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Boron doped hydroxyapatites in biomedical applications

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ABSTRACT

Hydroxyapatite has been widely used in biomedical applications as a coating material for implant surfaces, a drug carrier, a scaffold or composite for bone tissue engineering applications. The highly ionic structure of hydroxyapatite allows doping of various ions, resulting in an improvement in its properties. Boron is one of the elements which can be doped into hydroxyapatite structure by replacing phosphate (PO_4^{3-}) or hydroxyl (OH^-) sites to obtain scaffolds for bone tissue engineering applications or a coating material for metal substrates. Although the effects of supplemental boron on bone, liver, and brain metabolism have been shown to have important results as a nutrient, there are very few studies in the literature on the use of boron-doped hydroxyapatite in the biomedical field. In this review, the details of synthesis methods and functional groups of boron-doped hydroxyapatite were tabulated. Generally, the addition of boron leads to the formation of rod-like morphology, while the density and Vicker's microhardness of hydroxyapatite decrease. Thermal stability and electrical insulation properties were observed to improve with boron doping. Boron was also shown to increase biodegradability, bioactivity as well as cell proliferation and differentiation of different cell types on the surface of hydroxyapatite.

1. Introduction

Hydroxyapatite (HA,$Ca_{10}(PO_4)_6(OH)_2$), has been widely studied as a biomaterial in hard tissue applications. The structural, mechanical, biological, thermal, and electrical properties of HA can be tuned by doping different ions. HA was previously doped with various single elements, such as strontium [1], selenium [2], silicon [3], magnesium [4], silver [5]; also with binary elements, such as zinc and fluoride [6], yttrium and fluoride [7], iron and selenium [8], zinc and silver [9]; and also with ternary dopants, such as magnesium, strontium, and zinc [10].

Boron is a member of the 3rd periodic group and it is a non-metal element. According to current estimates, 73.4% of the world's boron reserves are located in Turkey [11]. It is becoming increasingly important as an additive in polymer and ceramic composites to provide high strength, lightweight, and thermal stability. Moreover, boron is widely used as a dopant in bioactive glasses not only due to its ability to form a glass network but also its crucial role in biological functions. It was previously shown to increase the rate of degradation and release of Si from a bioactive glass which facilitates HA precipitation in the simulated body fluid (SBF) [12].

Although it is widely used in the composite and glass industry, there is a limited number of studies that focus on the synthesis and characterization of boron-doped HA (borohydroxyapatite). The applications of boron-doped HA in literature are mostly limited to being a component of bone tissue engineering scaffolds and having a supportive function in phosphors for fluorescent lamps. Boron is normally present as boric acid and borate ions [13] in geothermal water and there are many studies that utilize HA for the removal of boron from geothermal and wastewater [11,14]. Moreover, boric acid is used as a neutron absorbent in nuclear reactors. Since the removal of boric acid, which is a neuron absorbent used in nuclear power plant accidents, is of great importance, a method based on precipitation in the form of HA by addition of $Ca(OH)_2$ was studied [15]. The methods used for the synthesis of boron-doped HA were solid-state reaction, sol-gel, and wet precipitation methods based on acid-base reactions in general. Microwave-assisted biomimetic methods were also used to obtain boron-doped HA.

The research profile of boron doped hydroxyapatite was analysed. The analysis was done by search engine of "Web of Science" with keywords "boron",

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"doped", "containing" and "hydroxyapatite". According to the results; in last five years, the number of research articles increased with about 2.5 fold of the total number of research items published since 2001 (Figure 1).

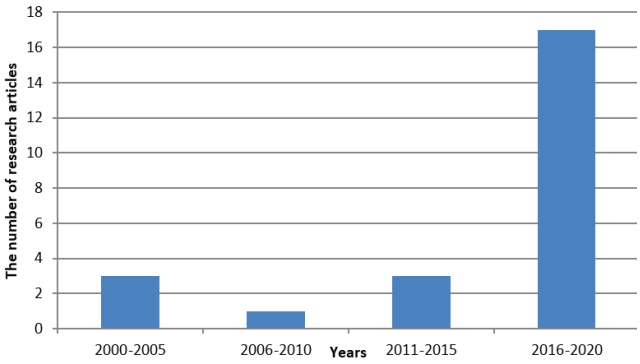

Figure 1. The number of research items published in periods consisted of five years.

The topics investigated were also analyzed. The results showed that new topics emerged such as mechanical properties and electrical properties in last five years. The weight of the research in biological properties did not differ much between the periods 2011-2015 and 2016-2020, whereas, the percentage of investigation related to structural properties decreased in last five years (Figure 2).

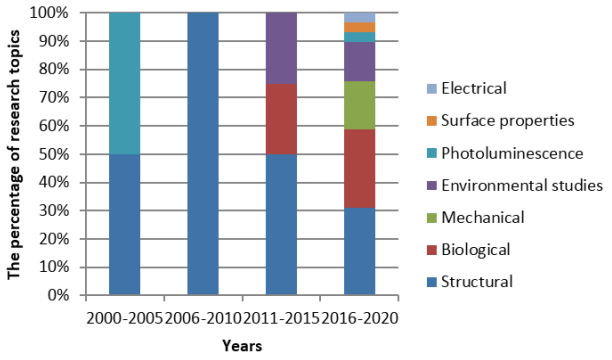

Figure 2. Percentage of research types based on periods consisted of five years.

In this review, the synthesis methods and the effects of boron doping into the structure of HA were evaluated based on studies published in the period from 2002 till now. The biological, mechanical, thermal, and electrical properties of HA affected by the addition of boron were summarized. Additionally, the influence on co-doping of boron with ions such as Sr^{2+}, Eu^{3+}, and Ce^{3+} was also reviewed.

2. The role of boron on biological functions

There are many pieces of evidence showing that boron can be a trace element for the human body [16]. The investigations related to the effects of boron on biological functions were mostly based on nutritional studies. The relationship between boron administration and biological responses in animals was widely documented [17]. The supplementary effect of boron on functions in brain and liver, bone metabolism, immune response, wound healing, and activities of hormones made boron a crucial element for metabolism [18].

Boron plays an important role in bone development, especially in terms of mineralization and bone growth. The studies related to osteogenesis revealed that boron has an important role in the mineralization function of osteoblasts by effecting related gene expressions and hormones such as 17β-estradiol (E2) and testosterone [18]. Moreover, the expression of mRNA, especially those encoding growth factors involved in angiogenesis and wound repair [19] is stimulated by boron. The affected proteins are not only bone-related proteins, such as bone morphogenic proteins (BMP-4, BMP-6, BMP-7), collagen type 1, osteopontin, bone sialoprotein, osteocalcin, but also wound healing enzymes such as elastase, collagenase, and alkaline phosphatase that are found in fibroblasts [20]. Boron induces absorption of magnesium which is an essential trace element and interacts with calcium and vitamin D, all of which play a role in bone metabolism [21].

Other functions of boron include increasing antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, and decreasing the amount of inflammatory biomarkers. Furthermore, boron enhanced the formation and activity of essential biomolecules, such as nicotinamide adenine dinucleotide (NAD^+) [20].

3. Boron-doping in synthetic apatites

Boron is not one of the essential trace elements in bone [22]. However, as mentioned earlier, it has numerous functions in the regulation of bone metabolism. Therefore, researchers focused on synthesizing boron-doped HA and the investigation of its structural, mechanical, and biological properties as a candidate for biomedical applications.

The proposed formula of borohydroxyapatites is as follows:

$$Ca_{10}[(PO_4)_{6-x}\ (BO_3)_x]\ [(BO_3)_y\ (BO_2)_z\ (OH)_{2-3y-z}] \qquad (1)$$

There are three different sites of occupation in HA. These are (1) PO_4^{3-} site as triangular BO_3^{3-}, (2) OH^- site as triangular BO_3^{3-} which formed AB-type borohydroxyapatite and (3) OH^- site as two-fold coordinated linear BO_2^- (Figure 3) [23]. In a study aiming at removing boron in geothermal water with the use of HA, boron was reported to be located in OH^- site [11].

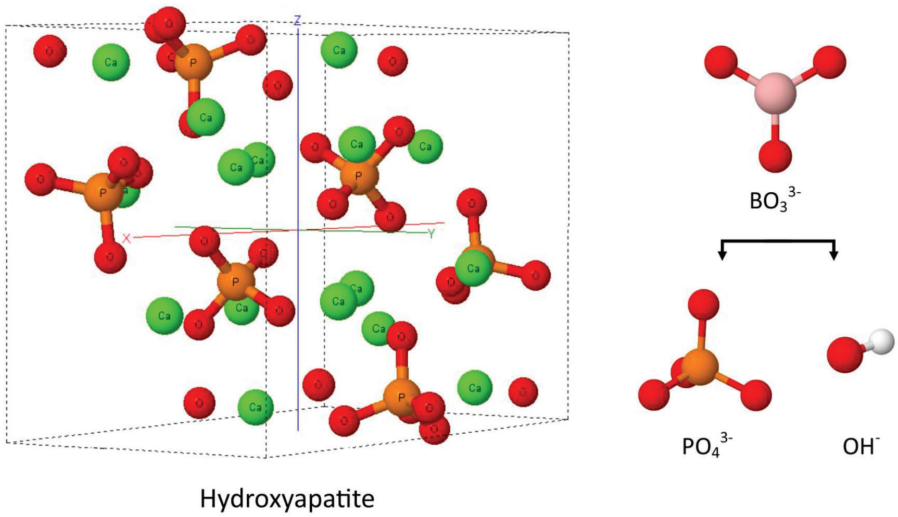

Figure 3. The lattice structure of hydroxyapatite with possible locations of BO_3^{3-}.

3.1. Synthesis and morphology of B doped HA

The synthesis methods of boron-doped HA were solid-state reaction, sol-gel, microwave-assisted biomimetic method, wet precipitation method, and acid-base method which was defined as a type of wet chemical synthesis method. Table 1 summarizes the production methods and boron amounts doped in HA structure.

Boron-doped HA with Ca/(P+B) molar ratio of 1.67 was previously synthesized by ultrasonic spray pyrolysis route for the incorporation of functional BO_2 groups [29]. BO_2^- ions were reported to replace OH^- ions and PO_4^{3-} ions were substituted with BO_3^{3-} after sintering. The dissociation of BO_2 groups into $B(OH)_3$, increased the amount of OH^- which resulted in the changes in surface properties, such as static contact angle of boron-doped HA.

Wet chemical precipitation method was also applied to synthesize boron-doped HA [30]. However, solid-state reaction was more effective in terms of the incorporation of boron ions when compared to wet chemical precipitation [24].

In addition to the precursors listed in Table 1, $Ca(OH)_2$ was used for the immobilization of borate with the aim of removing it from solutions [31]. The mechanism was explained in two stages which consisted of nucleation/crystallization in the presence of phosphate ions

Table 1. The synthesis methods and precursors for boron doped HA.

Method	Precursors for production			Molar ratio Boron / Phosphate (B/P) (%)	Temperature and duration of heat treatment
	Calcium	Phosphate	Boron		
Solid-state reaction [23],[24]	$CaCO_3$	$(NH_4)_2\ HPO_4$	H_3BO_3	3.4 5.3 9.1 13.2 15.4 20 50	1000°C / 24 h
Solid-state reaction [25]	$CaCO_3$	$Ca_2P_2O_7$	B_2O_3	9.1 18.2	1200°C / 6 h
Sol-gel [26]	$Ca(NO_3)_2.4H_2O$	$(NH_4)_2HPO_4$	H_3BO_3	1.7 3.3 5 6.7 8.3	800°C / 2 h
Sol-gel [27][a)]	$Ca(NO_3)_2{\cdot}4H_2O$	$(NH_4)_2HPO_4$	$B(OC_3H_7)_3$	26.7	1000°C / 2 h
Microwave-assisted biomimetic [28]	$CaCl_2{\cdot}2H_2O$	$NaH_2PO_4{\cdot}H_2O$	H_3BO_3	3.8 4.4 5 6.6 8.6 21	-
Wet precipitation [24]	$Ca(NO_3)_2{\cdot}4H_2O$	$(NH_4)_2HPO_4$	H_3BO_3	20	-

[a)] Approximate values are represented as % of B/P ratio

and co-precipitation/co-sorption when there were no phosphate ions left in the system. In the nucleation/crystallization stage, the trigonal form of boron was preferable, whereas co-precipitation/co-sorption stage provided immobilization of boron in tetragonal form by co-sorption of $B(OH)_4^-$. Additional mechanism supported HA precipitation in nucleation/crystallization phase by dissociation of $(CaB(OH)_4)^+$ and $B(OH)_4^-$ ions as a result of decomposition of trigonal borate and $Ca(OH)_2$ complex. Maximum sorption density as denoted by B/Ca ratio was found to be 0.40 to keep single-phase HA. Above this ratio, amorphous CaB_2O_4 and HA were likely to form. It was also observed that when the borate species were captured in HA, the morphology changed from fibrous nano-sized rods to a more swollen structure.

For the samples synthesized by the biomimetic method, the size of the agglomerated spherical HA particles was previously reported to decrease from 50 nm to 30 nm with the addition of boron [28]. The HA nanoparticles exhibited a needle-like morphology while boron-doped HA had a rode-like form similar to biological apatite. However, in another study [15] where wet precipitation method was used to utilize boric acid as the boron source and $Ca(OH)_2$ as the calcium source, the particle size of boron-substituted HA was reported to increase approximately twofold in both dimensions with respect to HA without borate. The average length of the particles increased from 10 nm to nearly 20 nm and the width increased from 5 nm to 10 nm. Similarly, boron-doped samples synthesized by sol-gel method showed higher average particle size when compared to pure HA. Particle size increased from 537 nm to 1710 nm with the addition of boron [27].

3.2. Boron-doped HA as a coating material

Calcium phosphates, especially HA, are widely used in the coating of metallic biomaterials to combine their biocompatibility and bioactivity properties with the mechanical strength of metals. There are also numerous studies about various ion-doped HA coatings on metallic substrates, some examples are strontium-doped [1], selenium-doped [2], magnesium and silver co-doped [32], magnesium and fluoride co-doped [33] HA coatings on titanium or its alloys. However, the use of boron-incorporated HA as a coating material has been addressed in very few publications.

One study was reported that B_2O_3 doped HA was used as a coating material for Ti6Al4V substrate by applying high-velocity oxy fuel as the coating technique [34]. B_2O_3 doped HA was first synthesized by sol-gel method by using boron isopropoxide $[B(OC_3H_7)_3]$ as a precursor and the resulting B_2O_3 powder was mixed with HA during the HA production via sol-gel route, and then calcined before the coating process at 900°C for 2 hours. It was claimed that boron addition decreased deformation and delamination at the edges when compared to samples coated with pure HA. When the amount of boron increased from 1 wt.% to 3 wt.%, the scratch resistance of the coating also increased. Another important parameter for surface properties for biomedical applications is the contact angle due to cell to surface interaction. B_2O_3 addition decreased contact angle from 106.5 to 37.6 degrees when 3 wt.% B_2O_3 was added and the surface became highly hydrophilic when compared to pure HA.

3.3. Boron-doped HA as a scaffold

Since boron is an important trace element for bone metabolism, scaffolds including boron-doped HA were studied in order to observe its effects on bone repair. Tuncay et al. [28] coated boron-doped HA by using the microwave-assisted biomimetic method on a chitosan scaffold prepared by freeze-drying. The porosity of the boron-doped HA/chitosan scaffold was around 85.7%. No significant reduction in porosity of the scaffold was observed due to boron-doped HA coating. By using a similar synthesis method for boron-doped HA, a scaffold consisting of poly(butylenes adipate-co-terephthalate) and 5 wt.% of boron-doped HA was produced [35]. It was detected that boron doping increased fiber diameter by decreasing pore size. In another study, a bacterial cellulose/boron-doped HA/gelatin scaffold was produced by lyophilization technique [27]. A pore size between 45 to 210 μm was achieved. The pore size was in the range of 100 and 350 μm which was asserted as ideal pore size for tissue ingrowth.

3.4. Effect of Boron on microstructural characteristics of HA

Microstructural characteristics of boron-doped HA were investigated in terms of phase composition, crystallinity, lattice parameters, and functional groups in the structure.

3.4.1. Phase composition and crystallinity

The phase composition of HA ceramics is one of the most important properties because it has a determining effect on their biocompatibility, bioactivity, and mechanical characteristics. Phase transformations can occur due to the heat treatments applied during solid-state synthesis of boron-doped HA. For example, after heat treatment at 700°C for 24h, $CaCO_3$, $Ca_3(BO_3)_2$, CaO and β-TCP phases appeared in the system [23]. However, sintering at 1000°C for 24h resulted in X-ray diffraction (XRD) peaks that are only related to the apatite space group of $P6_3/m$ since the reaction is complete. Besides, β-TCP phase was detected at above 700°C in samples synthesized with wet precipitation method due to the thermal decomposition of HA [30]. Boron incorporation into HA occurred at above 900°C via the chemical reaction between HA and $B(OH)_3$ and β-TCP appeared as a second phase.

The phase transformation from β-TCP to α-TCP was detected at above 1200°C. In another study, boron-doped HA samples with 9.1 mol % boron synthesized by solid-state reaction and heated up to 1200°C for 6 h, a trace amount of CaO and α-TCP phases were detected [25]. The increase in borate content in co-precipitation of borate with HA resulted in the formation of different calcium metaborates such as $CaO{\cdot}B_2O_3$ (CaB_2O_4), $2CaO{\cdot}B_2O_3$($Ca_2B_2O_5$) and $3CaO{\cdot}B_2O_3$($Ca_3B_2O_6$) after calcination at 850°C [15]. It was also stated that calcination improved the crystallinity of HA without any mass loss of immobilized boron species. When wet and dry production methods were compared, wet precipitation method yielded less crystalline products. The XRD peaks in samples synthesized with wet precipitation were broadened as boron addition was increased [24,25]. However, no significant change in crystallinity was observed with the addition of boron in samples synthesized with solid-state reaction [24].

Another important factor for the formation of different phases was the P/B ratio. It was determined that a complete apatitic structure was formed at P/B=7.22 while $Ca_3(BO_3)_2$ was observed below this value [23]. At P/B=11, the phases detected were biphasic borohydroxyapatite, and $Ca(OH)_2$ and boron incorporation was limited to a ratio of P/B=7.22. $Ca_3(BO_3)_2$ and CaO phases occurred above P/B=7.22 [23]. Biphasic calcium phosphate samples consisting of HA and β-TCP were obtained by addition of boron with approximate amounts between 0.5 and 2 wt.% after sintering at 1000°C, 1100°C and 1200°C for 2h [36]. It was detected that as boron content increased from 0 to 2 wt.%, the amount of HA phase decreased from 85 to 69.7 wt.% and the remaining phase was β-TCP. Similarly, the percentage of HA phase decreased as the sintering temperature increased from 1000°C to 1200°C while keeping the amount of boron constant.

3.4.2. Lattice parameters

The process of removing borate by co-precipitation with HA by using $Ca(OH)_2$ results in the formation of boron-doped HA. As the boron content increased in the calcined product, the lattice parameter *a* decreased, and the lattice parameter *c* increased up to a point where the amount of boron was 1.41 mmol/g [15]. Since the decrease in *a* was more dramatic than the increase in *c*, the unit cell volume showed a decreasing trend. Calcination at 850°C resulted in dehydration of borate and the formation of BO_2 trigonal structure which increased lattice parameter *c* and decreased lattice parameter *a*. When boron content was further increased, the lattice parameters *a* and *c* in boron-doped HA gradually approached the values of pure HA due to the formation of calcium borate compounds as secondary phases.

Table 2 lists the changes in lattice parameters and unit cell volumes based on the amount of boron added to HA. When wet precipitation and solid-state reaction methods were compared in terms of lattice parameters of boron-doped HA obtained, wet precipitation method yielded products with no significant difference with HA [24]. However, the lattice parameter *a* decreased and the lattice parameter *c* increased with boron addition in samples synthesized with solid-state reaction. Moreover, Barheine et al. stated that *a* slightly decreased and *c* slightly increased with the increased amount of boron in samples synthesized by solid-state reaction [25]. However, due to the formation of trace amounts of CaO and α-TCP as second phases, the lattice parameter a did not follow *a* linear relationship.

3.4.3. Functional groups

The functional groups of boron-doped HA as detected by Fourier transform infrared (FTIR) spectroscopy analysis are summarized in Table 3. Small shifts in IR bands were observed according to some factors such as the amount of boron and the synthesis method.

Some of the bands were detected due to impurity phases in the structure such as $Ca_3(BO_3)_2$ and $Ca(OH)_2$. Bands detected at 717 (ν_2), 794 (ν_2), 903 (ν_1), 1228-1229 (ν_3) and 1280 (ν_3) cm^{-1} were assigned to BO_3^{3-} in $Ca_3(BO_3)_2$.

3.5. Effect of Boron on biological properties of HA

Boron addition increased in vitro biodegradability of HA and boron-doped HA reported to degrade quicker than β-TCP. Moreover, apatite forming ability in SBF was improved with boron addition [25].

Table 2. Lattice parameters and unit cell volume of boron-doped HA.

Production method	Nominal composition Ca/P+B molar ratio	Boron content[a)]	Lattice parameter *a* (Å)	Lattice parameter *c* (Å)	Unit cell volume (Å^3)
Solid-state reaction [23]	1.665	x=0.2	9.413	6.888	528.5
	1.664	x=0.3	9.409	6.893	528.4
	1.638	x=0.5	9.399	6.900	527.9
Solid-state reaction [25]	1.67	B/P=0.091	9.389	6.904	527.1
	1.54	B/P=0.182	9.389	6.927	528.8
	1.50	B/P=0.091	9.400	6.917	529.3

[a)] where x is the amount of borate (BO_3) substituted in HA structure

Table 3. FTIR bands detected for the boron-doped HA.

Functional groups	Vibration mode	Wavenumbers (cm^{-1})	Ref
PO_4^{2-}	ν_2	472	[37]
BO_3^{3-}	ν_4	571 616	[23]
PO_4^{2-}	ν_4	600-603	[23, 25, 34]
OH^-	librational	630	[23]
BO_3^{3-}	ν_2	743-744 755 770-772 782-784	[23, 25, 26, 34, 37]
PO_4^{2-}	ν_1	960- 963	[23, 26, 34, 37]
PO_4^{2-}	ν_3	1040-1050 1090-1100	[23, 25, 26, 34]
BO_3^{3-}	ν_3	1204-1208 1250-1253 1304 1312	[23, 25, 26, 34, 37]
BO_2^-	ν_3	1930-1933 2002-2005	[23, 25, 34]
OH^-	stretching	3570	[23]

Osteoinductive property of bone tissue engineering scaffolds with boron-substituted HA, which was synthesized by the biomimetic method and containing 1.15 wt.% boron, was studied previously [38]. The chitosan hydrogel scaffolds with boron-doped HA claimed to have higher mineralized matrix formation rates when compared to the scaffolds with bone-like HA precipitated in SBF. In another study about the effect of nano boron-doped HA on the adhesion, proliferation, and differentiation of human bone marrow-derived mesenchymal stem cells (MSC), boron addition was found to enhance cell adhesion and proliferation [39]. On the other hand, no change was observed in adipogenic or osteogenic differentiation of MSCs cultured with nano-sized pure HA and boron-doped HA with the molecular formula of $Ca_{10}(PO_4)_5{\cdot}8(BO_4)_{0.2}(OH)_{1.6}$.

When compared with scaffolds containing only gelatine and bacterial cellulose, the addition of boron-doped HA to the scaffold composition, in which the ratio of boron-doped HA/gelatin was 1/5 wt./wt., slightly decreased the degradation rate after 4 weeks in phosphate buffered saline (PBS) solution [27]. Boron-doped HA, which was coated on chitosan by using the biomimetic method, significantly increased the proliferation of MC3T3-E1 osteoblastic cells on the 5^{th} and 7^{th} days of culture compared to pure chitosan [28]. Gene expression related to collagen type I as a marker of the initial stage of differentiation increased significantly on boron-doped HA/chitosan scaffold on day 14. Late-stage differentiation marker osteocalcin, which is related to the mineralization, was measured the highest for boron-doped HA/chitosan sample. Other differentiation markers, such as RunX2 and osteopontin gene expression, showed no significant differences on both HA/chitosan scaffold and boron-doped HA/chitosan scaffold [28]. Moreover, nanometer-sized boron-doped HA affected Saos-2 human osteosarcoma cells' Wnt and transforming growth factor-β (TGF-β) signaling pathways, which have a crucial role to overcome cell stress, by releasing boron within 1h [40]. According to the results of ALP activity and intracellular calcium amount determination tests, boron-doped HA in gelatin/bacterial cellulose scaffolds increased osteogenic activity of Saos-2 cells significantly after 14 days in cell culture [27].

In poly(butylenes adipate-co-terephthalate) (PBAT)/boron-doped HA composite scaffold, although all scaffolds improved attachment and proliferation, an enhancement in the differentiation of human bone MSCs was observed for scaffolds containing boron-doped HA when compared to scaffolds with pure HA [35]. The study on Saos-2 cell line also showed that the addition of boron-doped HA brought a proliferative effect to the scaffolds consisting of gelatin and bacterial cellulose [27]. Mitochondrial activity, lactate dehydrogenase activity, and DNA quantity of Wharton's jelly-derived MSCs were not affected by boron substitution into the structure of HA [24].

3.6. Effect of Boron on thermal properties of HA

There is a very limited number of studies that directly examine how boron affects the thermal properties of HA. Thermogravimetric analysis was previously performed on the scaffolds consisting of bacterial cellulose, gelatin, and boron-doped HA [27]. When the samples were compared with pure HA, the samples included boron-doped HA had higher thermal stability after heating above 200°C. Moreover, the total mass loss of boron-doped HA was approximately 0.81%, whereas it was detected as 1.89% for HA. According to differential scanning calorimetry (DSC) analysis, the evaporation temperature range of absorbed water increased from 100-150°C to 170-200°C with boron addition which claimed that water retention capability of the scaffolds increased.

3.7. Effect of Boron on mechanical properties of HA

Ion substitutions generally change the structural, mechanical, and biological properties of HA. Not only biocompatibility but also mechanical properties of HA biomaterial play a crucial role in fulfilling the desired task within the body. Suchanek et al. tested several sintering additives in HA, such as H_3BO_3, $CaCl_2$, KCl, and $Na_2Si_2O_5$, and they determined that the density of the HA decreased significantly with the addition of 5 wt.% H_3BO_3 after sintering at 1000°C and 1100°C for 2h [41]. The inhibition of densification in groups with reduced density was attributed to the inhomogeneous distribution of the additive and the larger particle size of the additive compared to sintered HA. However, according to scanning electron microscopy (SEM) results, no inhomogeneous distribution was observed for samples with 5 wt.% H_3BO_3. Moreover, transgranular fracture was observed in the samples that are sintered at 1000°C. Similarly, a gradual decrease in density was observed in 0.5, 1, and 2 wt.% boron-doped HA [37]. The density of pure HA, which was sintered at 1200°C, was measured as 2.92 g/cm^3 and decreased to 1.93 g/cm^3 with the addition of 2 wt.% boron. From the SEM images of the fractured pellets of samples synthesized using 0.5% boron by weight, it was observed that a denser structure was achieved with the increase of sintering temperature, as expected. The decrease in density with the increasing amount of boron was linked to voids formed in HA structure by boron substitution.

The effect of boron-doped HA on compressive strength and Young's Modulus was examined previously in the scaffolds consisting of bacterial cellulose, boron-doped HA, and gelatine [27]. Compressive strength increased from 75.3 MPa to 94.9 MPa with boron addition. On the other hand, Young's modulus decreased from 11.3 MPa to 10.0 MPa. According to the compression test results of 0.5, 1, and 2 wt.% boron-doped HA, compressive strength decreased from 39.5 MPa to 29.1 MPa with boron addition for samples sintered at 1000°C [37]. As sintering temperature increased to 1200°C, compressive strength values of 0.5 wt.% boron-doped HA samples highly improved and reached to 161.2 MPa [37].

Vicker's microhardness test was applied to samples doped with 0.5, 1, and 2 wt.% boron [37]. Vicker's microhardness values decreased drastically with boron addition. Increasing sintering temperature from 1000°C to 1200°C resulted in an improvement of approximately 16-fold as a maximum.

3.8. Co-doping of Boron and other ions in HA

Co-doping of boron with other ions, such as Ce^{3+} and Eu^{3+}, was studied for applications as phosphors in fluorescent lamps. Rare-earth ions showed suitable spectroscopic properties for use in lasers [42,43]. Two different cerium ion concentrations, namely 1 and 0.8 mol% Ce^{3+}, were added to HAs with 3.3% and 5.5% moles of boron, respectively. [42]. Nuclear magnetic resonance (NMR) studies revealed that boron increased disorder in the structure and resulted in shifts of wavelengths to longer values in laser-induced emission bands. Moreover, luminescence intensity increased due to the addition of boron. In another study, in which the photoluminescence excitation (PLE) spectra of boron introduced Ce^{3+}-doped HA was studied, the mechanism behind the increase in the intensity of blue emission was explained with a decrease in OH^- ions around Ce^{3+} ion due to boron substitution [44]. Ternane et al. also studied co-doping of 1 mol % Eu^{3+} and 5.5 mol % B^{3+} into HA and showed that boron substitution increased perturbations in luminescence features of Eu^{3+} [43].

Co-doping of Sr^{2+} and BO_3^{3-} into HA was carried out by both wet precipitation and solid-state reaction routes [24]. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used to detect the strontium and boron contents in the structure of HA. Wet precipitation yielded 0.22 mol boron and 0.52 mol strontium while the dry method was more efficient in terms of the incorporation of ions as the boron content was 0.92 mol and the amount of strontium was 0.88 mol. The comparison between the production methods revealed that BO_3^{3-} was located on the hydrated surface layer in the samples synthesized by wet precipitation method and only Sr^{2+} influenced the lattice parameters. On the other hand, in samples synthesized by solid-state reaction, lattice parameter c increased significantly whereas lattice parameter a increased less than that of only Sr^{2+}-doped HA which indicated the incorporation of BO_3^{3-} in the structure.

3.9. Effect of Boron on electrical properties of HA

The electrical properties of bioceramic materials are of great interest due to the increased biological response on the polarized surfaces. Like the structural properties of HA, its electrical properties can be improved by adding foreign cations [45]. The dielectric constant of boron-doped HA was investigated and a decrease in the electric dipole moment of OH^- ions, which were substituted by boron ions, was observed [26]. Oscillation due to electric field and polarization decreased which resulted in a decrease in the dielectric constant of HA. In terms of alternating electrical conductivity, boron addition enhanced the insulation properties of HA. Therefore, it can be proposed as a material for bioelectronics.

3.10. Future perspectives

There is a limited number of studies related to boron-doped HA as a candidate for biomedical applications. Researchers mainly focused on the elimination of boron from geothermal water by precipitation in the form

of boron-doped HA and supportive effect of boron on luminescence properties of Ce^{3+} and Eu^{3+} doped HA. In terms of incorporation mechanism two sites were proposed (OH^-, PO_4^{3-} sites). No detailed information about the substitution mechanism at atomic level was found in the literature. Moreover, the information related to lattice parameters is very limited. Most of the limited number of studies concentrated on phase composition, FTIR studies and biological properties such as bioactivity in SBF, cell proliferation and differentiation. There is a literature gap in many topics, such as the anti-bacterial effect of boron-doped HA and the maximum amount of doping in terms of biological response. Since it was known that boron compounds has an antibacterial effect via direct contact or as a constituent of dental composites, boron-doped HA may be investigated in terms of its anti-bacterial properties as a biomaterial for dental applications [46,47].

Generally, boron-doped HA was used as a constituent of tissue engineering scaffolds. However, the use of boron-doped HA as a coating material or a constituent of a composite is not very common. As stated before, boron affects the biological functions of many organs, especially bone. Therefore, more biomaterial prototypes in the form of scaffolds and coating materials are needed to be studied. The number of *in vivo* studies performed on boron-doped HA should be increased since its positive effect on cell differentiation was known [38]. Boron was also used as a supplementary element for many organ metabolisms other than bone, like liver and brain. Therefore, boron-doped HA can be integrated into a system for drug delivery purposes.

4. Conclusion

In this review, the studies on boron-doped HA were summarized by focusing on biomedical applications. The synthesis methods were evaluated and the effects of boron doping on phase composition, crystallinity, lattice parameters, and functional groups were analyzed. The phase composition was dependent on the P/B ratio and heat treatment during synthesis. It was also noted that boron incorporation occurred after calcination at above 900ºC. The phases detected other than HA were CaO, $Ca_3(BO_3)_2$, β-TCP, α-TCP, and metaborates. In general, boron substitution decreased the lattice parameter *a* and increased the lattice parameter *c*. The unit cell volume decreased depending on the lattice parameter *a*. Crystallinity has been shown to be affected by the synthesis method, for example, crystallinity decreased with boron addition in wet precipitation method whereas no change in crystallinity was observed with boron addition in samples synthesized by solid-state reaction. There was also a difference in morphology between pure and boron-doped HA. Rod-like morphology was observed in boron-doped HA. Boron doping increased the thermal stability and insulation properties of HA. Biological properties of boron-doped HA have been studied relatively more extensively in the literature. Boron doping into HA was reported to increase biodegradability, apatite forming ability, cell proliferation, and osteogenic activities in general. However, cell proliferation and osteogenic activities can change based on the cell type. Boron addition decreased the density of HA due to the formation of voids in the structure. Moreover, Vicker's microhardness also decreased with boron substitution. The most studied forms of boron-doped HA in the biomedical field were listed as a constituent of a scaffold and a coating material on the titanium alloy (Ti6Al4V) substrates. There were no studies that utilized boron-doped HA in a drug delivery system although the supportive effect of boron on metabolic functions of bone, liver, and brain.

5. Conflict of interest

The authors declare no sources of support or conflict of interest.

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