

Mechanical properties and cell viability of MgO-reinforced biografts fabricated for biomedical applications

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In the present study, biografts were produced by sol gel method by adding different rates of MgO which has bone-like crystal structure and high endurance into different proportions of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, KOH, NaNO_3 , and P_2O_5 compounds. The biografts were investigated in terms of mechanical and biocompatibility properties. FTIR, SEM and XRD analyses were carried out to examine the chemical characteristics and changes in structural morphology. Mechanical properties were also investigated by conducting hardness and compression tests. In addition, cytotoxicity tests were conducted by using osteoblast cells. While results of FTIR and XRD analyses revealed that all biografts had HA (hydroxyapatite) and β -TCP contents, MgO peaks were also observed in biografts. In SEM images, grains of Non-MgO and MgO-10 biografts had sharper edges, pores formed between grains and grain size increase with increasing MgO amount (MgO-20 and MgO-30). It was found that compression stress and hardness values increased as MgO content elevated. From the cytotoxicity tests, no any toxic effect was observed in the synthesized biografts.

Key words: MgO, sol-gel, biograft, cytotoxicity

1. Introduction

In recent years, biografts with high biocompatibility, which can substitute bone tissues in the case of trauma, infection, and fracture have been started to be produced particularly as bioceramic materials [1], [13]. Calcium phosphate bioceramics, one of the most known bioceramics, are among the most preferred compounds for bone tissue repair thanks to their similarity with mineral composition of the bone, high bioactivity, and osteoconductivity properties [10], [11]. Among them, hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, HA) phase is commonly utilized because of its chemical resemblance to the bone mineral. However, application area of HA is also limited since it has low rate of biodegradability [27]. Beta-tricalcium phosphate [$\text{Ca}_3(\text{PO}_4)_2$, β -TCP], which is another calcium phosphate bioceramic, is a high response phase and displays well biodegradability [9], [12].

Negative influence of HA and β -TCP phases have been tried to be reduced by using bioceramics produced from the elements such as Ag, Mg, Sr, and Zn and their compounds as bone graft [1], [2], [6], [17], [22], [23]. It was also determined that bioceramics newly produced with these elements facilitated formation of apatite which is the major component material of the bone and bioactivities of those produced by compounds with oxide content in particular were high [2]–[4], [17], [22], [23]. Especially MgO compound has always been considered as a secondary oxide and involved within formulations of wollastonite-based glass-ceramics and some bioceramics [25]. Studies conducted by adding MgO- Na_2O - P_2O_5 sintering material into β -TCP bioceramic material indicated that it improved bending strength even when added in small amounts and increased densification, hardness, compression strength, and fracture toughness of calcium phosphate [7], [15], [16], [18]. HA and magnesium

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Table 1. Chemical composition of MgO reinforced biografts

Biografts	Ca(NO ₃) ₂ ·4H ₂ O(%)	KH ₂ PO ₄ (%)	Na ₂ CO ₃ (%)	P ₂ O ₅ (%)	MgO(%)
Non-MgO	45	20	20	15	–
MgO-10	45	15	15	15	10
MgO-20	45	10	10	15	20
MgO-30	45	5	5	15	30

phosphate cement-based sodium phosphate added composite materials were analysed. As a result of such analysis, it was determined that micropores were formed in composite materials, and good surgical handiness and sufficient mechanical strengths were obtained [24]. It has been observed that MgO has developed antibacterial properties in the cements produced with MgO and sodium dihydrogen phosphate (NaH₂PO₄) or ammonium dihydrogen phosphate (NH₄H₂PO₄) [19]. In another study, ceramic oxides with a mixture of Zirconia-yttria and magnesia were produced by sol-gel method [21].

It has been reported that high homogeneous powders with low porosity and cracky fine grain structures were obtained. In another study on ZrO₂-acetylaceton-2-proponal and CeO₂ biomaterials produced by sol-gel method, it was determined that agglomeration structure was exhibited with very stable thermal properties [20].

The present study investigated the effects of MgO on biografts produced by sol-gel method adding different rates of MgO into Ca(NO₃)₂·4H₂O, KH₂PO₄, Na₂CO₃, and P₂O₅ compounds, performing FTIR, XRD and SEM analyses and hardness and compression tests were carried out to examine mechanical properties. It was also analyzed whether or not biografts produced by sol-gel method had any toxic effect performing cytotoxicity tests on osteoblast cell.

2. Materials and methods

2.1. Materials and sol-gel process

MgO compound was used to reinforced HA which is the major component of the bone and the effects of Mg element contained within the bone structure was observed. Such chemicals as Ca(NO₃)₂·4H₂O (Sigma-13477-34-4) powder in micron size was used to obtain bone graft, KOH (99% purity, Cas no-1310-73-2), NaNO₃ (99% purity, Cas no:7631-99-4), P₂O₅ (97,1% purity, Merck-K33152940.418) are preferred as additive material owing to their properties to increase densification and sinterability. Besides, MgO (Tekkim,

191113.702, Turkey) was used to obtain results that were similar to or the same as chemical and mechanical properties of the bone. Table 1 shows the codes and percentages by weight of biografts produced by sol gel method. To syntesizing biografts by sol gel method, all samples were stirred in magnetic stirrer at 35 °C for 3 hours and homogenized (Cole Parmer-750 W) at 35 °C for 30 minutes. pH of the solutions were adjusted to 7.5 using phosphoric acid and NH₄OH. The sol mixtures were filtered by a filter paper with 100 µm pores and aged at room temperature overnight. Then, they were dehydrated at 120 °C for 24 hours and sintered under argon atmosphere at 1180 °C for 3 hours.

2.2. FTIR, XRD and SEM analysis

FTIR, XRD and SEM analyses were performed for different rates of MgO (10, 20, and 30%). Compound structures and phase structures of MgO-reinforced biografts were also analyzed using FTIR (Alti Unicam Wattson 1000) and XRD (X-Ray Diffraction (XRD) (Bruker D8 Advance, $\lambda = 1.5406 \text{ \AA}$) devices. Structural evaluation of syntesized biografts were examined by using SEM (JEOL JSM-7001F brand) device.

The chemical bond formation of the synthesized samples were determined by FTIR (Alti Unicam Wattson 1000) analysis. The spectroscopy was used in a wavelength range of 2800–650 cm⁻¹. The pulverized X-ray diffraction patterns of the produced biografts were recorded using a brand diffractometer. The diagrams were produced by conducting the measurements recorded in the range of $2\theta = 3-70^\circ$ at a scanning rate of 2 °/min and 1-s constant time gap.

Mechanical properties of as-sintered dense MgO-reinforced biografts were evaluated for Ultimate Stress and % Compression Displacement under compressive loading. The ultimate stress of the biograft structures were evaluated by using a universal testing machine (Shimadzu, 5 kN) with a constant crosshead speed of 5 mm/min. Vickers microhardness of the samples were evaluated by using a Leica testing machine with a load of 20 N for 5 seconds. Four cylindrical samples were tested for each of the selected compositions (Non-MgO, MgO-10, MgO-20 and MgO-30).

Cytotoxicity analyses were performed to investigate cell viability of the fabricated biografts. To this end, produced biografts were dissolved and diluted in DMEM solution to reduce their concentrations to 1mg/ml. Osteoblast cell line is used for cytotoxicity tests, toxicity of cells was evaluated in 96-well culture plates by MTS (cell proliferation assay) (3-(4,5-dimethyliazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl) tetrazolium, (CellTiter 96 Aqueous One Solution Assay) test. Cells were firstly inoculated within 100 μ l of culture medium into 96-well culture plates, each of which contained 5000 cells, and were allowed to growth for 24 hours. The next day, this culture medium was removed and concentrations of what were added onto cells within culture medium (DMEM). DMEM negative control culture medium and 20% DMSO positive control culture medium were used. When the incubation ended, culture medium in the wells was removed and 10 μ l MTS+100 μ l of a culture medium was ensured. Cells were allowed for incubation with MTS at 7 °C for 2–3 hours at 5% CO₂ and 37 °C, and cell viability was measured using automated Petri reader (Elisa plate reader) at 490 nm following the incubation. Change of cell count in the wells was calculated at 0.1–0.5 μ m/ml concentrations and within 24–72 hours of periods. Whether or not biograft materials had any toxic effect on the cells they were applied on, the effects of different grafts on cellular change were investigated.

3. Results

3.1. FTIR

Figure 1 shows FTIR spectra of MgO-reinforced (10–30 wt%) with Ca(NO₃)₂·4H₂O, P₂O₅, KH₂PO₄, and Na₂CO₃ additions. Biografts were produced using sol–

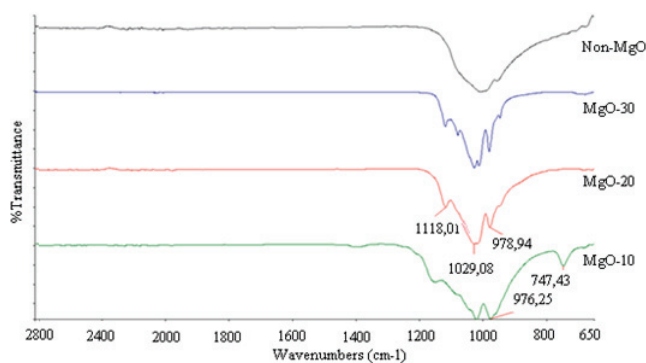


Fig. 1. FTIR spectra of the synthesized biografts (Non-MgO, MgO-10, MgO-20, MgO-30)

gel method. From the spectra, all biografts were observed to yield peaks of PO₄³⁻ compound in the range of 1118.01–747.43 cm⁻¹. This indicates that HA and β -TCP phases occurred in all MgO-reinforced biografts. PO₄³⁻ compound was detected to form at intensities of similar peaks of all MgO-reinforced biografts. In addition, peak number and intensity increased as MgO content elevated in MgO-10, MgO-20, and MgO-30 biografts and the sharpest peak was obtained from MgO-30 biograft.

3.2. XRD

XRD analysis given in Fig. 2 compared different biografts fabricated by sol–gel method. It was observed that while HA and β -TCP phases occurred dominantly in all biografts, the peaks yielding MgO compound occurred also in MgO-reinforced biografts (Fig. 2). The spectra also show that peak intensity of all biografts was high and peak intensity increased with increasing MgO content.

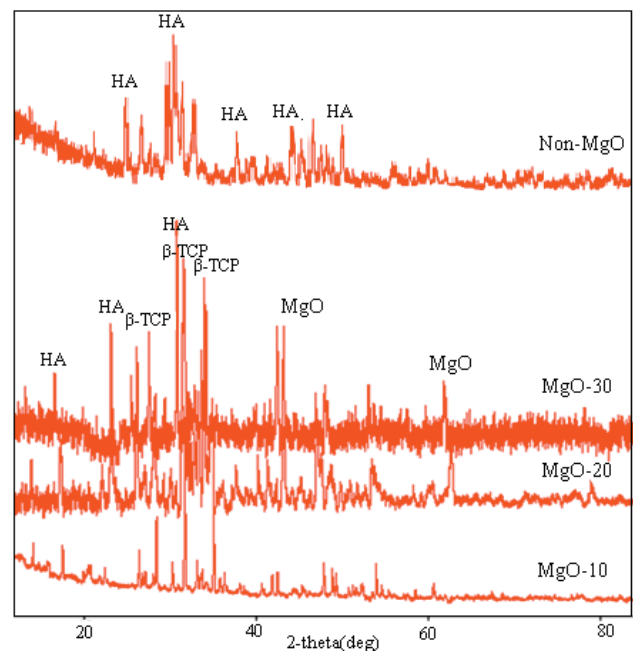


Fig. 2. XRD spectra of the synthesized biografts (Non-MgO, MgO-10, MgO-20, MgO-30)

3.3. SEM

SEM images of the fabricated biografts were given in Figs. 3a–d. The SEM views indicate that a mixed grain distribution occurred in all biografts. Moreover, grain borders were not observed clearly depending on

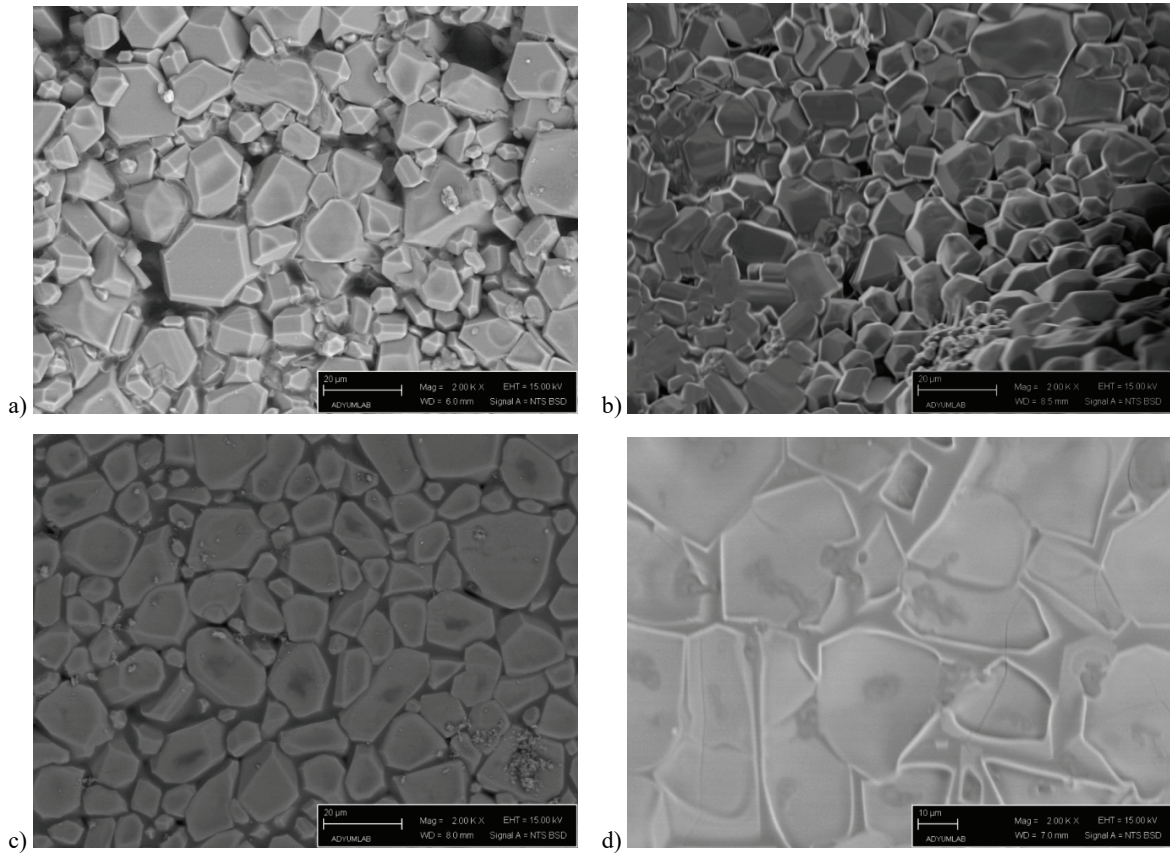


Fig. 3. SEM views for different MgO reinforcements: (a) Non-MgO, (b) MgO-10, (c) MgO-20 and (d) MgO-30

liquid phase sintering of MgO-20 and MgO-30 biografts and that liquid phase sintering increased as MgO content increased. It was determined that grains were irregular and observed clearly with inter-grain spaces occurred following the sintering process in Non-MgO biografts (Figs. 3a–d). Furthermore, from the SEM images taken at X2000 magnification, for each biograft, 3 different grain sizes were measured and the effect of MgO on grain size was examined. As a result, while grain size of Non-MgO has a value of $10.43 \mu\text{m}$ as MgO-20 has a value of $16.50 \mu\text{m}$. It was observed that the lowest grain size of MgO-10 biografts ($4.80 \mu\text{m}$) was observed in all biografts. As the amount of MgO increased, the grain size increased and the highest grain size MgO-30 ($31.17 \mu\text{m}$) was observed due to the increase of liquid phase sintering in biografts.

3.4. Compression tests

Figures 4a–b show results of ultimate compression stress (a) and displacement (b) values for different MgO additions biografts (Non-MgO, MgO-10, MgO-20, and MgO-30). Examinations revealed that while the material with the minimum ultimate stress was found in the Non-MgO biograft with 53 MPa and the maxi-

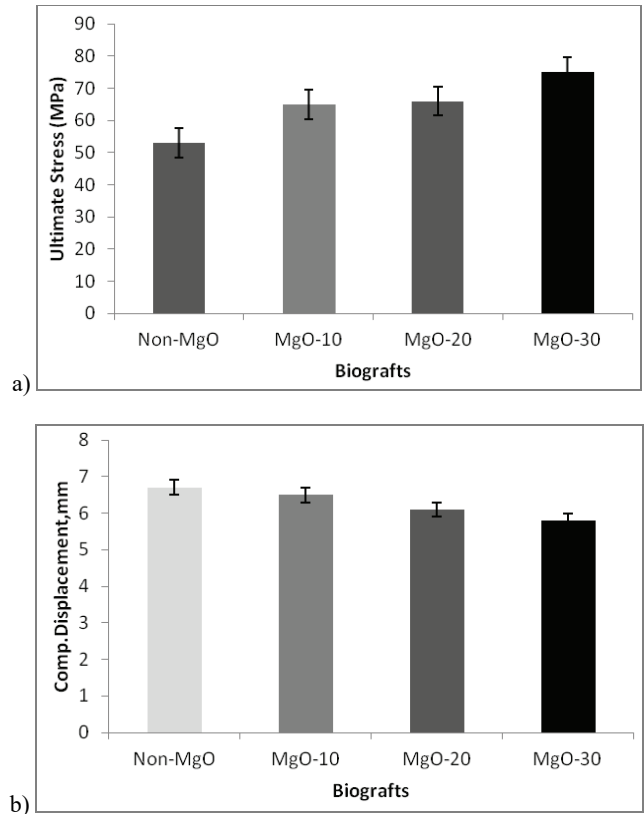


Fig. 4. Compression test results of MgO-reinforced biografts

imum value was obtained 75 MPa in MgO-30 samples. In addition, the values of %compression displacement were inversely proportional considering ultimate stress values and increase the ultimate stress value of biografts caused values of %compression displacement to decrease.

3.5. Hardness tests

Figure 5 shows the results of vickers micro-hardness test performed on all biografts under 20 kgf of load within 5 sec. From the results, it was observed that the maximum hardness value was yielded by MgO-30 biograft with 6.89 GPa, the minimum hardness value was yielded by Non-MgO biograft with 3.2 GPa. It was also determined that the amount of additive and values of hardness were directly proportional in MgO-reinforced biografts and value of hardness increased with increasing MgO amount.

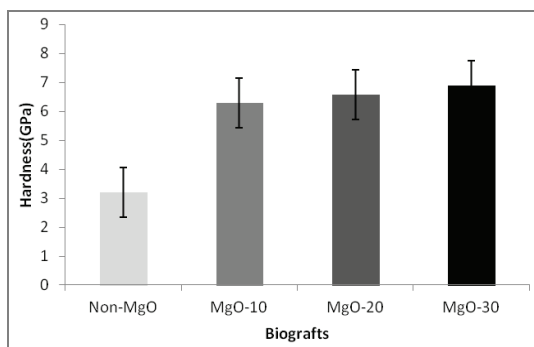


Fig. 5. Vickers microhardnesses for MgO-reinforced biografts

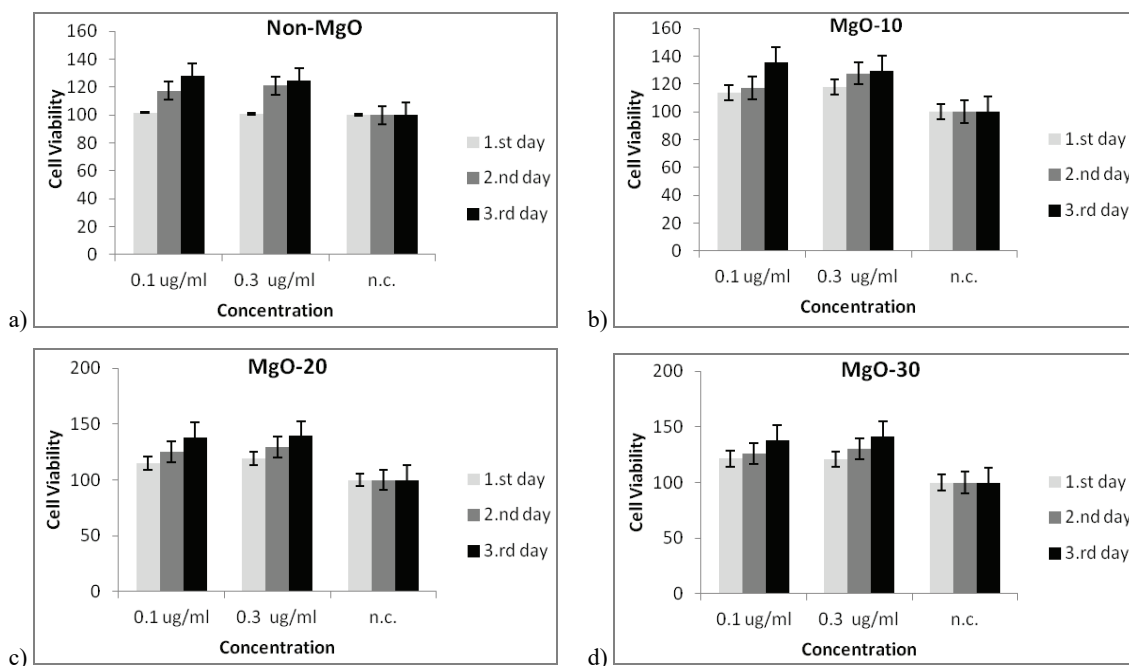


Fig. 6. Cell viability results of samples: (a) Non-MgO, (b) MgO-10, (c) MgO-20 and (d) MgO-30 for 1 and 3 incubation days

3.6. Viability tests

Figures 6a–d show the results of in vitro experiments (cytotoxicity) carried out with osteoblast cells on the fabricated biografts by adding 10–30 wt. % MgO into $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, P_2O_5 , KH_2PO_4 , and Na_2CO_3 .

After 3-day cytotoxicity experiments performed at concentrations of 0.1–0.3 $\mu\text{g}/\text{ml}$, it was observed that percentage rates of viability in all MgO concentrations of all biografts increased proportionally (Figs. 6a–d).

4. Discussion

In the present study, the effects of MgO compound on biografts were investigated. In contrast to literature studies, high MgO was added and MgO-containing biografts were performed with the mechanical properties and cytotoxicity tests. MgO added at different ratios to biografts increased both the mechanical properties and the cell viability. FTIR results showed that biografts with different rates of MgO (10–30 wt. %) content formed peaks of PO_4^{3-} in the wavelength range of $1118.01\text{--}747.43\text{ cm}^{-1}$ based on HA and $\beta\text{-TCP}$ compounds (Fig. 1). The results in previous studies [5], [26], [29] supports the present study in which the peaks PO_4^{3-} was observed in the range of $962\text{--}1087\text{ cm}^{-1}$. Figure 1 also shows that peak intensity increased with increasing MgO content in biografts.

As a result of XRD analysis it was determined that HA and β -TCP phases with calcium phosphate content appeared in all biografts (Fig. 2). XRD analysis revealed the MgO compound as well as HA and β -TCP phases in the biografts with different MgO values in the same composition (Fig. 2). It was also observed that as the rate of additives increased, the peak became more regular and stable, accordingly width of the peak reduced and length of the peak increased. When results of FTIR and XRD analysis were compared, the results of FTIR and XRD were observed to support each other based on the appearance of HA and β -TCP phases obtained from XRD results and PO_4^{3-} peaks in FTIR analysis of all biografts (Figs. 1, 2).

SEM images of Non-MgO, MgO-10, MgO-20, and MgO-30 biografts showed that the fabricated biografts displayed different morphological and chemical property based on chemical content. SEM images at different magnifications indicate formation of a mixed grain distribution in all biografts (Figs. 3a–d). Compared to MgO-reinforced and Non-MgO biografts, while grains of Non-MgO biograft clearly had grains with sharper edges, micro-pores also occurred between grains (Fig. 3a). MgO based biografts were identified to have a liquid phase-like sintering along with irregular grain distribution and sinterability seemed to increase as MgO amount increased (Figs. 3 b–d).

FTIR, XRD and SEM analyzes were carried out to determine whether the chemical, morphological and mechanical properties of the biografts (hardness and compression test) were the same or similar to those of the human bone. In order to achieve this purpose, the maximum ultimate stress value was obtained from MgO-30 with a value of 75 MPa, the minimum from the Non-MgO biograft with 53 MPa, amongst $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, P_2O_5 , KH_2PO_4 , and Na_2CO_3 based and 10–30 wt. % MgO-reinforced biografts. As compression displacement (%) values of all biografts were examined, it was observed that the values are inversely proportional to ultimate stress value. The compression displacement (%) values decreased with increasing MgO amount. In the study, when the hardness values of biografts were examined, it was determined that MgO-containing biografts gave similar results and the hardness values increased in direct proportion to the compressive stress values. Accordingly, the maximum hardness value was 6.89 GPa, obtained from 30% MgO-reinforced biograft (MgO-30) and the minimum hardness value was 3.2 GPa, obtained from the Non-MgO biograft. Phase structures, crystallinity, and mechanical properties of biografts may vary based on the chemical compounds, production method and sintering temperature used in produc-

tion of biografts [30]. Mechanical properties of HA and β -TCP compounds occurring as sintering temperature increased in biograft production increased as well as their increasing crystallinity [8], [14]. Besides, in MgO-reinforced biografts, MgO (0.05–0.1 wt. %) was determined to improve Young's Modulus, Fracture Toughness and hardness (HV) of HA and densification behavior [28]. In the present study, It has been observed that both sinterability is increased and its mechanical properties are closer to those of the human bone in $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, P_2O_5 , KH_2PO_4 , and Na_2CO_3 based on higher MgO (10–30 wt. %) added biografts. Furthermore, unlike the literature studies conducted in this area, it has been determined that biografts are biocompatible with cytotoxicity tests performed with osteoblast cells and cell viability increases as the amount of MgO increases.

When mechanical properties and FTIR, XRD and SEM results of all biografts were compared, they were seemed to support each other. In other words, FTIR and XRD analysis revealed that crystallinity increased because the peak intensity increased with increasing MgO amount and therefore mechanical properties improved. In addition, results supported each other since ultimate stress and hardness values were minimum based on porous and sharp edged structure of Non-MgO and the maximum ultimate stress and hardness values were obtained by MgO-30 depending on high liquid phase sintering in SEM images.

In similar work previously conducted it was reported that MgO was observed to influence HA cytotoxicity and degradation of osteointegration positively and to aid improving *in vivo* biocompatibility of HA in biological environment [3], [9], [28]. S.S. Banerjee et. al. [3], reported that SrO (0.25–1.0 wt. %) and MgO (0.25–1.0 wt. %) added to β -TCP, it was observed that it decreased the compressive strength and increased cell attachment and growth. In the study, it was determined that $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, P_2O_5 , KH_2PO_4 , and Na_2CO_3 based on higher MgO (10–30 wt. %) added biografts increased the mechanical properties (ultimate stress and hardness) and cell viability. Cytotoxicity tests performed to analyze toxic effect of all biografts showed a type of immature cell which is precursor of bone cell and transforms into immature osteocyte by proliferation. Thus, in other words, it can be resulted that it plays a role in formation and regeneration of bones. As a result of 3-day cytotoxicity tests on osteoblastic cell cultures at 0.1–0.3 ug/ml concentration, all biografts were observed to have no toxic effect and such additions increased the rate of cellular viability. It was also determined that cellular viability rate of the fabricated biografts with

addition of MgO increased, compared to Non-MgO biografts.

5. Conclusions

As a result of examinations carried out on MgO-reinforced biografts fabricated by sol gel method it was observed that:

- XRD analysis results revealed that HA and β -TCP phases occurred dominantly in all biografts and MgO peaks also occurred in biografts with MgO content;
- FTIR and XRD results supported each other and peak intensity increased with increasing MgO amount and crystallinity increased based on increased peak intensity;
- hardness and ultimate stress values increased and % Displacement values decreased with increasing MgO amount. The maximum value of ultimate stress and hardness was obtained from MgO-30 and the minimum value of ultimate stress and hardness was obtained from Non-MgO biografts;
- when mechanical test results and FTIR, XRD, and SEM results were compared, they supported each other. In other words, as MgO amount increased, peak intensity of FTIR increased and accordingly crystallinity also increased. In SEM images it was observed that grain size shrinkage (tane boyutunda küçülme) for MgO-10 or liquid phase sintering and grain size increase occurred with increasing MgO amount (MgO-20 and MgO-30);
- after 3-day *in vitro* test on biografts, cellular viability rate of all biografts increased and cellular viability rate increased with increasing MgO amount.

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