



Naturally Derived α Tricalcium Phosphate Based Porous Composite Bead Production

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Abstract

In this study, a simple, innovative approach is applied to produce porous α -TCP-CeO₂-Al₂O₃ composite beads via using bovine bone-derived hydroxyapatite, cerium oxide, and alumina ceramics. Bovine-bone derived hydroxyapatite was obtained via calcination of bones at 950°C for 3 hours. Hydroxyapatite is a thermally unstable biomaterial at high temperatures, and depending on its stoichiometry decomposes at 800°1200°C. Sodium alginate was successfully used as an in situ gelling templates for the production of the ceramic beads and starch, an environmentally friendly and economic pore-forming agent, is used to achieve interconnected, highly open porosity containing composite beads. Sintering of the ceramic–starch–alginate green composite beads at 1200°C for 1 hour resulted in the decomposition of the hydroxyapatite phase and formation of α -TCP. XRD analysis revealed that α -TCP-CeO₂-Al₂O₃ composite beads were achieved. SEM investigations of the produced composite beads revealed that bimodal pore size distribution, fine and coarse, was achieved.



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Introduction

Accidents, falls, sports injuries or other causes such as low bone density and osteoporosis may

lead to bone injuries/fracture.¹ Implantation of bone autografts or allografts is considered an ideal strategy to heal bone defects. However, some drawbacks

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such as limited availability, extended surgical time and complications related to its harvesting process like donor site morbidity and pain limit the widespread usage of these strategies. Increasing advances in technology enabled researchers to develop synthetic, bioceramic, and polymer based bone substitute materials.²⁻⁵ Bioactive calcium phosphate (CP) ceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP) are attractive materials for bone tissue applications because of their similarity to natural bone, osteoconductivity, bioactivity, biocompatibility and chemical stability in body fluid.⁶⁻⁷ HA and TCP ceramics are the most commonly used CP ceramics for bone replacement.

There are two main production methods for the calcium phosphate ceramic powders such as HA and TCP. These are relatively high cost conventional chemical methods⁸⁻¹² and relatively low-cost natural resources.^{8,13-17} Natural sources such as bovine bones,¹⁸⁻²³ fish scales,²⁴⁻²⁶ egg shells,²⁷⁻²⁹ fruit wastes,²⁷ waste kina shells,³⁰ sea snail,³¹ and oyster shells^{32, 33} are used to produce micro and nano CP ceramic powder synthesis.

Despite excellent biocompatibility properties, low strength and brittle nature limits the use of HA [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] especially in load-bearing applications.^{34, 35} HA decomposes into CP phases such as TCP and tetracalcium phosphate (TTCP). Low mechanical strength of HA is attributed to these calcium phosphate phases which are brittle and have weaker strength.³⁶ Several strategies such as making composites and using different pressing/sintering methods like hot press sintering,³⁷ spark plasma sintering,^{38, 39} underwater shock compaction⁴⁰ and microwave⁴¹ have been employed to improve the mechanical properties of HA.

Reinforcing porous HA and TCP with materials such as alumina,^{42, 43} zirconia,⁴³⁻⁴⁵ titania^{46, 47} and calcium silicate^{48, 49} have been applied to enhance strength and fracture toughness of HA ceramics. Alumina is an attractive bio-inert material with high chemical and mechanical strength however it can not form biochemical interfacial bond with the natural tissue.^{42, 50} On the other hand HA is a highly bioactive

ceramic able to form a sound, firm bond with bone tissue. Therefore, highly bioactive composite scaffolds with a relatively enhanced mechanical strength can be produced by reinforcement of porous HA with alumina powder.⁵¹ The pores in the structure of these bioceramics provide enough space for cell ingrowth and nutrient uptake facilitating bone tissue formation.⁵² Due to these advantages porous materials are potentially suitable for various hard tissue applications such as bone cement, total hip joints and tooth implants.⁵³⁻⁵⁶

So far, several methods have been reported for the preparation of HA and TCP composites such as sol-gel process,⁵⁷ chemical precipitation process,⁵⁸ spark plasma sintering,⁵⁹ and high energy ball milling.⁶⁰ In this study, a facile innovative approach is applied to produce porous α -TCP-CeO₂-Al₂O₃ composite beads via using bovine bone derived HA, cerium oxide, and alumina ceramics and pressureless sintering. Starch, an environmentally friendly and economic pore-forming agent, is used to achieve interconnected, highly open porosity containing composite beads. Starch also has a controlled quality, simple processing, commercial availability, easy handling and low burnout temperature without generation of toxic materials.⁶¹

Experimental Studies

Bovine bones, collected from a local meat shop in Konya/Turkey, were initially manually cleaned and then boiled in hot water to remove the adherent meat and other organics. Cleaned bones were rinsed with distilled water for many times and then dried in an oven at 100°C overnight before calcination.

Bovin bones were calcined at 950°C for 3 hours at a heating rate of 10°C/min in air atmosphere. After calcination bones were ground to the powder form in an agate mortar. Al₂O₃ (Esan Eczacibasi Group, Turkey) and CeO₂ powders were used to produce three different α -TCP-CeO₂-Al₂O₃ composite beads (Table 1). Corn starch (Gunes, Turkey) was obtained from a local market and used as a pore forming agent. Sodium alginate (SA) and calcium chloride (CaCl₂) were obtained from Katki Dunyasi, Turkey.

Table 1: Designed compositions

Designation	HA Amount, g	Al ₂ O ₃ Amount, g	CeO ₂ Amount, g	Starch Amount, g
30H10A5C	30	10	5	10
20H20A5C	20	20	5	10
10H30A5C	10	30	5	10

H: HA, A: Al₂O₃, C: CeO₂

Composite bead production was performed by the following method. SA was dissolved in distilled water by magnetic stirring at room temperature for 24 hours to achieve 3 wt. % SA solution. To prepare a homogenous slurry 45 g ceramic powders (HA + Al₂O₃ + CeO₂), 10 g starch, and 150 mL SA solution was mixed in a planetary ball mill at a rotation speed of 300 rpm for 50 minutes. The cross-linking solution was prepared via dissolving 1 wt.% CaCl₂ in distilled water by magnetic stirring at room temperature for an hour. Achieved aqueous

solution was added dropwise into the CaCl₂ aqueous solution at room temperature using a 60 mL hypodermic syringe through a needle under a constant stirring condition at room temperature. The alginate–ceramic composite beads are obtained via cross-linking in the CaCl₂ solution for at least an hour. Subsequently the beads are removed from the CaCl₂ solution by sieving and washed for many times with distilled water. Fig. 1 depicts the fabricated spherical ceramic–starch–alginate green composite beads.

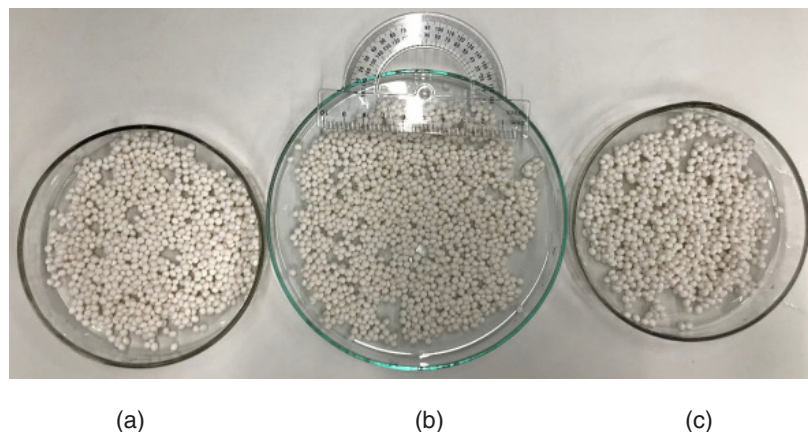


Fig. 1: Spherical ceramic–starch–alginate green composite beads.
(a) 30H10A5C, (b) 20H20A5C, and (c) 10H30A5C

The thermal behavior of the starch and sodium alginate was investigated via thermogravimetry (TG) analysis (TA, SDT650) in an air atmosphere at a heating rate of 10°C/min. Ceramic–starch–alginate composite beads were pressureless sintered at 1200°C for an hour. Applied heating and cooling rate was 10°C/minute. Porosity content, pore size and distribution, grain size and distribution of the porous

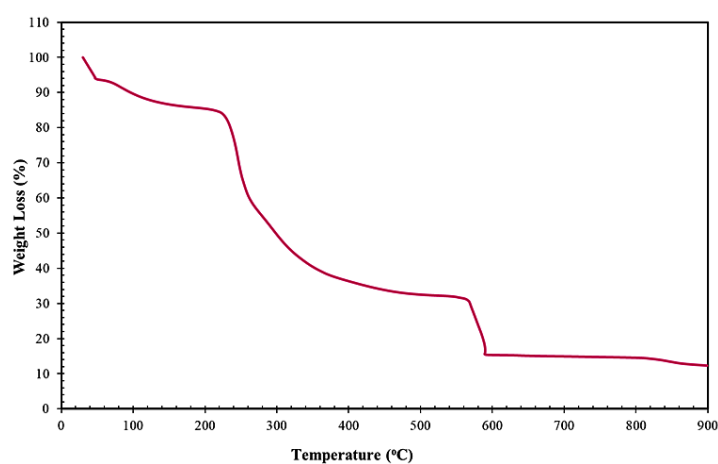
composite beads were investigated with a scanning electron microscope (SEM, Carl Zeiss/Gemini 300) in a secondary electron (SE) image mode. Phase distribution and elemental compositions of the grains were investigated via an energy dispersive X-ray spectrometer (EDX, Bruker). Phase content investigation of the calcined bovin bones and produced composite beads were carried out by X-ray

diffraction (XRD, Bruker AXS/Discovery D8), using monochromatic Cu-K α radiation ($\lambda=1.5406 \text{ \AA}$)

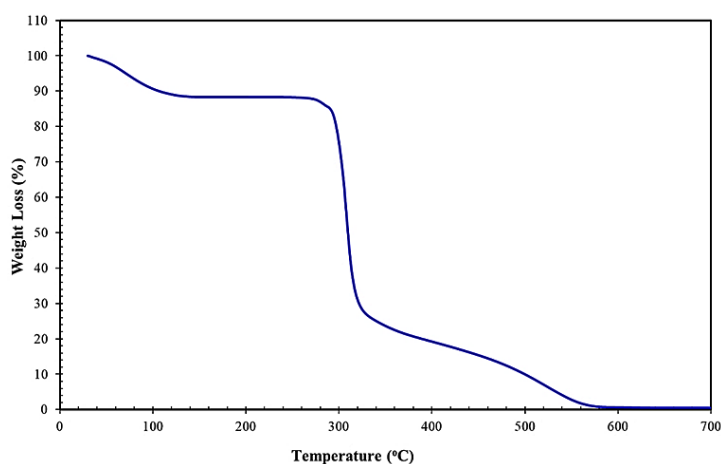
Results and Discussion

According to TG analysis, the corn starch demonstrated three significant weight change stages (Fig. 2-b), while five weight change reaction was occurred when sodium alginate was analyzed (Fig. 2-a). The first stage (room temperature-190°C) in the TGA thermogram was related to the release of the adsorbed water for both materials. At higher

temperatures, decomposition of the materials was observed. Corn starch decomposed in two-stage, while sodium alginate decomposed in four stages. Sodium alginate loses almost 80 wt. % and 85 wt. % of its weight at 600°C and at 872°C, respectively. The corn starch loses approximately 100 wt. % of its weight at 600°C. The corn starch undergoes decomposition suddenly in the beginning stage, which undergoes up to 326°C, and after this temperature, the weight loss speed was decreased.



(a)



(b)

Fig. 2: TG analysis result of (a) alginate, and (b) corn starch

SEM investigations of the corn starch (Fig. 3) revealed that the particle size distribution of the

starch is relatively narrow. Particle size of the starch is changing from ~5 to ~25 μm range.

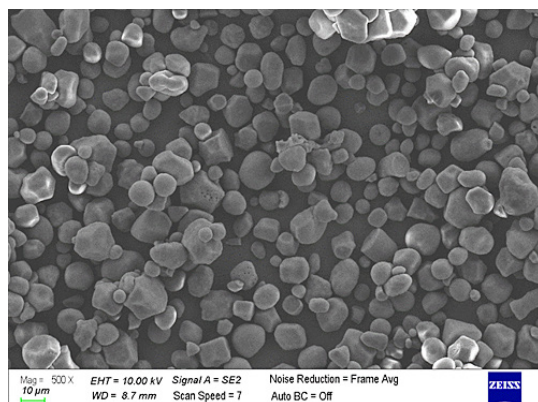


Fig. 3: SEM image of the corn starch

XRD analysis result of the calcined bovine bone and sintered composite beads are given in Fig. 4. XRD

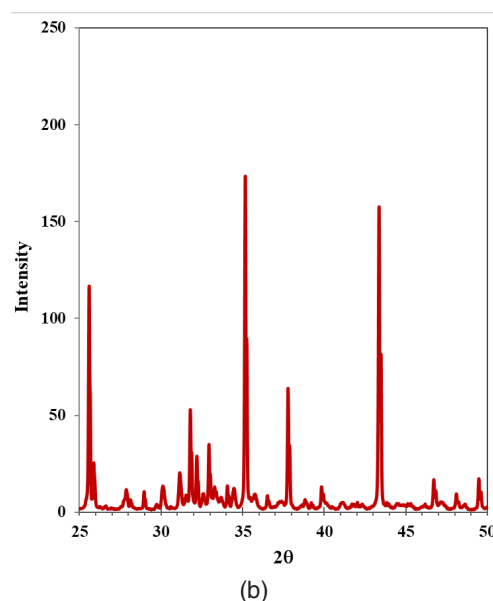
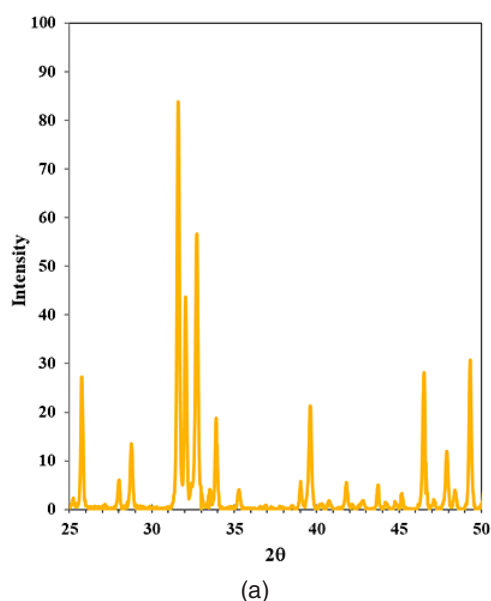
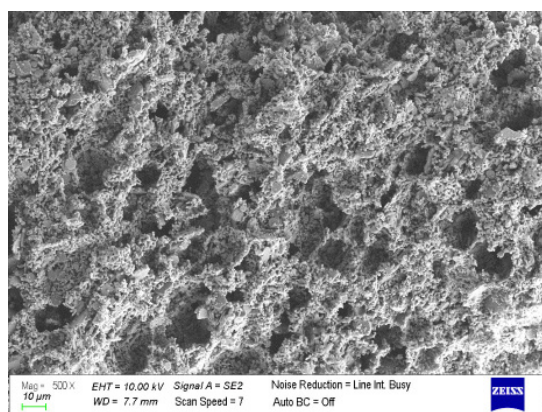


Fig. 4: XRD analysis result of the (a) calcined bovine bone, and (b) sintered composite beads

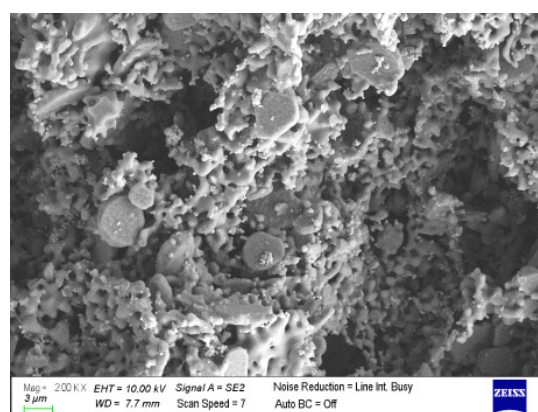
SEM investigations of the produced composite beads (Fig. 5-7) showed that highly interconnected and open porous structure was obtained for all compositions. It is also determined that bimodal pore size and distribution was achieved. Removal of sodium alginate and also relatively low pressureless sintering temperature resulted in small pores between the ceramic particles. Also, different morphologies of the starting materials enhanced the fine porosity development in the microstructure of the composite beads. On the other hand, removal of the

analysis revealed that only HA phase was existing in the calcined bovine bones. This result is consistent with the literature reporting that after calcination of bovine bones at 600-1000°C for 2-3 hours, the only stable phase is HA.^{62,63} XRD analysis result of the sintered composite beads revealed that HA phase is not existing in the composite beads. Hydroxyapatite is a thermally unstable material at high temperatures, and depending on its stoichiometry decomposes at 800-1200°C.⁴⁴ Sintering of the ceramic–starch–alginate green composite beads at 1200°C for 1 hour resulted in the decomposition of the hydroxyapatite phase and formation of α -TCP. XRD analysis revealed that α -TCP-CeO₂-Al₂O₃ (Fig. 4) composite beads were achieved.

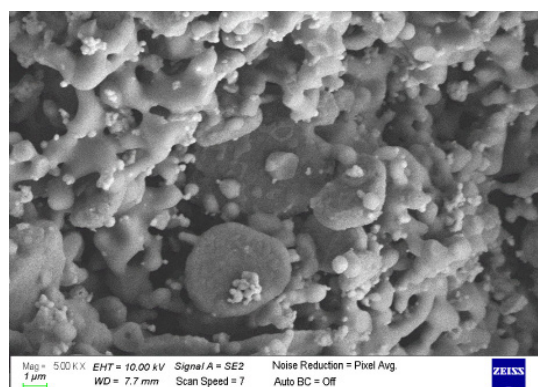
corn starch from the structure during the sintering process resulted in the formation of big pores (~5-25 μ m). SEM investigations showed that particle size of the corn starch was changing from ~5 to ~25 μ m range (Fig. 3), and achieved porosity sizes are in good agreement with this finding. It was also observed that a good bonding was achieved between the ceramic particles during the sintering process via liquid phase sintering and neck formations took place.



(a)

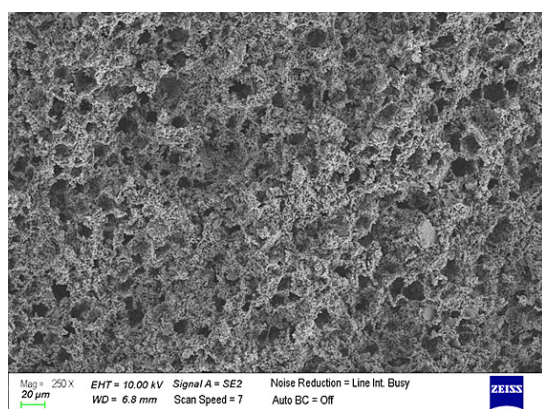


(b)

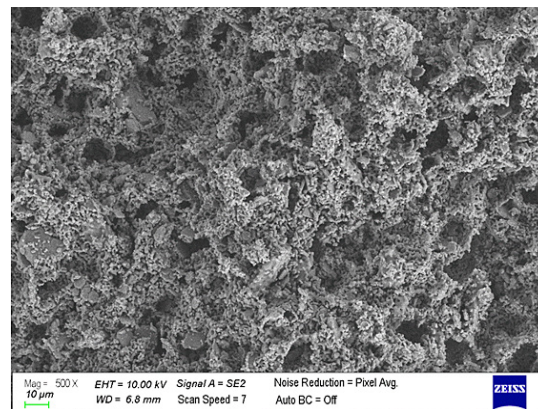


(c)

Fig. 5: SEM images of 20H20A5C sample at (a) 500X, (b) 2000X, and (c) 5000X



(a)



(b)

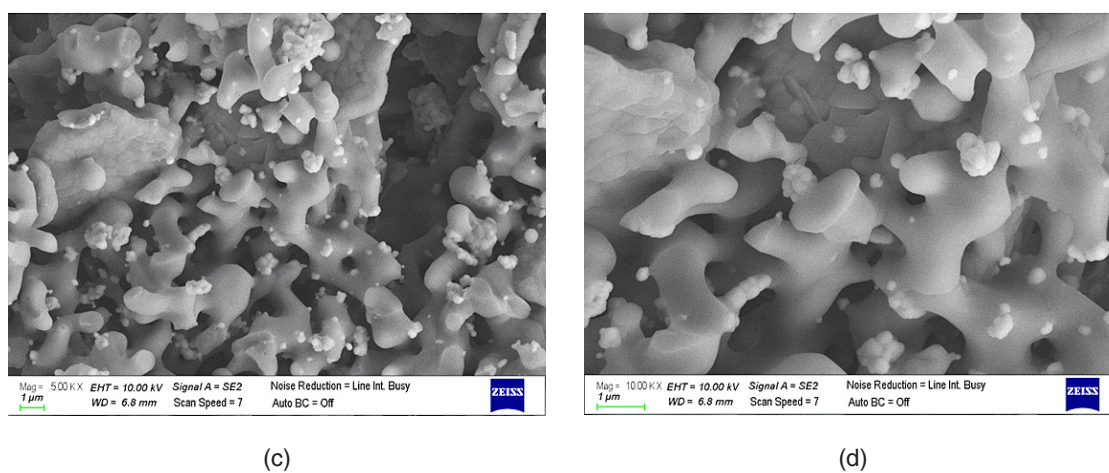


Fig. 6: SEM images of 30H10A5C sample at (a) 250X, (b) 500X, (c) 5000X, and (d) 10000X

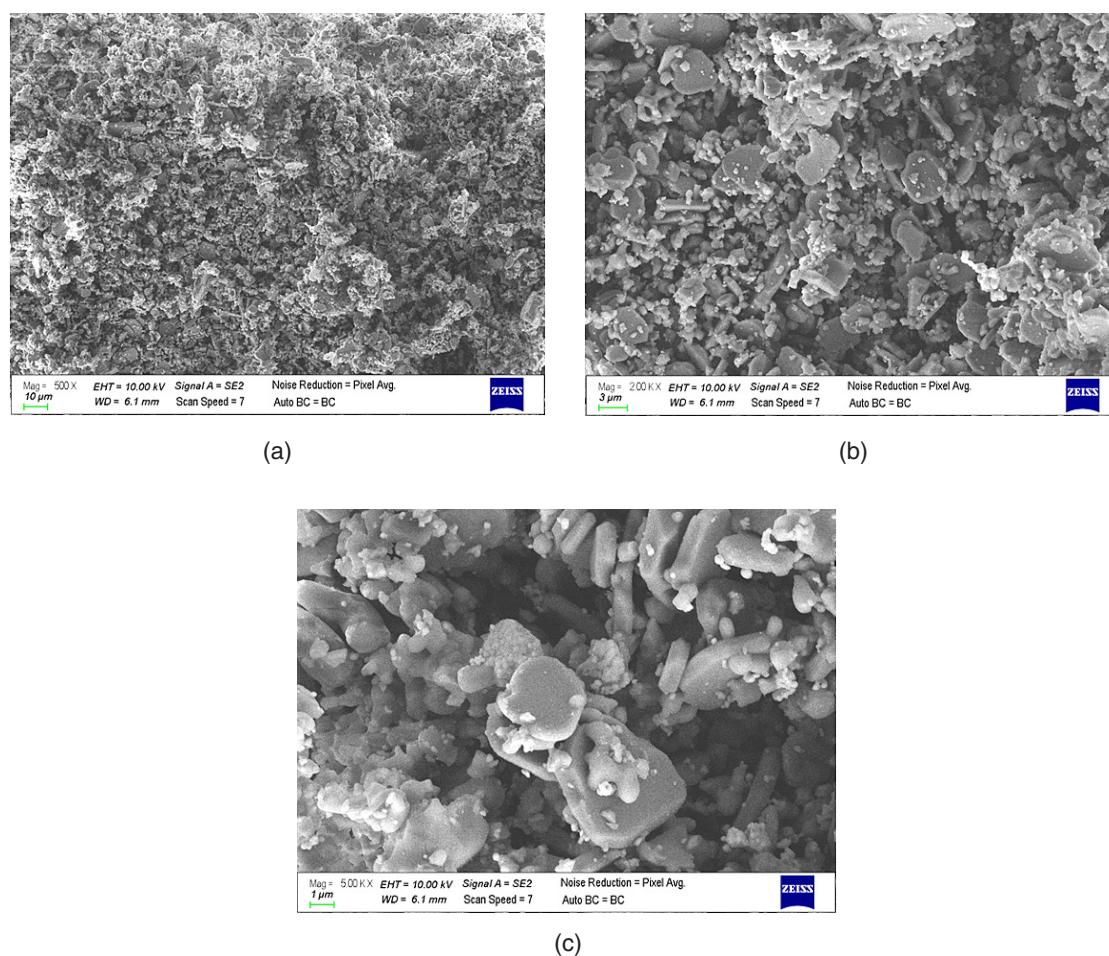


Fig. 7: SEM images of 10H30A5C sample at (a) 500X, (b) 2000X, and (c) 5000X

SEM image of sample 30H10A5C and SEM-EDX K_{α} maps of O, Al, P, Ca, and Ce are given in Fig.8. Elemental distribution maps of the investigated

sample confirmed that the produced composite beads are homogenously prepared.

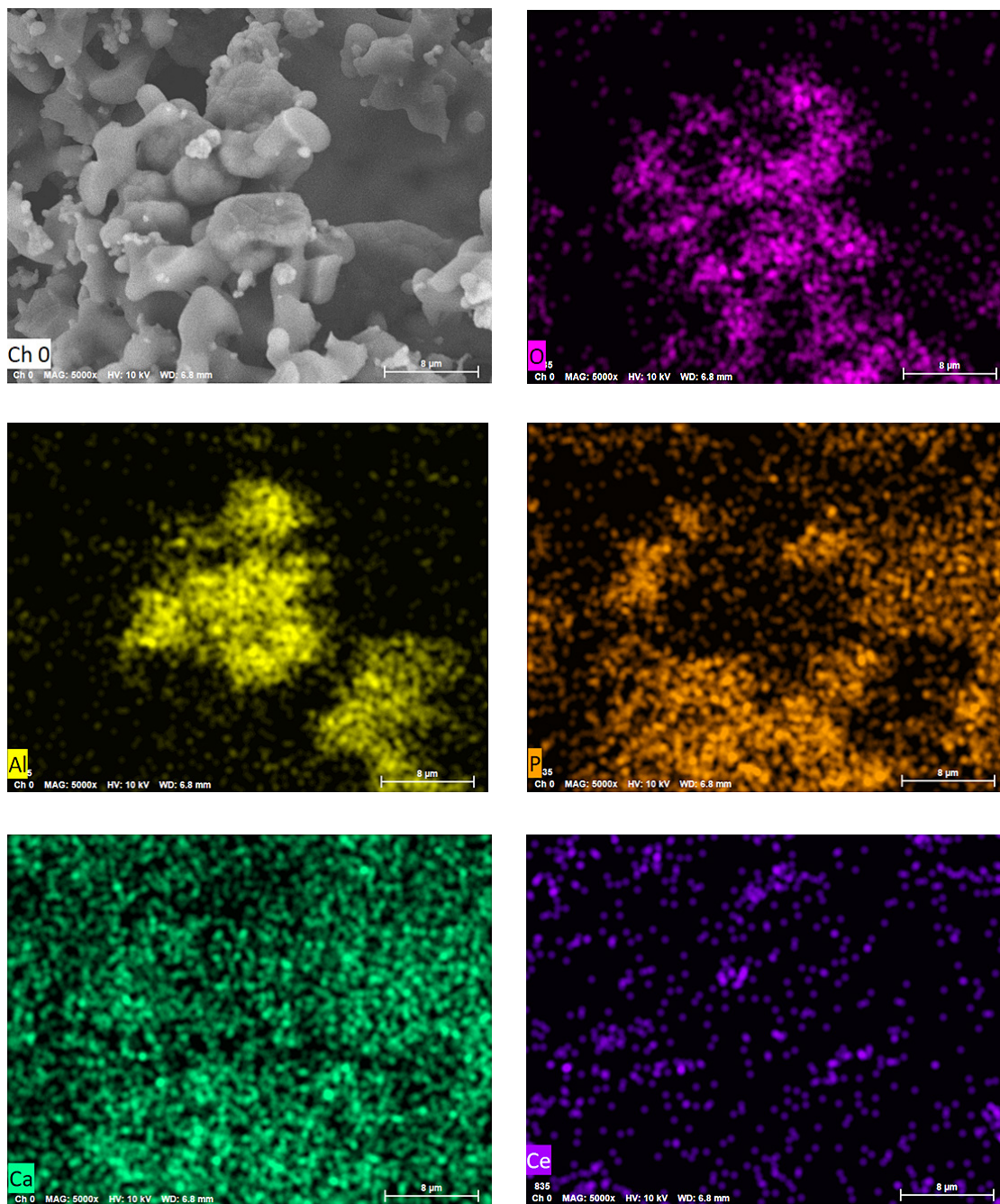


Fig. 8: SEM image of sample 30H10A5C and SEM-EDX K_{α} maps of O, Al, P, Ca, and Ce

Conclusions

- A simple, eco-friendly, and innovative approach is applied to produce porous α -TCP-CeO₂-Al₂O₃ composite beads via using bovine bone-derived HA, CeO₂, and Al₂O₃ as starting materials.
- Starch, an environmentally friendly and economic pore-forming agent, is used to achieve highly porous composite beads. Highly interconnected and open porous structure was obtained for all three compositions. It was also determined that a bimodal pore size and distribution was achieved.
- HA and TCP based porous beads are very essential materials, especially for bone tissue engineering applications. Due to low mechanical properties of CP ceramics, oxide reinforced CP ceramics can enlarge the applications of these materials due to better mechanical performances. Fabricated highly porous α -TCP-CeO₂-Al₂O₃ composite beads are promising candidates for various biological and biomedical applications, as well as various applications such as adsorption of dyes, heavy metals, desalination, water

treatment. Produced beads also can be used to reinforce different matrix materials to achieve innovative composite materials.

- These are the initial findings of the newly started research. New composition designs will be performed, and cytotoxicity, antibacterial and drug release properties of the produced spheres will be examined in the future studies.

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Conflict of interest

The authors declare that she/he has no conflict of interest regarding the publication of this article.

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