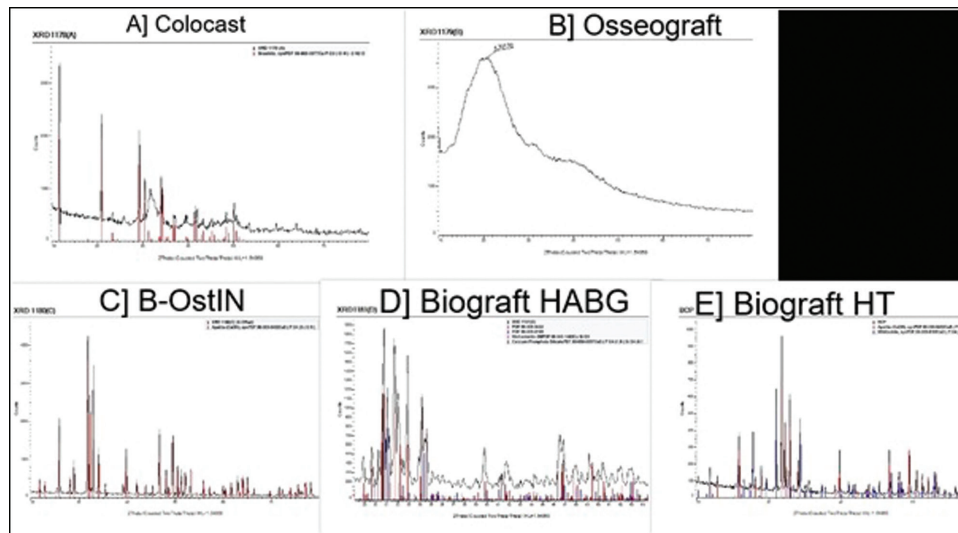


Figure 6: XRD analysis (colored stick indicates peak positions of standard, and black pattern indicates those of samples): (A) colocast, (B) osseograft, (C) B-OstIN, (D) biograft HABG, (E) biograft-HT.

incorporating both HA and β -TCP, which improves the resorption profile needed for periodontal regeneration. A composite biomaterial should have adequate mechanical properties, have suitable degradation kinetics, and safely dissolve without producing any toxic residues.^[22]

In our study, biograft HT is an example of a biphasic BGS consisting of 60% HA and 40% β -TCP. New bone formation by osteoblasts is related to osteoclastic resorption during bone remodeling; decreasing the percentage of HA would improve the dissolution rate of biphasic composites. The dissolution of biphasic BGS forms a chemical bond between native bone and apatite that forms on the surface of ceramic. The mechanical properties of a bone-grafting material, therefore, depend on its composition (increasing the percentage of HA), surface geometry (porosity), and processing parameters (sintering temperature and time). Sintered HAP ceramics, which are manufactured using high temperatures (1000–1200°C), showed very slow biodegradation than precipitated HAP ceramics. A higher sintering temperature will result in a more perfect crystal structure, resulting in a lower degradation rate. If the crystallite size of HAP ceramics is very small

and if there is carbonate incorporated, there is faster biodegradation due to a higher solubility.^[2] Bioactive glasses undergo specific surface reactions *in vivo*, which leads to the formation of a HA-like layer that forms a strong bond between the graft material and host bone. They release therapeutic ions such as silicate and calcium ions, which is said to promote cell differentiation into osteoprogenitor cells by affecting gene expression, thereby promoting osteoinduction.^[23] Among the BGS studied, biograft HABG is an example of a composite BGS that incorporates HA and bioactive glass.

The results of this study show that there are wide variations among the different BGS available in the market with respect to their particle size, surface characteristics, and chemical compositions as reported by the manufacturers. In this study, we described the particle size, surface morphology, mineral phases, and crystallinity of five BGS available for dental applications. A further evaluation of other physicochemical characteristics such as hydrophilicity, viscoelasticity, dimensional changes, porosity analysis, and composition needs to be done for a complete understanding of the BGS. A successful periodontal regeneration will depend on the correct classification of bone defect, proper surgical planning, and

precise selection of a BGS, necessitating a thorough understanding of its properties and characteristics.

CONCLUSION

Periodontal regeneration is a tissue engineering concept that relies heavily on the role of a scaffold. A wide range of biomaterials are now commercially available, including xenografts and alloplastic materials. This study was an attempt to physicochemically characterize five different commercially available BGS. A wide range of surface characteristics, chemical composition, and crystalline structures were observed. A clinician should take into consideration these properties while selecting a BGS for clinical application.

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Nil

CONFLICTS OF INTEREST

The authors report no conflicts of interest in the study.

AUTHORS CONTRIBUTIONS

Not applicable.

ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT

The study was approved by the research and ethical committee of Sree Mookambika Institute of Dental Sciences, Kulashekaram, Tamil Nadu.

PATIENT DECLARATION OF CONSENT

Not applicable.

DATA AVAILABILITY STATEMENT

Not applicable.

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