

Comparative Evaluation of Bovine-Derived Hydroxyapatite and Commercial Hydroxyapatite for Potential Hard-Tissue Bioceramic Applications

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ABSTRACT

Hydroxyapatite (HA) derived from biological waste remains an attractive calcium-phosphate bioceramic, owing to its chemical similarity to the mineral phase of human bone and its potential use in hard-tissue repair. The bovine-bone-derived HA has been widely reported, however, a comparative assessment of thermally treated bovine-derived HA to commercial HA is provided in the present study with emphasis on particle size, phase composition, functional groups, morphology and elemental features for preliminary bioceramic screening. The femur bone of the cow was cleaned, defatted, ground, dried and thermally treated with two-step heating to eliminate organic components and to promote HA crystallization. The obtained powder was characterized using particle size analysis, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). The bovine-derived powder was characterized by nanoscale particle.

Keywords- Bovine bone, Bioceramic, Compressive strength, Hydroxyapatite.

I. INTRODUCTION

Multiple therapies have been researched for the cure of bone defects surpassing the boundaries of critical bone injury. One of the therapies most dominantly accepted is bone grafting. Autograft bone surgery is often accompanied by insufficient bone availability [1], as well as, voicing pain and suffering, and the increased chance of infections. Allograft surgery has also had to deal with the fundamental question of disease transmission. There is an increased and overgrowing demand for alternative biomaterials [2, 3]. At present, the bioceramics' chemistry along with their thermal stability, high strength and abrasion, aesthetically pleasing looks plus remarkable biocompatibility makes them apt candidates for hard tissue substitution[4]. They are utilized in different configurations like monoclinic crystals, polycrystalline, composite and coatings [5]. Calcium phosphates hold a considerable amount of potential and have been explored for a number of projects. Biomedically, tri-calcium phosphate and hydroxyapatite extensions have been very beneficial. Hydroxyapatite, a salt compound, is of strong interest for its thermo-stability and for its constituent biosynthesis with bones and teeth [6]. Hydroxyapatite's involvement with vertebrates placed it at the forefront among other candidates for biomineralized materials due to its abundance. Given its function's role in the biological calcification processes, HA serves as the model chemical for biomineralization studies [7, 8]. Increased utilization of HA in face reconstruction, orthopedics, the ear, eye, and dental

implants has led to a numerous slated processes of production and manufacture of HA [9, 10]. As always, the first concern is the ease of production at the lowest cost and maximum effectiveness. Harvesting HA from bone deposits is the easiest and cheapest method. Also, his approach is more biologically safe than any other way [10]. There are three methods for this type of extraction: the first one subcritical water process where the bone is immersed in the water 'd' at 250 degrees Celsius 'C' and the organic phase is gotten rid of by deionizing the water bone with the bone dried for recovery the mineral phase later [11, 12] the second method is alkaline hydrothermal hydrolysis, where the hydrothermal investigation was accomplished by using a sodium hydroxide solution with 25% (wt) at 250 degrees 'C' for 5 hours 'h' at which temperature the organic phase is destroyed [11, 12] and the last way where the process of thermal decomposition occurs. During the phosphate bone lost by the process of heat behaved mineral wherein the remaining phase can be characterized [11]. The novelty of this work is therefore concentrated on three points: (i) the preparation of bovine-derived HA by a simplified thermal-treatment pathway from waste femur bone, (ii) direct comparison with commercial HA by particle-size, XRD, FTIR, SEM and EDX characterization and (iii) a more careful assessment of its potential for hard-tissue applications based on material properties. This difference is relevant because phase purity, crystallinity, carbonate substitution, particle agglomeration and morphology may have a strong influence on the resorption rate, surface bioactivity and processing behavior of HA-based bioceramics.

II. MATERIAL AND METHOD

Preparation of bovine bones

Cortical bone was obtained from fresh bovine femur by scraping off attached soft tissue, periosteum, medullary tissue and bone marrow. This cleaning step was necessary in order to reduce the amount of nonmineral organic material which could impair the purity of the obtained hydroxyapatite powder. The cleaned bone was cut into smaller pieces to allow penetration of solvent and to make the defatting and washing processes more efficient. The defatting of the bone pieces was performed using chloroform to remove the lipid residues and fatty components present within the bone structure. After the defatting procedure, the samples were sequentially rinsed with acetone, distilled water and deionised water to remove residual organic material, traces of solvent and soluble impurities. These washings were done in order to reduce the contamination and to obtain a cleaner mineral rich bone precursor before the thermal treatment.

The pieces of bone were washed to remove residual moisture and then dried as excess water may affect the grinding efficiency and thermal decomposition behavior during calcination. The dried bone samples were then mechanically ground to a fine powder to increase the exposed surface area and to allow for a more uniform heat distribution during the subsequent thermal treatment. A planetary ball mill with zirconia balls was used for further size reduction and homogenization. Zirconia milling media were chosen to minimize external contamination during grinding. The bone powder was milled at 120 rpm for 2 h to obtain a finer and more homogeneous powder. This preparation step was important because the reduction of the size of the particle increases the surface area of the bovine-derived powder, increases the efficiency of organic matter removal during calcination and increases the reproducibility of the subsequent characterization analyses such as the particle size measurement, XRD, FTIR, SEM and EDX.

The cleaning, defatting, washing, drying, grinding and milling were generally carried out to obtain a more homogeneous bovine bone powder precursor to extract hydroxyapatite by thermal decomposition.

Thermal decomposition

The bone powder was prepared from bovine bone and then submitted to thermal treatment in two main stages, drying/dehydration and high temperature calcination. The powder was washed and air-dried under normal laboratory conditions for 24 h to allow slow evaporation of moisture from the surface. This pre-drying was necessary to reduce the excess water prior to oven drying and to prevent a rapid release of moisture during subsequent heating. The air-dried powder was then heated in an electric oven at 170 °C for 24 h to remove any residual moisture and physically adsorbed water from the bone powder. At this point, full dehydration was needed to improve the powder stability during calcination and to reduce the possibility of particle agglomeration caused by the moisture remaining in the powder. The dried powder was re-milled to break down the soft agglomerates formed during the drying process and to increase the uniformity of the powder. The step was useful in order to get a more homogeneous precursor powder, which is important to get uniform heat transfer and uniform decomposition of the organic components during calcination. The first calcination step was done in air at 650 °C for 6 h using a Carbolite Gero furnace. At this stage the powder has changed from light or white to grey, indicating that there has been partial thermal decomposition and combustion of organic constituents such as collagen, residual fat, and other carbon containing constituents. This temperature was chosen as an intermediate treatment step to allow for gradual removal of organic matter without causing too large structural changes on the mineral phase.

A second thermal treatment was carried out afterwards, at 1000 °C for 2.5 h, to improve the removal of the remaining organic residues and to improve the crystallinity of the hydroxyapatite phase. After this step, the powder looked white which indicated that the carbonaceous materials had been more completely removed and more pure calcium phosphate ceramic phase was formed. High-temperature treatment also improved crystal growth and phase stabilization, which are important to obtain hydroxyapatite powder with suitable physicochemical properties for further characterization. The treated powder was

cooled to room temperature and stored in sealed containers prior to analysis to prevent moisture uptake and contamination. The entire sequence of thermal treatments, drying, re-grinding, first calcination and final high temperature treatment is shown in Fig. 1 and Fig. 2.



(c) fragmentation of bone

Fig. 1 Bovine bone preparation.



(a) removal organic bone and whitening by heat treatment



(b) bone grinding step 1



(c) bone grinding step 2



(d) bone grinding using high speed electric grinder



(e) final draying powder



(f) heating the powder to 650°C



(g)final powder after heating 1000°C

Fig. 2 Steps to prepare bone powder for hydroxyapatite extraction.

III. CHARACTERIZATIONS

The physicochemical, structural, morphological and compositional properties were characterized and compared with commercial hydroxyapatite powder for the bovine-bone-derived hydroxyapatite powder. This comparison was made to find out whether the extracted material had characteristics consistent with hydroxyapatite and to see if it could be useful as a bioceramic material for hard tissue applications. Complementary information about the prepared powder was obtained using several characterization techniques. The identification of the main functional groups in the samples was done by FTIR. Special attention was paid to the characteristic bands of absorption of phosphate groups, hydroxyl groups, carbonate substitutions and adsorbed water. The presence of phosphate and hydroxyl bands is important for confirmation of hydroxyapatite formation, while the bands associated with carbonate may indicate the presence of carbonated hydroxyapatite, generally associated with apatite materials of biological origin. The FTIR spectra of the bovine derived powder were compared to the spectra of commercial HA to identify similarities and potential differences in chemical bonding. Analysis of X-ray diffraction was done to determine the crystalline phases and crystallinity of prepared powder. The analysis was performed in a range of $2\theta = 20-80^\circ$ with Cu K α radiation. The diffraction peaks obtained were compared with the standard hydroxyapatite reference patterns to confirm the formation of the HA phase and to identify any possible secondary calcium phosphate phases. The position, intensity and sharpness of the diffraction peaks were considered as the indicators of phase formation, crystallinity and structural changes occurred during the thermal treatment. The powder particles surface morphology was studied by SEM. This analysis provided information on particle shape, surface texture, agglomeration behavior and features associated with porosity. These morphological characteristics are important because they can affect surface area, cell attachment behavior, and the potential interaction of the material with surrounding biological tissue. Elemental composition of the prepared powder, especially the presence of calcium, phosphorus and oxygen was also determined by Energy Dispersive X-ray spectroscopy (EDX). The Ca/P ratio was calculated to assess the chemical composition of the extracted hydroxyapatite and to compare it with the theoretical Ca/P ratio of stoichiometric HA. Moreover, powder-size distribution and degree of polydispersity were evaluated by particle-size analysis. This analysis was important because the particle size and distribution can affect the powder reactivity, sintering behavior, surface area and potential biomedical performance.

The particle size results were used together with SEM observations to provide a more complete understanding of the powder morphology and homogeneity. Overall, the combined characterization methods enabled a systematic comparison between bovine-derived HA and commercial HA and provided useful information regarding the suitability and limitations of the extracted powder for further bioceramic development.

IV. RESULT

Particle size results

Particle size analysis was performed to assess the particle size distribution of the extracted bovine-derived hydroxyapatite powder and to compare the particle characteristics with that of commercial hydroxyapatite. The particle size is an important parameter for bioceramic powders, as it can influence surface area, reactivity, dispersion behavior, sintering response and possible interaction with biological fluids. The average effective particle diameter of the present bovine-derived HA sample was 83.5 nm, while the commercial HA sample was larger with an average effective particle diameter of 424.1 nm. This result shows that the extraction and milling process generated bovine-derived HA particles in the nano scale range.

The nanoscale size of the HA powder of bovine origin is considered as an advantage for preliminary bioceramic development. In general, the smaller the particle, the larger the specific surface area compared to a larger particle. Such increased surface area can improve the surface reactivity, ion exchange and interaction with biological fluids which are important factors for bioactive calcium phosphate materials. Furthermore, nanosized HA particles could be used in implant coatings, bone substitute fillers, composite reinforcement and scaffold manufacturing, where good surface interaction and powder dispersion are needed. However, the average particle size of bovine derived HA was in nanoscale, but the sample showed relatively broad size distribution. The bovine derived sample showed a polydispersity index of 0.388, which indicates a moderate heterogeneity of the particle-size distribution. This indicates that the powder is not uniform and may contain aggregated particles or groups of particles that have been formed during drying, calcination or milling. Fine ceramic powders often show agglomeration due to the high surface energy of small particles and tendency of agglomeration. Such behavior may be detrimental to the effective dispersion of the powder and may affect the reproducibility of subsequent processing steps. On the other hand, the commercial HA sample showed an average effective particle diameter of 424.1 nm with a PDI value of 0.335. The commercial HA (larger average particle size) had a slightly lower PDI, indicating a slightly narrower particle-size distribution than the bovine-derived sample. This comparison indicates the advantage of smaller particle size of bovine-derived HA powder, but needs to improve in particle uniformity and dispersion stability. Thus, the particle-size results indicate that the bovine-derived HA powder possesses promising nanoscale characteristics for the first stages of bioceramic development. However, additional post-processing steps may be necessary before the powder can be used for scaffold fabrication, implant coating or other biomedical processing routes. The steps can be sieving, centrifugation, ultrasonic dispersion, controlled milling or the optimization of the drying and calcination conditions, so as to decrease the agglomeration and improve the homogeneity of the powders. Improving the uniformity of particle size would be beneficial for the reproducibility, processability and performance of the extracted HA powder. Fig. 3 displays the comparison of particle-size distribution of bovine-derived HA and commercial HA samples.

Particle Size Analysis Result of Bovine-Derived Hydroxyapatite Powder

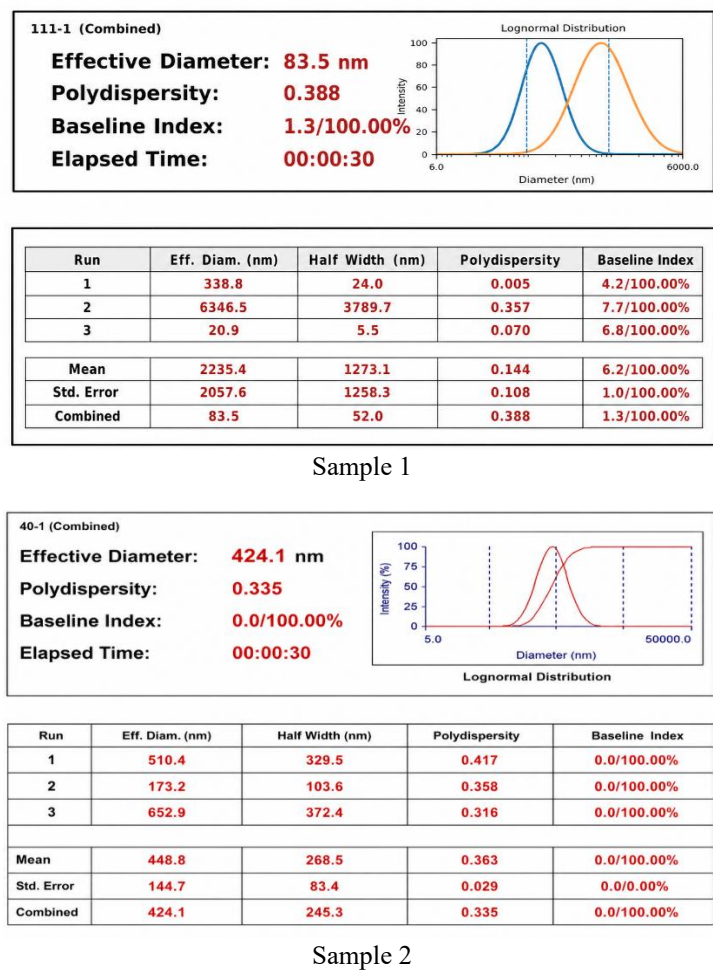


Fig. 3 Examination result Particle Size of powder for sample 1, 2.

X-ray Diffraction (XRD)

X-ray diffraction (XRD) analysis was performed to determine the crystalline phases present in the thermally treated bovine-derived powder and to compare with the commercial hydroxyapatite (HA) reference sample. The XRD pattern of the sample from bovine showed diffraction peaks characteristic of hydroxyapatite, especially around 26° and in the range 31.7° – 33.1° 2θ . These peaks are generally related to the principal apatite reflections, including the planes related to crystalline HA. The presence of these peaks confirms that the thermal treatment has removed the majority of the organic constituents of the bovine bone and induced the formation of a crystalline calcium phosphate phase. The bovine derived HA shows sharp and well defined diffraction peaks which suggest an increase in crystallinity after high temperature treatment. This indicates that the calcination process had an important role in the transformation of mineral phase of bovine bone into a more stable and crystalline HA structure. Moreover, the position of the bovine-derived HA peaks is generally in agreement with the standard HA reference pattern reported in JCPDS 09-0432 further supporting the successful formation of hydroxyapatite. The diffraction profile of the bovine-derived sample was similar to that of commercial HA, suggesting that the two samples were mainly composed of HA phase. Minor differences in the intensity and sharpness of the peaks could be due to differences in the crystallinity, size of the particles, thermal history and the natural origin of the material from bovine sources.

In general, the XRD results confirmed that the extracted bovine derived powder was successfully converted into crystalline hydroxyapatite after thermal treatment. The similarity between the bovine-derived HA and commercial HA patterns indicates that the prepared material is promising to be used as a bioceramic precursor for biomedical applications. Fig. 4 shows the XRD patterns of bovine-derived HA and commercial HA.

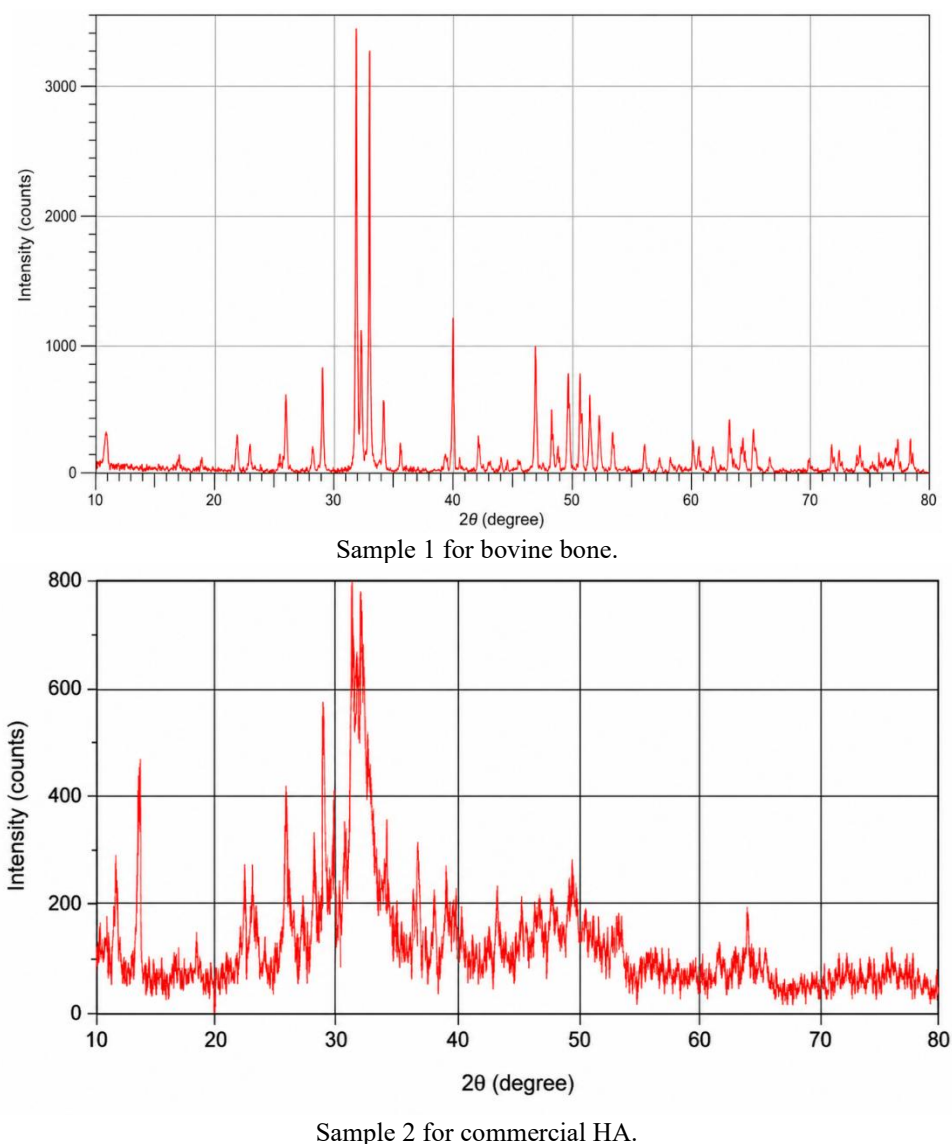


Fig. 4 Examination result X-ray Diffraction (XRD).

The maximum intensity reflections for sample 1 at:

- $2\theta = 31.98^\circ \rightarrow d = 2.7967 \text{ \AA} \rightarrow (211)$
- $2\theta = 33.12^\circ \rightarrow d = 2.7025 \text{ \AA} \rightarrow (112)$
- $2\theta = 40.01^\circ \rightarrow d = 2.2517 \text{ \AA} \rightarrow (300)$

The maximum intensity reflections for sample 2 at:

- $2\theta = 32.05^\circ \rightarrow d = 2.7898 \text{ \AA} \rightarrow (211)$
- $2\theta = 29.19^\circ \rightarrow d = 3.0561 \text{ \AA} \rightarrow (112)$
- $2\theta = 26.02^\circ \rightarrow d = 3.4209 \text{ \AA} \rightarrow (300)$

The absence of sharp peaks that could be attributed to major secondary phases like CaO or β -TCP suggests that HA was the predominant phase under the thermal conditions used. However caution is needed in interpretation, because minor phases are hard to detect when they are in small amounts or overlap with HA peaks. The small differences of 2θ values between bovine-derived HA and commercial HA can be attributed to carbonate substitution and lattice variation, which are common in biogenic apatite. Partially substituted carbonate-containing HA could be useful since it is more similar to the mineral phase of natural bone than fully stoichiometric HA. High crystallinity, however, may also decrease rates of dissolution and resorption. Therefore, the XRD results indicate the powder’s initial suitability for bioceramic production, but alone cannot determine biological performance.

The outcomes of the comparison between hydroxyapatite sourced from bovine bone and commercial hydroxyapatite, as well as the comparison to previous studies [13], are illustrated in the Table. 1.

Table. 1 Peak data list comparison for HA from different sources.

Peak	2 theta degree			Peak	2 theta degree		
	HA from Bovine bone	Commercial HA	HA previous study [13].		HA from Bovine bone	Commercial HA	HA previous study [13].
1	11.0454	10.4163	11.1250	15	44.0618	45.5105	44.17821
2	21.9881	11.8077	22.1125	16	46.8970	46.7098	45.3342
3	23.0774	13.6594	23.1004	17	48.2814	48.0292	47.0102
4	26.0818	16.9302	25.9975	18	48.8133	49.6484	48.5217
5	28.3204	18.4474	28.3520	19	49.6806	50.8279	49.0112
6	29.1367	20.9800	29.4581	20	50.6860	53.2668	50.4987
7	31.9752	22.9253	31.6661	21	51.4719	56.1657	51.2501
8	32.3959	26.0258	32.3327	22	52.2766	59.6444	53.4675
9	33.1211	29.1977	32.5321	23	53.3914	61.8837	55.9998
10	34.2610	32.009	34.26660	24	56.0682	64.1130	57.5232
11	35.6665	33.9589	35.3999	25	57.3195	65.3826	59.9987
12	39.3910	36.7264	39.3300	26	58.2381	68.3817	61.9898
13	40.0092	39.4343	39.9981	27	60.1380	69.4814	63.1867
14	42.3199	43.3917	42.1849	28	60.6156	71.8207	64.0012

In the bovine-derived HA, the commercial HA and the referenced previous study [13] we observed apatite structure related reflections including peaks at around 26° (002), $31.7\text{-}33.1^\circ$ (211/112/300) and 34.3° (202). This agreement is in support of the formation of HA as the main phase. Calcium phosphate residues such as brushite or octacalcium phosphate could account for the low angle features seen in the commercial HA. In the other hand, the pattern of HA from bovine origin shows the prevalence of a crystalline apatite phase after heat treatment. These results are consistent with the phase-identification result, but more refinement, for example, Rietveld analysis, would be helpful in future work to quantify phase fractions and crystallite size more accurately. [14].

Fourier Transform Infrared Spectroscopy (FTIR) result

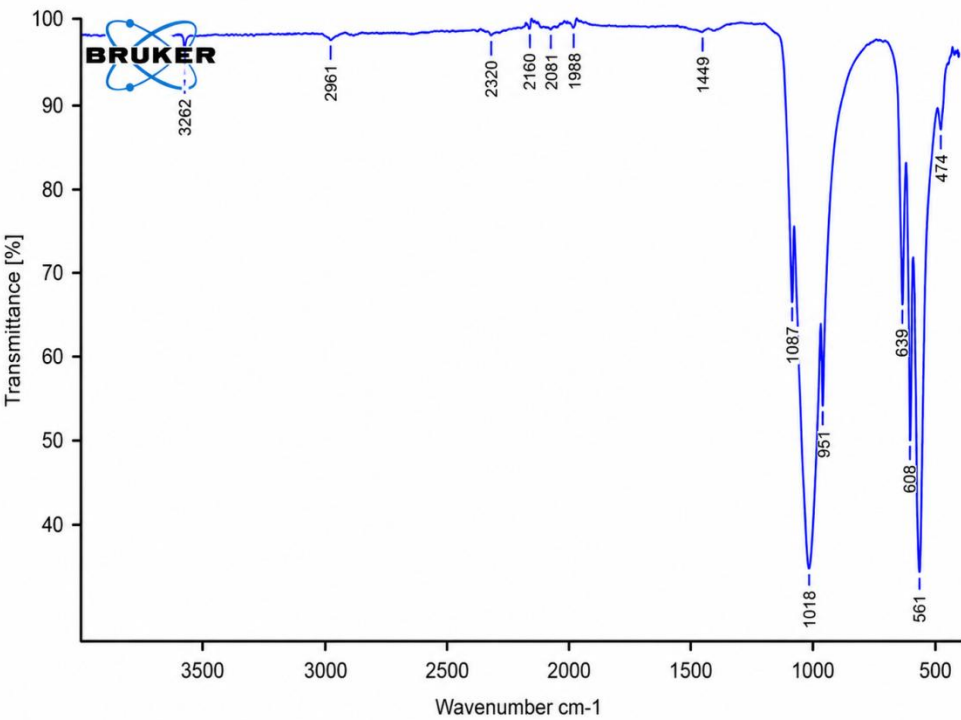
FTIR spectroscopy was performed to identify the main functional groups present in the prepared bovine-derived hydroxyapatite (HA) and to compare with those observed in the commercial HA reference sample. The FTIR spectrum of the bovine-derived sample showed several characteristic absorption bands related to the phosphate group (PO_4^{3-}), the main structural unit of hydroxyapatite. These bands related to phosphate support the successful formation of HA after thermal treatment and confirm the presence of calcium phosphate bonding in the apatite structure. In addition to the phosphate vibrations, bands related to hydroxyl groups (OH^-) and adsorbed water molecules were also observed. Those bands could be attributed to the structural hydroxyl groups of HA and moisture physically adsorbed on the powder surface. The bovine-derived HA spectrum also showed the presence of carbonate-related bands. A carbonate contribution such as this is expected in biologically derived hydroxyapatite inasmuch as natural bone mineral is not generally pure stoichiometric HA but is in

fact carbonated apatite. The carbonate ions may substitute partially the apatite lattice, which shows that the prepared material has retained some biological properties of the natural bone mineral.

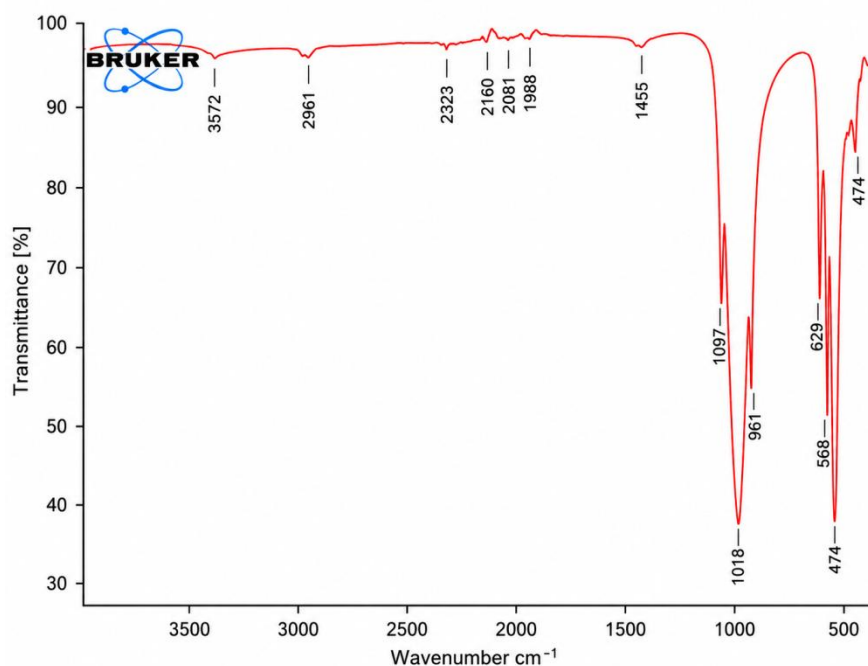
The bovine-derived HA exhibited similar phosphate-related absorption features compared to the commercial HA sample, suggesting that the calcination process was effective in transforming the bovine bone mineral phase into hydroxyapatite. However, small differences in band intensity or width can be due to differences in crystallinity, carbonate content, residual moisture, particle size and natural origin of the bovine-derived material. The FTIR results in general show the formation of HA in the prepared bovine-derived powder and confirm the XRD results with respect to the successful conversion of the thermally treated bone material into a hydroxyapatite phase. The main FTIR spectra and the corresponding band assignments are given in Fig. 5 and Table 2, respectively.

Table. 2 Bond vibration peaks for HA compound.

HA	P-O	P-O-P	C-O	O-H(H ₂ O)	O-H(HA)
	Stretching vibration mode	Bending mode			
[15]	962 1038 1095	565 603	1420	1650	3570
Commercial HA	908 1373 1252 1029	526 542 560 600 624	1574	1800	3700
HA bovine bone	961 1087 1018	561 598 629	1988	1458	3572



(a) FTIR for sample 1.



(b) FTIR for sample 2.

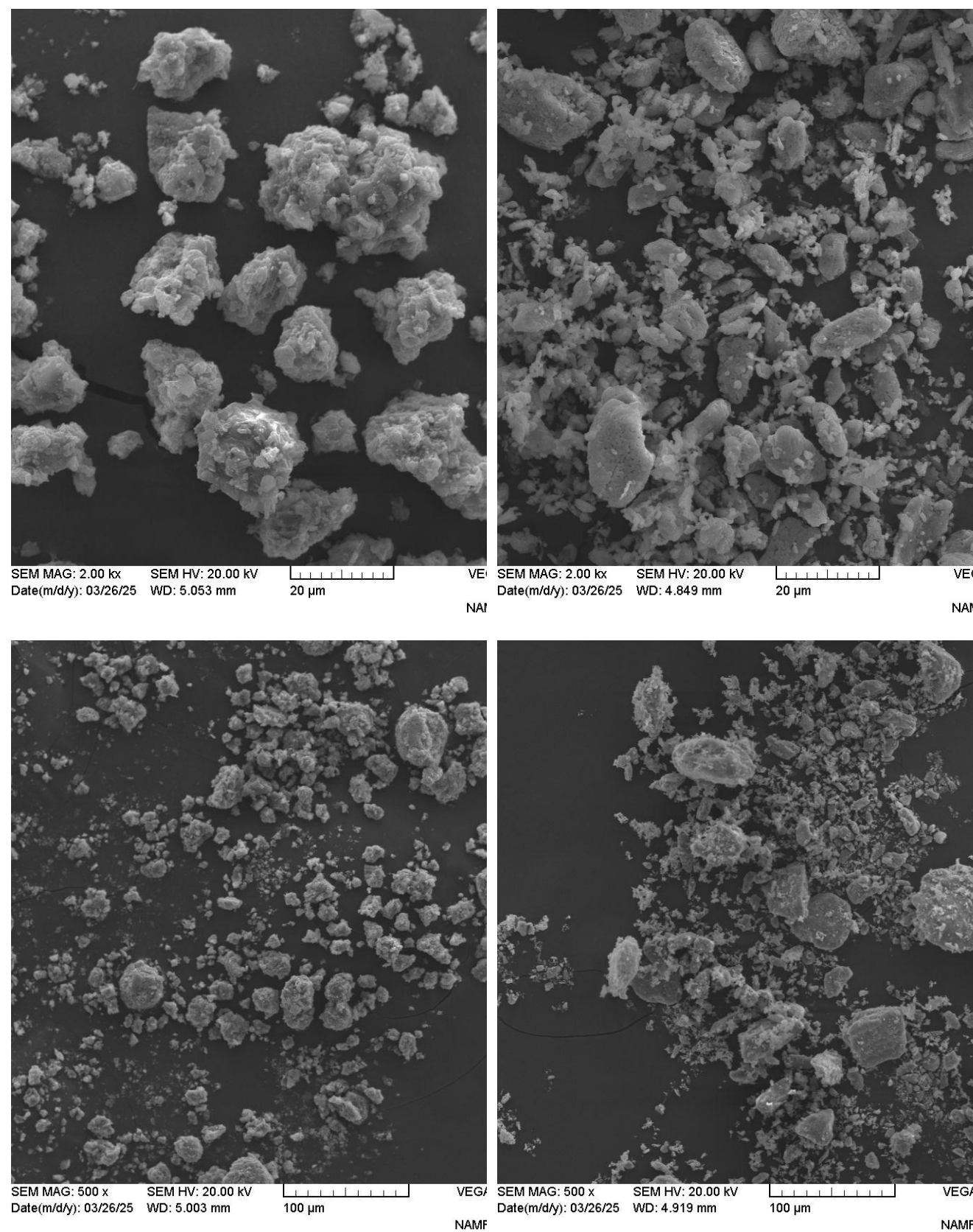
Fig. 5 Examination result FTIR.

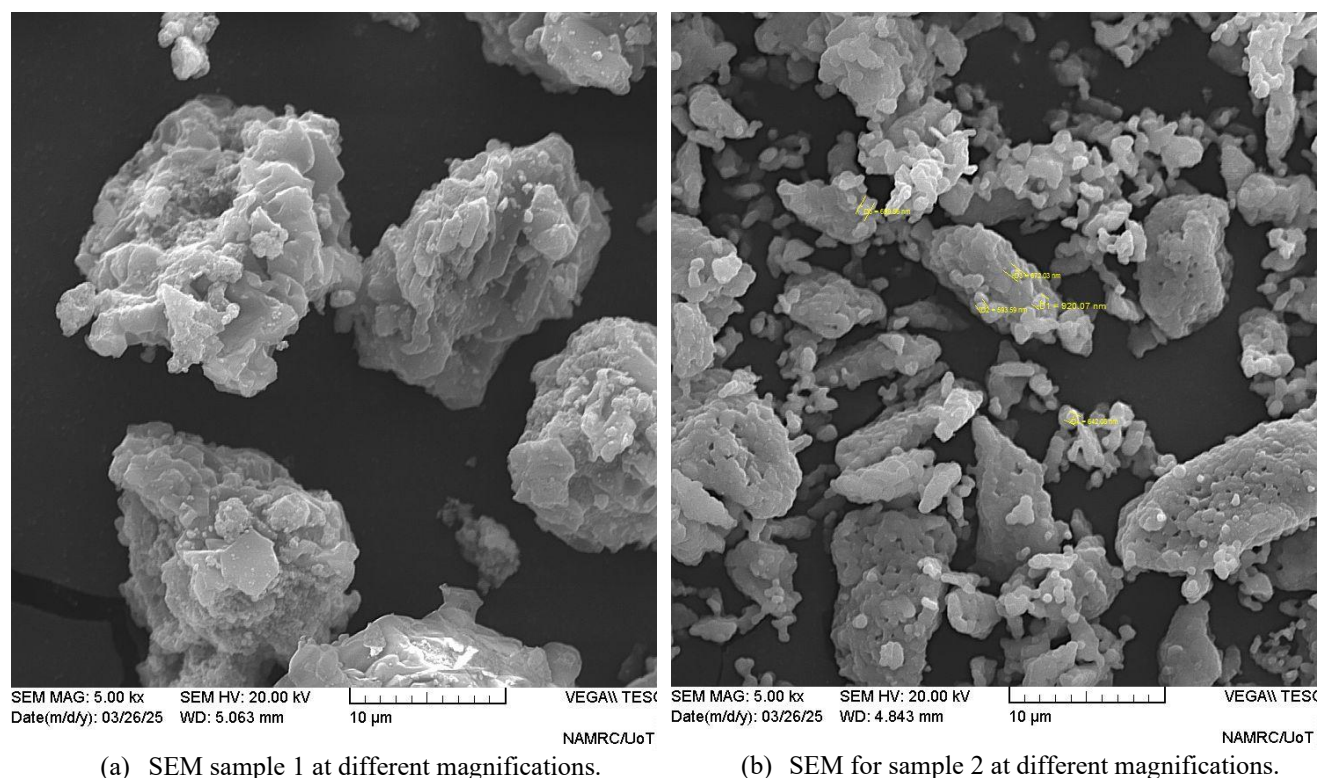
The bands at ~561, 598 and 629 cm^{-1} are assigned to phosphate bending vibrations, while the bands at ~961, 1018 and 1087 cm^{-1} are related to phosphate stretching vibrations. These bands confirm the apatite phosphate framework presence. The band near 3572 cm^{-1} is assigned to structural hydroxyl groups in HA and features such as the band near 1458 cm^{-1} are assigned to carbonate incorporation. It is important that carbonate is present because biological apatite is often non-stoichiometric and carbonate substituted. However, excess carbonate or residual organic contamination may influence crystallinity and dissolution behavior, hence FTIR results should be interpreted together with XRD and EDX, and not alone, to claim biomedical suitability.

Scanning Electron Microscope (SEM) analysis

The surface morphology, particle shape and microstructural features of the prepared bovine derived hydroxyapatite (HA) powder were studied by SEM analysis and compared with that of commercial HA sample. SEM micrographs of bovine derived HA exhibited irregular shape, rough surface, porous features and significant agglomeration of particles. This morphology is typical of HA powders obtained from natural bone sources and especially after heat treatment and subsequent milling. The agglomerated structure of the bovine derived HA may be because of the nanoscale nature of the particles and their high surface energy. During calcination the organic components are eliminated and the mineral phase becomes more crystalline. However, the thermal process can also lead to particle bonding or partial sintering, which may result in the formation of clustered particles. Moreover, the particle size can be reduced in the milling step, but the fine particles may not be completely separated and agglomerates can be formed. The roughness and porosity of the HA surface obtained from bovine is considered as an advantage for biomedical applications especially for bone tissue engineering and bone repair. The porous and irregular surface may provide a larger contact area for interaction with biological fluids, and may promote protein adsorption, cell attachment and early bone-like apatite formation. These surface characteristics are important as they can affect the biological response of the material when used as a scaffold, coating, filler or bioceramic precursor.

But the strong agglomeration can cause some processing problems as well. Agglomeration of particles can cause a loss of powder homogeneity and influence the uniformity of scaffold fabrication, ceramic forming, coating deposition and sintering behavior. If particles are poorly dispersed, the final product may have a non-uniform microstructure, uneven porosity, poor mechanical performance or inconsistent biological response. Therefore, further powder processing steps may be needed before the bovine-derived HA can be used in a final biomedical product. Such steps include controlled milling, sieving, ultrasonication, optimization of dispersion or use of appropriate binders and dispersing agents. The bovine origin HA presented a more irregular and porous morphology than the commercial HA sample that could be attributed to its natural origin and the conditions of processing during calcination and milling. The SEM results indicated that the prepared bovine-derived HA is promising in terms of surface characteristics for bone-related applications. But further optimization of particle dispersion and forming processes is needed to improve the powder uniformity and processing reliability. SEM micrographs of HA samples derived from bovine and commercial HA are shown in Fig. 6.





(a) SEM sample 1 at different magnifications.

(b) SEM for sample 2 at different magnifications.

Fig. 6. SEM examination.

V. DISCUSSION

The results of the present study confirmed that hydroxyapatite (HA) can be successfully obtained from bovine bone using a simplified thermal-treatment route. But the scientific contribution of the present work is not limited to the extraction of HA from bovine bone, as this has been reported in previous studies. However, the main contribution of this work is in the comparative evaluation of bovine-derived HA with commercial HA and in the evaluation of the properties of the obtained material with respect to possible hard-tissue bioceramic processing.

The particle size analysis showed the average effective diameter of bovine derived HA to be 83.5 nm indicating the powder to be in the nano-scale range. This nanoscale particle size is good for bone related biomaterials as it can improve the surface area and possibly enhance the interaction with proteins, cells and physiological fluids. However, the PDI value of 0.388 indicates a moderate broad particle size distribution. This indicates that the powder was not perfectly homogenous and contained some degree of aggregation. Such heterogeneity can arise from incomplete particle separation during milling, high surface energy of fine particles or clustering after thermal treatment. Therefore, despite the promising particle size, further optimization by controlled milling, sieving, centrifugation, ultrasonication or dispersion enhancement is required before the powder can be applied in scaffold fabrication, implant coating or other biomedical processing routes. In this study, the XRD pattern indicated that hydroxyapatite was the main crystalline phase after thermal treatment. The major peaks appeared at about 26° and in the range of 31.7° – 33.1° , which are generally related to apatite structure. The peak positions were close to the HA reference pattern, which suggested that the bovine bone mineral was successfully converted into HA. No intense peaks belonging to CaO or β -TCP were detected, suggesting that the heat-treatment condition was adequate and did not lead to evident decomposition of the HA phase. The relatively sharp peaks also indicated the good crystallinity of prepared powder. This is important for HA-based bioceramic materials since crystallinity can play a role on phase stability and material performance. However, the existence of very small secondary phases cannot be totally eliminated from this analysis alone. Further quantitative phase analysis for more accurate evaluation of prepared HA purity and stability is recommended for future work.

The FTIR analysis also confirmed the formation of HA in the prepared sample. The main bands associated with phosphate groups are clearly observed and these bands are usually associated with apatite structure. Also observed are bands associated with hydroxyl, which confirms the formation of hydroxyapatite after calcination. In addition, some carbonate bands were detected in the spectrum. This could be that the HA obtained is not fully stoichiometric, but has carbonate substitution. This result is plausible, since carbonated apatite is often found as a natural bone mineral. The presence of carbonate may be beneficial in that it makes the bovine derived HA more similar to natural bone. But it should be also

considered carefully, as carbonate substitution can influence the crystallinity, solubility and degradation behavior of HA. For this reason, future work should assess the content of carbonates and their influence on the final materials properties.

The SEM images revealed that the HA powder derived from bovine consisted of irregular particles with rough, porous, and agglomerated surfaces. This type of morphology could be beneficial for bone-related applications as the rough and porous surface may improve the contact with biological fluids and provide suitable sites for cell attachment. However, the agglomeration observed in the sample has to be considered as a limitation. Strong agglomeration of the particles can reduce the uniformity of the powder and affect the following processing steps such as scaffold preparation, coating formation and sintering. This might also affect the final mechanical performance of the material. Hence, the surface morphology of the prepared HA is promising but further optimization of the processing is required before practical biomedical use. The physicochemical characteristics of bovine derived HA are promising as shown by the particle-size analysis, XRD, FTIR, SEM and EDX results together. These findings indicate that the prepared powder could be a potential material for hard-tissue bioceramic applications. However, the present characterization is not sufficient for proving its clinical applicability. More tests are needed before the material can be considered suitable for practical applications. Future work should include mechanical evaluation such as compressive strength and hardness, as well as porosity and density measurements. In addition, in vitro bioactivity testing in simulated body fluid, degradation studies, coating adhesion tests and cytocompatibility tests using bone related cells should be performed. Further investigations of these studies will be useful to confirm the mechanical reliability and biological safety of the produced bovine-derived HA for future hard-tissue repair applications.

VI. CONCLUSIONS

The thermal decomposition method is cheap and simple for extracting HA from wasted materials. Due to its biocompatibility and normal ossification conduction, it most probably can be used and substituted in the human body. It has been reported that bovine femur was heated at 1000°C for 2 hours to extract HA. This extraction is of great interest to the medical field. Rendering bone samples to 1000°C also produced quite TCP-like calcium phosphate compounds for use in biodegradable applications. Therefore, it can be concluded that the three variables of temperature, heat treatment duration and particle size of bone powder are important in defining the composition for the extracted phosphate of calcium and hence its application.

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