BULLETIN OF STOMATOLOGY AND MAXILLOFACIAL SURGERY
Volume 21, Issue 3

DOI: 10.58240/1829006X-2025.3-23

RESEARCH ARTICLE

INFLUENCE OF TEMPERATURE AND SINTERING DURATION ON HYDROXYAPATITE'S STRUCTURAL AND MORPHOLOGICAL CHARACTERIES EXTRACTED FROM OVINE BONE

Khushali K Shah[1], Deepak Nallaswamy Veeraiyan[1], Dhanraj Ganapathy[1,*], Rajalakshmanan Eashwarmoorthy[2] Ravleen Nagi[3], Junad Khan[3], Suresh Venugopalan[1],Saravanan Sekaran[1,*]

[1]Department of Prosthodontics and Implantology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.

[2]Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.

[3]Department of Orofacial Pain and TMJ Disorders, Eastman Institute for Oral Health, University of Rochester, NY, USA

Corresponding Author: Dhanraj Ganapathy MDS, PhD, Professor and Head Department of Prosthodontics and Implantology Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences Saveetha University,Chennai, India,E-mail: dhanraj@saveetha.com
Saravanan Sekaran PhD,Associate Professor,Department of Prosthodontics and Implantology,Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences,Saveetha University,Chennai, India. E-mail: saravanans.sdc@saveetha.com
Received: *Feb. 12, 2025;* ***Accepted:*** *Feb. 25, 2025;* ***Published:*** *Mar. 20, 2025*

ABSTRACT

Bone grafts are widely used in dentistry worldwide. Still, none of the products possess all the desirable qualities for a bone substitute material, including low immunogenicity, low patient morbidity, affordability, and angiogenic potential. This study evaluated the effect of temperature and sintering time on the hydroxyapatite derived from ovine bone. The ovine bone samples were categorized based on their temperature and sintering time into six groups: 120°C for 4 hours, 120°C for 8 hours, 240°C for 1.5 hours, 240°C for 3 hours, 360°C for 1.5 hours, and 360°C for 3 hours. Physiochemical properties using scanning electron microscopy, X-ray diffractometry, and Microculture Tetrazolium Assay were compared to commercially available bone grafts of BioOss and Osseograft, serving as a benchmark for the sintered ovine bone samples and highlighting their potential superiority. BioOss showed increased proliferation initially, but a decline was observed after 72 hours. In contrast, the sample sintered at 360°C for 3 hours exhibited increased cell aggregation after 72 hours, potentially enhancing osteogenic differentiation. In addition, the samples sintered at 360°C for 3 hours displayed the most distinctive and interconnected pore structure, suggesting higher sintering and consolidation effects. The unique and intriguing qualities of the sintered ovine bone samples, such as their distinctive pore structure and potential for strengthening osteogenic differentiation, could open new and promising avenues for bone graft materials in dentistry and orthopedics, offering optimism for their future use. The ovine bone showed physicochemical properties like BioOss with increased sintering time and temperature. Processing ovine bone with solvents like toluene and ethylene di amine and subjecting it to a temperature of 360° C for 3 hours showed similar results to commercially available xenograft-BioOss.

Keywords: Hydroxyapatite, Osteoblasts, Bio-Oss, Differentiation

INTRODUCTION

Bone grafts are a critical component in modern medical and dental treatments, facilitating the repair and regeneration of bone tissue. These grafts can be sourced from the patient's body or a donor or synthetically created, each type offering unique benefits and challenges. They are pivotal in treating fractures, bone defects, conditions like osteoporosis, and dental implant procedures. The ability to effectively replace or augment bone tissue not only aids in physical recovery but also enhances patients' overall quality of life, underscoring the significance of advancements in bone grafting techniques and materials.[1] Autogenous bone grafting has been considered the "gold standard." Autografts offer osteogenic, osteoconductive, and osteoinductive qualities. However, several disadvantages exist to using autologous bone transplants, including their restricted availability, donor site morbidity, and longer recovery times.[2,3] Owing to the rising need to correct cranial bony abnormalities and the improvements in dental implantology, the use of bone grafts and replacements in dentistry has expanded significantly in recent years.[4] These skeletal or bone abnormalities can result from oral cancer, trauma, periodontal conditions, surgical excision, cranioplasty, infection, or congenital deformities.[5] In dentistry, the most common indicator of insufficient bone following tooth loss is the rapid resorption of alveolar bone due to the absence of intraosseous stimulation typically provided by periodontal ligament fibers. For the successful placement of dental implants, adequate alveolar bone dimensions are crucial, requiring a minimum height of 10 mm and a diameter of 3 mm to 4 mm.[6,7] According to estimates, bone grafts will be used in as many as 50% of dental implant surgeries.[8] The development of innovative bone grafting materials, such as alternatives for bone grafts, has gained momentum despite the need for evidence-based studies on safety and indications. Consequently, in conjunction with the global aging population and the continuous, discernible rise in the need for bone graft materials, these issues make it imperative to conduct further comprehensive research into creating novel materials with ideal properties for diverse bone grafting techniques.[9,10] Ovine bone grafts, sourced from sheep, are increasingly employed in medical and dental sectors due to their advantageous properties and availability. These grafts are a viable alternative to human bone, providing a natural scaffold for bone regeneration.[11]

Ovine bone closely resembles human bone structurally and compositionally, making it an adequate substitute. Using ovine bone grafts can reduce the risks associated with human donor grafts, such as disease transmission and immune rejection. Additionally, they are readily available and can be processed to ensure biocompatibility and safety. The osteoconductive properties of ovine bone grafts promote new bone tissue formation, aiding in healing fractures, bone defects, and dental implant integration.[12] With ongoing research, the application of ovine bone grafts is expanding and promising, with improved bone repair and regeneration outcomes. Their efficacy and safety profile make them an asset in bone grafting. The temperature and sintering time are critical parameters in the processing of hydroxyapatite derived from ovine bone. These factors significantly influence the material's crystallinity, phase composition, mechanical properties, and bioactivity. Proper control of temperature ensures the stability and purity of hydroxyapatite. At the same time, optimal sintering time affects the densification and grain growth, ultimately impacting the performance of the bone graft in clinical applications. Understanding and optimizing these parameters can improve bone repair and regeneration outcomes, making the grafts more effective and reliable. Thus, the present study assessed the impact of temperature and sintering time on hydroxyapatite derived from ovine bone.

MATERIALS AND METHODS

The present in-vitro study was conducted at Saveetha Dental College and Hospitals, Chennai, India. The Scientific Review Board (SRB) approved the study on 26 August 2022. (SRB/SDC/PROSTHO-20/22/TH-073).

Ovine Bone Processing

Samples from a local slaughterhouse were obtained from the cortical hind limb-femur goat bones. They were cut into smaller pieces, and pretreatment with hydrothermal and wet and dry processing was done.[13] A portion of the organic phase was eliminated by pretreatment, which involved cutting broad portions of ovine bone from the middle of the femur bone and physically removing any attached soft tissue. The bone marrow and residual soft tissues were emptied by simmering the slices for 12 hours at 100° C, then

changing the water. This cycle was carried out for 96 hours. The samples were then cleaned and stored at 4° C for 12 hours and placed in a hot air oven at 100° C for 12 hours. This cycle was carried out for 48 hours. The samples were cleared of all visible soft tissue and debris (Figure 1 a).

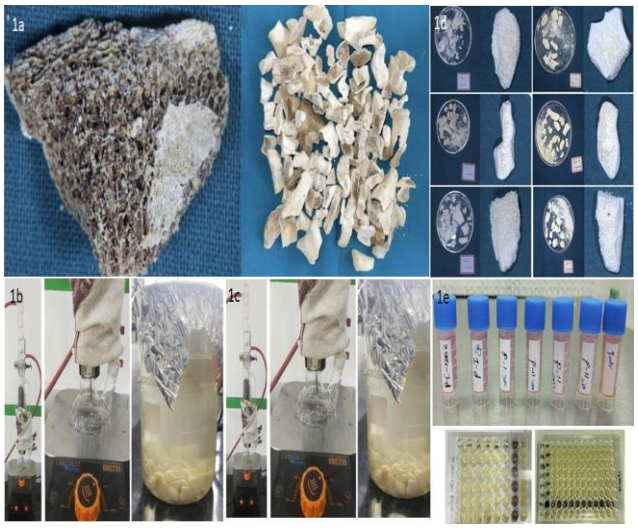

Figure1.1a) Presence of residual organic components-fats, proteins and debris post boiling the samples at 100° C for 12 hours followed by a water change every 12 hours for 96 hours; **1b)** Chemical processing using hot extraction method. Soxhlet extractor along with Toluene to aid in removal of organic contents; **1c)** Soxhlet extractor along with Ethylene di amine to aid in removal of organic contents; **1d)** Samples sintered at 120° C for 4 hours; Samples sintered at 120° C for 8 hours; Samples sintered at 240° C for 1.5 hours; Samples sintered at 240° C for 3 hours; Samples sintered at 360° C for 1.5 hours; Samples sintered at 360° C for 3 hours; **1e)** Samples prepared for MTT analysis.

The chemical processes for removing lipids, fat, and protein were carried out using the hot extraction method. 150 ml of toluene and the soxhlet extractor were used as an organic solvent. This process was carried out for 48 hours. The samples were soaked for 12 hours and then washed using ethanol to remove traces of the organic solvent.[13,14] The ethanol wash samples were heated in a hot air oven at 100° C for 12 hours (Figures 1b and c). For a thorough removal of organic components, 150 ml of ethylene di amine was used along with the soxhlet extractor as an organic solvent. This process was carried out for 48 hours. The samples were soaked for 12 hours and then washed using ethanol to remove traces of the organic solvent. The samples were then boiled at 100° C of distilled water for 12 hours. The samples were then washed twice in ethanol to remove any traces of organic components and the chemicals used for processing.

Sintering Time and Temperature

After being processed with chemical solvents, the ovine bone samples were categorized based on their temperature and sintering time into 6 groups: 120°C for 4 hours, 120°C for 8 hours, 240°C for 1.5 hours, 240°C for 3 hours, 360°C for 1.5 hours, and 360°C for 3 hours.[13,14] High and low sintering times and temperatures were applied in independent samples (Figure 1d).

The experimental group included these groups, whereas commercially available bone grafts- BioOss and Osseograft were grouped as the Control category. The sintering process is essential in material science, particularly for bone samples, as it influences the material's microstructure, mechanical properties, and biological behavior. Temperature and Sintering Time Ranges used in this study: 120°C for 4 hours and 8 hours, 240°C for 1.5 hours and 3 hours, 360°C for 1.5 hours and 3 hours. The reasons for choosing these ranges are thermal stability and phase changes, comparative analysis, and preservation of bone integrity.[13]

Thermal Stability and Phase Changes

120°C: This temperature is relatively low and is often used to remove organic components and water content without significantly affecting the mineral structure. Prolonged durations (4 and 8 hours) ensure thorough dehydration and initial decomposition of organic residues.

240°C: At this intermediate temperature, more organic material decomposes, and initial sintering processes begin, leading to the formation of a more cohesive structure. The two durations (1.5 and 3 hours) help study the impact of time on the intermediate phase of sintering.

360°C: Higher temperatures lead to significant sintering, densification, and possible bone mineral content phase changes (such as hydroxyapatite transformation). The chosen times (1.5 and 3 hours) allow for examining these changes and their effect on the mechanical properties of the bone samples.[13,14]

Comparative Analysis

Using different temperatures and times helps us understand the sintering kinetics and the role of thermal treatment in modifying the bone's structure. It allows for comparing low, intermediate, and high-temperature effects, comprehensively analyzing the sintering behavior, and optimizing parameters for desired properties.[13]

Preservation of Bone Integrity

Lower temperatures (120°C) preserve the bone's mineral structure while removing organic matter. Higher temperatures (240°C and 360°C) are applied to achieve significant sintering and modification of the bone structure while ensuring that the bone does not degrade excessively.[13,14]

Physicochemical characterization

After chemical processing and sintering, the samples were crushed and assessed for their physicochemical characterization. The experimental and control group samples were subject to physicochemical studies. The analysis included Scanning Electron microscopes (SEM) to evaluate their physical properties and X-ray diffraction (XRD) to analyze their mineral phases and crystallinity.[13]

Scanning electron microscopy

Particle size, structure, and surface topography were evaluated using SEM. The samples' modest size increased their electrical conductivity, and their stable placement on the specimen stage ensured their stability. The granules were air-dried, severely point-dried, and cleaned with acetone and distilled water to remove the moisture. The material was imaged utilizing a high-energy electron beam in an SEM. This interaction created secondary electrons, backscattering of electrons, and distinctive X-rays. Several detectors gathered these impulses to make pictures. The shorter wavelength of the electron beam allows for higher resolution and a greater depth of focus in SEM micrographs, which gives the material a three-dimensional aspect. Five scales were used to view the samples' topography: 1µm, 5µm, 10µm, 50µm, and 100µm. The investigation used a JSM-IT800 NANO SEM (JEOL Benelux, Netherlands). Using SEM pictures, the average particle size was calculated using Image J software.[13]

X-ray diffractometry

XRD was used to ascertain the materials' crystalline phases and crystallinity. The ground samples were placed on the analysis holder and subjected to a 2θ range of 10° to 80° using a D8 Advance (Bruker AXS GmbH, Karlsruhe, Germany). A cathode ray tube produced the X-rays, filtered out to generate monochromatic radiation directed toward the sample. This resulted in diffracted rays, which were detected, analyzed, and tallied. The sample contained enough material to fill an empty sample container. The X-ray's wavelength corresponded to the bond lengths between the atoms in the crystal, resulting in a pattern specific to the arrangement of atoms in the sample. The results of each material were compared to standards to determine their phase clarity and crystal structure, and the origin was used to plot the generated graphs.13

Compatibility analysis

Samples of the ovine bone were crushed and made into fine powder for the included Microculture Tetrazolium Assay (MTT) assay. The MTT assay was carried out to assess cell viability and proliferation(Figure1e).Eagle minimum essential medium F12 (1:1) containing 15% (vol/vol) heat-inactivated fetal bovine serum, two mM L-glutamine, 50 IU/mL penicillin, and 50 mg/mL streptomycin was used to cultivate MG-63 cells. It was cultured in T-25 cm2 culture flasks at 37°C with 95% air and 5% CO2 until it reached confluence, or 70% to 80%. Following two weeks, the cells were separated using a trypsin solution and multiplied at a density of 2.5 x 10^5 cells per well in 6-well plates. Each well received two milliliters of complete Dulbecco's Modified Eagle Medium F-12 (DMEM F-12) medium after the cells had been attached for 24 hours.

MTT assay

Each six-well plate was filled with one milliliter of complete culture media. 0.5 mg/mL of MTT was added to each well's bottom. The plate was subsequently incubated for four hours at 37°C. After incubation, the culture media was removed from the insert and the well, and 100µl of Dimethyl Sulfoxide (DMSO) solution was added to each well to dissolve the produced formazan crystals. The cell types were gently shaken for two minutes to ensure an even mixing of the solvent and the blue reaction product. Finally, 100µl of the colored DMSO solution was

transferred from each insert and well to a new 96-well plate to measure cell viability. Absorbance at 450 nm was measured using a microplate reader (Figure 1e). Percent cell viability was calculated using the following equation: Cell viability (%) = 450od of (sample)/A 450 od of (control) x 100

RESULTS

This study investigated the effect of sintering time and temperature on hydroxyapatite derived from ovine bone. The sintering parameters included six groups: 120°C for 4 hours, 120°C for 8 hours, 240°C for 1.5 hours, 240°C for 3 hours, 360°C for 1.5 hours, and 360°C for 3 hours. The synthesized HA samples were evaluated and compared with commercially available bone graft materials, BioOss and Osseograft, through scanning electron microscopy (SEM), X-ray diffraction (XRD) analysis, and 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay.

Scanning Electron Microscopy

The Scanning electron microscopy (SEM) analysis of BioOss depicts a well-interlaced matrix with dense inter-articles and certain bioactive surface coatings on the surface of the bone graft. More interconnected pore structures need to be identified in the SEM analysis of commercially available bone graft-Osseograft. The samples sintered at 120° C for 4 hours and 120° C for 8 hours showed poorly connected pore structure and matrix and a decreased porosity level compared to BioOss. Similar results were also obtained for samples sintered at 240° C and 360° C for a shorter enjoyable time of 1.5 hours. The samples sintered at 240° C and 360° C for 3 hours showed a well-defined pore structure and a dense structure with a well-defined surface morphology compared to BioOss. SEM analysis revealed a well-defined porous structure in all the sintering groups, indicative of successful synthesis of HA derived from ovine bone. Notably, the samples sintered at 360°C for 3 hours displayed the most distinctive and interconnected pore structure, implying a higher degree of sintering and consolidation. The samples sintered at 360° C showed a relatively smooth surface with a high level of inter-particle bonding and increased consolidated structure of Hydroxyapatite (Figure 2 a -e).

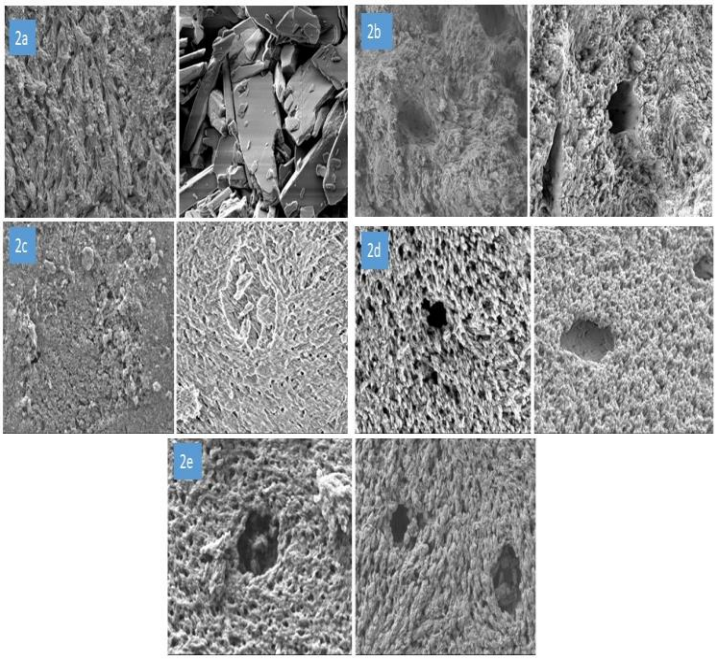

Figure 2. 2a) SEM analysis of commercially available xenograft- BioOss; Osseograft; **2b)** samples post Soxhlet extraction with Toluene; Ethylene di amine; **2c)** Samples samples sintered at 120° C for 4 hours; sintered at 120° C for 8 hours; **2d)** Samples sintered at 240° C for 1.5 hours; sintered at 240° C for 3 hours; **2e)** samples sintered at 360° C for 1.5 hours; sintered at 360° C for 3 hours**.**

X-ray Diffraction

The XRD patterns reveal the crystalline structures of hydroxyapatite [Ca4.854(H0.126(PO4)3)(OH)0.834] (01-074-9776) and calcium oxide phosphate [Ca5(PO4)3O0.5] (04-023-8826), respectively. The calcium oxide phosphate phase was predominantly observed, with peaks at 25.8° (002), 31.8° (211), 32.2° (112), 32.9° (300), 46.7° (222), and 49.5° (213). In addition, peaks in the region around 34.2° (202), 40° (221), 42.5° (302), and 43.6° (113) indicate the presence of the hydroxyapatite crystalline phase, which corresponds to the hexagonal crystal structure, as confirmed by the hkl indices. The XRD analysis suggests the primary presence of calcium oxide phosphate, with secondary contributions from the hydroxyapatite crystalline phase. From the XRD data, it was observed that hydroxyapatite (PDF 01-074-9776 Ca4.854(H0.126(PO4)3) (OH)0.834) and Calcium oxide phosphate (PDF 04-023-8826 CA5(PO4)3O0.5) crystalline phases were observed with the slight attribution of silica. In terms of the

graph, the High-intensity peak was observed first at around 31.7 deg. The two theta analyses observed a shift towards the right concerning increasing sintering temperature from 31.7 to 32.1 degrees. The maximum right shift was noted from 31.7 to 32.1 degrees at the highest sintering temperature at 360° C for 3 hours. The graph for BioOss also revealed three highly distinctive peaks suggestive of the presence of Hydroxyapatite in its crystalline form. The peaks ranged from 30 to 36 degrees, almost like ovine bone samples sintered at 360° C for 3 hours. However, the graph for Osseograft samples showed no distinctive peaks, indicating the absence of Hydroxyapatite and only the presence of Collagen(Figures 3 A, B, and C).

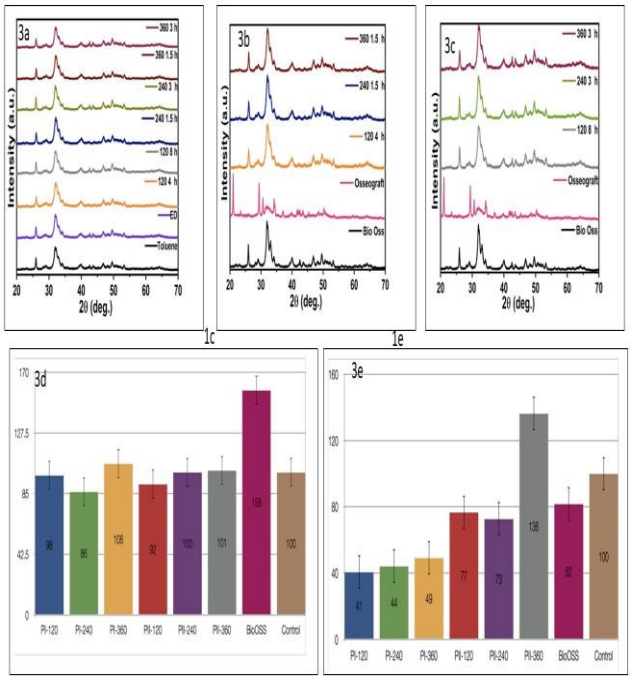

Figure 3. XRD peak data showing hydroxyapatite peaks when comparison made **3a)** between sample treated with Toluene, Ethylene di amine and sintered at 120°C for 4 hours, 120°C for 8 hours, 240°C for 1.5 hours, 240°C for 3hours, 360°C for 1.5 hours, and 360°C for 3 hours; **3b)** between 120°C for 4 hours, 240°C for 1.5 hours, 360°C for 1.5 hours and commercially available bone graft BioOss and Osseograft; **3c)** between 120°C for 8 hours, 240°C for 3 hours, 360°C for 3 hours and commercially available bone grafts BioOss and Osseograft; **3d & e)** Bar graph depicting the percentage of MG 63 cells proliferation for samples sintered at sintered at 120°C for 4 hours, 120°C for 8 hours, 240°C for 1.5 hours, 240°C for 3 hours, 360°C for 1.5 hours, and 360°C for 3 hours and its comparison to that of BioOss after an incubation period of 24 hours and 72 hours respectively.

A comparison with the Osseograft cannot be made as XRD confirmed no presence of Hydroxyapatite, and even the SEM data revealed a lack of interconnected pore structure. Therefore, even with the XRD results, ovine bone samples sintered at a higher temperature for a longer time showed comparable results to those of commercially available xenograft BioOss. When considering the results of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, all the samples showed biocompatibility and growth of MG-63 cells at 24 hours. BioOss samples showed an increased proliferation and development of MG-63 cells (158 %) compared to the other samples. However, at 72 hours, there was a decline (82%) of up to 2 folds in the BioOss samples (Figure 4).

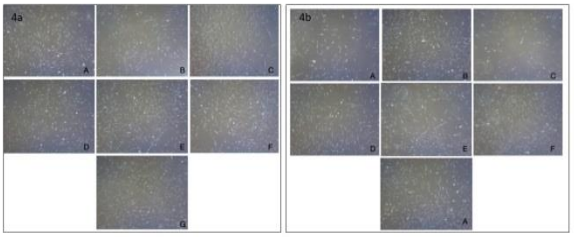

Figure 4a & b. Cell culture of MG-63 cells at 24 hours and 72 hours respectively for (A) commercially available bone graft- BioOss; (B) ovine bone graft sintered at 120° C for 4 hours (C) ovine bone graft sintered at 120° C for 8 hours; (D) ovine bone graft sintered at 240° C for 1.5 hours; (E) ovine bone graft sintered at 240° C for 3 hours; (F) ovine bone graft sintered at 360° C for 1.5 hours; (G) ovine bone graft sintered at 360° C for 3 hours.

The sample sintered at 360 ° C at 3 hours showed increased aggregation of the MG-63 cells of up to 1.2 folds after 72 hours, unlike the 2-fold decrease observed with that of BioOss. This can significantly affect and enhance the osteogenic differentiation potential of the ovine bone graft sintered at 360° C for 3 hours. The samples from other groups showed less than 50% cell viability, indicating toxicity (Table 1, 2 and Figure 3d, e).

Table 1. Sample groups based on Sintering Temperature and Time

GROUPS	SINTERING TEMPERATURE	SINTERING TIME
Group I	120° C	4 hours
Group II	120° C	8 hours
Group III	240° C	1.5 hours
Group IV	240° C	3 hours
Group V	360° C	1.5 hours
Group VI	360° C	3 hours

Table 2. The percentage of MG 63 cell proliferation for samples sintered at 120°C for 4 hours, 120°C for 8 hours, 240°C for 1.5 hours, 240°C for 3 hours, 360°C for 1.5 hours, and 360°C for 3 hours and its comparison to that of BioOss after an incubation period of 24 hours and 72 hours.

Groups	Cell Viability% After Incubation Period of 24 hours	Cell Viability% After the Incubation Period of 72 hours
PI-120	98	41
PI-240	86	44
PI-360	106	49
PII-120	92	77
PII-240	100	73
PII-360	101	136
BioOss	158	82
Control	100	100

DISCUSSION

This study observed the physicochemical properties of ovine bone, processed with solvents toluene and ethylene diamine and subjected to a temperature of 360°C for 3 hours to be like those of the commercially available xenograft BioOss. Improved results were observed with increased sintering time and temperature. Osseograft consists solely of collagen and lacks hydroxyapatite. The temperature and sintering duration affected the color of the ovine bone powder, which can range from grayish black to light yellow to white. Temperature differences caused changes in the organic compounds' vibrational states, which in turn caused changes in the chemical composition and the effective reflective index, which reflected variations in the sample's hue. The occurrence of ashes from the breakdown of the

organic component in the bone was indicated by the gray hue of the preprocessed samples. Therefore, further chemical processing is necessary to remove organic contents. Processing with toluene and ethylene di amine led to the additional removal of organic compounds. This was analyzed using data from SEM where certain impurities were present, and the pore structure was not as well defined as that of BioOss's SEM data.

The MTT analysis conducted using MG-63 cells indicates the impact of the tested substance on the viability and metabolic activity of these cells, which are commonly used as a model for bone-related studies. The MTT assay measures the conversion of MTT into a colored formazan product by mitochondrial enzymes, reflecting the overall cell metabolic activity. A higher intensity indicates increased cell metabolic activity and, thus, higher cell viability. Conversely, a lower intensity suggests reduced metabolic activity and decreased cell viability. However, the attachment of MG63 cells onto the surface of bone grafts could not be verified by optical microscopy. However, the proliferation of cells adjacent to the bone graft was confirmed. MTT assay can provide only a valuable initial assessment of the ovine bone graft's impact on cell viability. However, it should be supplemented with additional experiments and analyses to understand its effects on ovine bone comprehensively. Further investigations, such as gene expression analysis, alkaline phosphatase activity assays, and mineralization assays, can provide a more detailed understanding of the influence of ovine bone graft on bone cell differentiation and mineralization.

Previous literature and studies on the processing protocol regarding bovine bone-derived hydroxyapatite have been reported extensively.[15,16] Hydroxyapatite was also obtained while a three-step hydrothermal process processed bovine bone.[14] Studies on bovine bone have been previously carried out based on various processing methods.[17]

Alkaline hydrothermal hydrolysis was the method used by Barakat et al. to extract HAp from bovine bone.[18]

After heating the extracted HAp to 250 °C, a 1.86 Ca/P ratio nanoflake HAp was formed after five hours. In addition to cow bone, HAp has also been extracted from the bones of camels, horses, and pigs. Calcination of camel bone produced pure crystalline HAp with variable forms in the nanoscale range of 79-97 nm.[19]

Rahavi et al. reported asymmetrically shaped nano-sized HAp by calcining horse and camel bones for two hours at 700 °C.[20]

While in the present study, chemical treatment was carried out using a Soxhlet extractor along with toluene and ethylene di amine with a temperature range below 400° C, previous studies based on bovine bone employed a combination of alkaline heat treatment and calcination to extract Hap.[21]

However, there was limited evidence of obtaining hydroxyapatite and processing it associated with ovine bone. The literature revealed limited data regarding hydroxyapatite derived from fish bone, which was sintered at 800°C, 900°C, 1000°C, 1100°C, and 1200 °C.[22,23]

However, the only drawback observed was decreased mechanical properties, especially its fragility and decreased mechanical properties.[24]

The present study yielded significant insights into the impact of sintering time and temperature on hydroxyapatite derived from ovine bone; it is essential to acknowledge certain limitations. Firstly, the focus was on the sintering parameters without considering other factors, such as the chemical composition and impurities within the starting material. Future research should investigate the combined influence of sintering parameters and material composition for a more comprehensive understanding. Furthermore, although the employed characterization techniques (SEM, XRD, and MTT analysis) provided valuable information on the microstructure, crystallography, and biological aspects of sintered hydroxyapatite, there remains potential for more advanced characterization techniques. Techniques like FTIR or Raman spectroscopy could be incorporated to examine the material's chemical bonding and functional groups. Additionally, while the MTT assay offered insights into cell viability and proliferation, further investigations encompassing comprehensive biocompatibility assessments, including cell adhesion, differentiation, and gene expression analysis, are warranted. In vivo studies employing animal models would also be essential to evaluate the material's performance within a more realistic biological environment.

Further studies should explore advanced processing techniques like additive manufacturing or 3D printing to fabricate intricate structures using sintered hydroxyapatite. The incorporation of bioactive molecules, growth factors, or drug delivery systems could be investigated to enhance the material's regenerative properties. Furthermore, long-term degradation studies and mechanical testing under physiological conditions would provide crucial insights into the material's stability and mechanical integrity over time. The present study contributed to understanding sintering time and temperature effects on hydroxyapatite derived from ovine bone; there are still substantial opportunities for future research to address limitations and expand the scope of knowledge. These endeavors would further refine the understanding and application of hydroxyapatite derived from ovine bone for various biomedical purposes.

CONCLUSION

The influence of temperature and sintering duration on hydroxyapatite characteristics is well-documented. Still, this study offers new insights by focusing on hydroxyapatite derived from ovine bone, which is less extensively studied than other sources like bovine or synthetic hydroxyapatite. One essential contribution is using ovine bone as a novel alternative source, which could provide a more accessible or affordable option depending on regional availability and ethical considerations. Additionally, the study identifies an optimized sintering parameter of 360°C for 3 hours, which produced hydroxyapatite with highly interconnected pore structures, potentially enhancing biocompatibility and osteogenic potential. This specific sintering condition may not have been widely explored in previous studies. Furthermore, by directly comparing the ovine bone-derived hydroxyapatite to commercial bone grafts like BioOss and Osseograft, the study offers practical insights into the potential of ovine bone as a viable alternative for bone grafting in dental and orthopedic applications. The findings suggest that ovine bone-derived hydroxyapatite can achieve superior properties to or even superior to commercial products, particularly in biocompatibility and osteogenic differentiation. Also, the compositional or structural advantages of ovine bone hydroxyapatite over other sources would be valuable information in the future. This study unravels the intricate relationship between sintering time and temperature and their consequential impact on the processing of hydroxyapatite derived from ovine bone. The results unequivocally demonstrate that prolonged sintering time and elevated sintering temperature positively influence the material's properties, forming a remarkably uniform and interconnected porous network like the commercially available xenograft BioOss.

DECLARATIONS

Acknowledgments

None

Funding

None

Conflict of interest

None of the authors have any personal or financial conflict to disclose.

Author Contributions

Khushali K. Shah: Conceptualization, Methodology, Data Collection, and Manuscript Writing

Deepak Nallaswamy Veeraiyan: Study Supervision, Review, and Final Approval of the Manuscript

Dhanraj Ganapathy: Project Administration, Data Analysis, and Critical Revision of the Manuscript

Rajlakshmanan Eashwarmoorthy: Biochemical Analysis, Interpretation of Results, and Technical Guidance

Ravleen Nagi: Literature Review, Data Interpretation, and Manuscript Editing

Junad Khan: Statistical Analysis, Interpretation, and Critical Review of the Manuscript

Suresh Venugopalan: Experimental Design, Validation, and Manuscript Review

REFERENCES

1. Azangookhiavi, H., et al., The effect of platelet-rich fibrin (PRF) versus freeze-dried bone allograft (FDBA) used in alveolar ridge preservation on the peri-implant soft and hard tissues: a randomized clinical trial. BMC Oral Health. 2024;24(1):693.
2. Kishen, A., et al., Fabrication of hydroxyapatite reinforced polymeric hydrogel membrane for regeneration. Saudi Dent J. 2023;35(6):678-683.
3. Schmidt, A.H., Autologous bone graft: Is it still the gold standard? Injury, 2021; 52 (2): 18-22. doi: 10.1016/j.injury.2021.01.043.
4. Vaidya, G., et al., Injecting hope: chitosan hydrogels as bone regeneration innovators. J Biomater Sci Polym Ed.2024;35(5):756-797.
5. Guhan, B.B., et al., Utilization of non-vascularized bone graft with regional flap as an alternative for facial reconstruction. Natl J Maxillofac Surg. 2022;13(1):143-146.
6. Üner, D.D. and B.S. İzol, Analysis of whether or not the alveolar palatine bone thickness of the central incisors is sufficient for implants of 3 mm and 3.5 mm thickness using Cone-Beam Computed Tomography. International Dental Research.2019.doi: 10.5577/intdentres.2019.vol9.no2.3
7. Ravipati, V., et al., Accuracy of Dental Implant Placement using Robotics. A Systematic Review of in vitro Studies. The European Journal of Prosthodontics and Restorative Dentistry, 2024. doi: 10.1922/EJPRD_2669
8. Zhao, R., et al., Bone Grafts and Substitutes in Dentistry: A Review of Current Trends and Developments. Molecules, 2021. **26**(10). doi: 10.3390/molecules26103007.
9. Bhatt, R.A. and T.D. Rozental, Bone graft substitutes. Hand Clin. 2012; 28(4):457-68. doi: 10.1016/j.hcl.2012.08.001.
10. Haugen, H.J., et al., Bone grafts: which is the ideal biomaterial? J Clin Periodontol, 2019. **46(21)**:92-102. doi: 10.1111/jcpe.13058.
11. Sparks, D.S., et al., A preclinical large-animal model for the assessment of critical-size load-bearing bone defect reconstruction. Nature Protocols, 2020;**15**(3):877-924. doi: 10.1038/s41596-019-0271-2.
12. Wang, W. and K.W.K. Yeung, Bone grafts and biomaterials substitutes for bone defect repair: A review. Bioactive Materials, 2017. **2**(4):224-247.doi: 10.1016/j.bioactmat.2017.05.007.
13. Figueiredo, M., et al., Effect of the calcination temperature on the composition and microstructure of hydroxyapatite derived from human and animal bone. Ceramics International,2010;**36**(8):2383-2393. doi:10.1016/j.ceramint.2010.07.016
14. Ramirez-Gutierrez, C.F., et al., Effect of the temperature and sintering time on the thermal, structural, morphological, and vibrational properties of hydroxyapatite derived from pig bone. Ceramics International, 2017. **43**(10): p. 7552-7559. doi:10.1016/j.ceramint.2017.03.046
15. Odusote, J.K., et al., Synthesis and characterization of hydroxyapatite from bovine bone for production of dental implants. Journal of Applied Biomaterials & Functional Materials.2019;**17**(2): 2280800019836829.doi: 10.1177/2280800019836829.
16. Cao, H.T., V.L. Ha, and D.N.T. Mai, In vitro assessment of bovine-derived hydroxyapatite for bone xenografts. Journal of Materials Science,2024;**59**(23):10406-10418. doi:10.1007/s10853-024-09794-z
17. Mohd Pu'ad, N.A.S., et al., Syntheses of hydroxyapatite from natural sources. Heliyon.2019;**5**(5):01588. doi:10.1016/j.heliyon.2019.e01588
18. Barakat, N.A.M., et al., Extraction of pure natural hydroxyapatite from the bovine bones bio waste by three different methods. Journal of Materials Processing Technology, 2009;**209**(7):3408-3415. doi.org/10.1016/j.jmatprotec.2008.07.040
19. Jaber, H.L., A.S. Hammood, and N. Parvin, Synthesis and characterization of

hydroxyapatite powder from natural Camelus bone. Journal of the Australian Ceramic Society, 2018. **54**:1-10. doi: 10.1007/s41779-017-0120-0

20. Rahavi, S.S., et al., A comparative study on physicochemical properties of hydroxyapatite powders derived from natural and synthetic sources. Russian Journal of Non-Ferrous Metals, 2017;**58**(3): 276-286. doi:10.3103/S1067821217030178
21. Barakat, N.A.M., et al., Physiochemical characterizations of hydroxyapatite extracted *ve* from bovine bones by three different methods: Extraction of biologically desirable HAp. Materials Science and Engineering: C, 2008. **28**(8): p. 1381-1387. doi:10.1016/j.msec.2008.03.003
22. Granito, R.N., et al., Hydroxyapatite from Fish for Bone Tissue Engineering: A Promising Approach. Int J Mol Cell Med, 2018;**7**(2):80-90.doi: 10.22088/IJMCM.BUMS.7.2.80.
23. Aarthy, S., et al., Exploring the effect of sintering temperature on naturally derived hydroxyapatite for bio-medical applications. J Mater Sci Mater Med, 2019; **30**(2): 21. doi: 10.1007/s10856-019-6219-9.
24. Kamal, M.M., et al., Effects of sintering temperature on the physical, structural, mechanical and antimicrobial properties of extracted hydroxyapatite ceramics from Anabas testudineus bone and head scull for biomedical applications. Results in Materials.2024;**22**:100590. doi: 10.1016/j.rinma.2024.100590