Physico–Chemical and *In Vitro* Biological Study of Zinc-Doped Calcium Sulfate Bone Substitute

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Abstract: In the present study, series of Zn incorporated calcium sulfate bone cements, with different amounts of doped Zn(0, 0.74, 1.97, 3.05, 4.21 wt %) were prepared by mixing a calcium sulfate hemihydrate powder and solutions of zinc sulfate, and the effect of zinc-doping on some physical, physico–chemical, and biological properties of the cements were investigated. Pure calcium sulfate cement was also made as control, with the mentioned powder and distilled water as liquid phase. The initial setting time and compressive strength of the cement significantly changed from 17 min and 3.2 MPa for the pure calcium sulfate to 6 min and 6 MPa for the Zn-added calcium sulfate, respectively. Compared to pure calcium sulfate, more gypsum precipitates were formed in the zinc sulfate added samples with a morphology of thin, elongated, and rod-shaped crystals. The biological properties of the samples were analyzed in the terms of cell viability and cell activity on human osteosarcoma (G-292) using MTT assay and alkaline phosphatase (ALP) activity in the cell culture medium. The best increased cell density and ALP activity were achieved for the calcium sulfate cement with a content of 0.74 wt % Zn, whereas a toxic behavior was observed for the samples with Zn concentrations more than 1.97%. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 91B: 37–45, 2009

Keywords: bone graft; calcium sulfate; cell-material interactions; *in vitro*

INTRODUCTION

Calcium sulfate is highly biocompatible and bioresorbable synthetic bone graft, with a long clinical history, used for the treatment of bone and/or dental defects such as maxillofacial augmentation, Alveolar bone loss, periodontal disease, and endodontic lesions.[1–8] However, incorporation of some individual ions having stimulatory effects on bone formation into calcium sulfate implants may be increase the efficacy of the material by accelerating the healing process. Zinc is one of the ions that provides the mentioned requirements. The zinc ions have been reported that increased the alkaline phosphatase (ALP) activity and DNA content of the bone cells, leading to acceleration of osteogenesis of osteoblasts.[9] The characteristics of various zinc-containing calcium phosphate bioceramics have been studied heretofore. Ito et al.[10] introduced zinc-containing tricalcium phosphate/hydroxyapatite composites as biomaterials with a pharmaceutical effect on promoting bone formation. In another work,[11] they prepared zinc-doped tricalcium phosphate and reported its accelerating effect on the proliferation of osteoblast-like cells. Ishikawa et al.[12] showed that the proliferation of human osteoblast cells significantly improved the surface of an apatitic calcium phosphate cement (CPC) using zinc doped tricalcium phosphate in the cement composition. Lima et al.[13] developed zinc-doped hydroxyapatite bioceramics for clinical applications. Bandyopadhayay et al.[14] showed good adherence of osteoblast cells onto the surfaces of hydroxyapatite containing 1.5 wt % ZnO.

However, no evidence has been reported for the biological and other properties of zinc-containing calcium sulfate bone substitutes. The aim of the present work was to investigate the influence of zinc ions incorporated into calcium sulfate ceramics on the physical, physico–chemical, and biological properties of the material as an important bone substitute.

MATERIALS AND METHODS

Preparation of Calcium Sulfate Cements

Calcium sulfate cement was prepared by mixing calcium sulfate hemihydrate (CSH, $CaSO_4 \cdot 0.5\ H_2O$) powder and distilled water at a powder to liquid loading ratio of 1.7 g/mL. To have zinc-containing calcium sulfate specimens, different concentrations of zinc sulfate heptahydrate solutions (in distilled water) were prepared and used as liquid

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TABLE I. Amount of Zn Ions Doped into the Calcium Sulfate Structure

Name of the Samples	Zn (0)	Zn (1)	Zn (2.5)	Zn (4)	Zn (5.5)
Amount of $ZnSO_4.7H_2O$ (as wt % based on the amount of solid phase) used in the liquid phase	0	1	2.5	4	5.5
Zn (wt %)	0	0.74	1.97	3.05	4.21

phase of the cement. The specimens were named according to Table I, which the amount of zinc sulfate (by weight percent) has been indicated in parentheses. For example, Zn (1) represents cement containing 1 wt % (based on the weight of the powder phase) zinc sulfate heptahydrate. To remove the probable un-reacted zinc sulfate (un-doped ions), which may be remained in the cement composition, the specimens were washed with distilled water after the hardening. The amount of Zn ions incorporated into the final calcium sulfate product was determined using atomic absorption spectroscopy. It was in the range of 0 to 4.21 wt % (Table I).

Setting Time

The initial setting time was recorded according to the ASTM-C266-89 standard[15] using a Gillmore needle Instrument. The initial setting time is defined as the time that a light needle (113.4 g, 2.13 mm ϕ) does not form a visible indent onto the surface of the sample.

Compressive Strength

The mechanical strength of the set specimens was evaluated in the term of compressive strength. The cement paste was packed in split of a cylindrical Teflon mold (6 mm in diameter and 12 mm in height). After the initial setting, the specimen was removed from the mold and stored in a humidified chamber (100% humidity and 37°C) for 24 h. The compressive strength of the samples were recorded using a universal testing device (Zwick/Roell-HCR 25/400) with a crosshead speed of 1 mm/min. Five specimens of each composition were tested.

XRD Analysis

The phase evaluations were carried out on the samples incubated for 24 h and immersed in Ringer's solution for 1 day using X-ray diffractometry (Philips PW 3710) with Ni filtered Cu-Kα radiation generated at 30 kV and 25 mA. For this purpose, the incubated/soaked specimen of each cement composition was washed with double distilled water, quenched in acetone to stop the setting reactions, dried in air, ground to fine powder, weighed, and characterized.

The amount of gypsum phase in each calcium sulfate specimen is directly proportional to its peak intensity in related XRD patterns, because no amorphous phase was in the composition. It was semi-quantitatively calculated using the following expression[16]:

$$I_g = \frac{I_g}{I_g + I_h} \quad (1)$$

where I_g corresponds to the intensity of the gypsum maximum peak at $2\theta = 11.7^o$ and I_h corresponds to the intensity of maximum peak of another hydrated phase (CSH) at $2\theta = 25.7^o$. Four specimens of each composition were selected for semi-quantitative assessments.

FTIR Analysis

Fourier transform infrared spectroscopy (FTIR, BRUKER VECTOR 33 spectrometer) was used to identify the functional groups in the cement composition. The spectrum was recorded in the 4000–400/cm region with 2/cm resolution.

SEM and EDXA Analysis

Morphologies of the fractured surfaces of the specimens were analyzed using scanning electron microscopy (SEM, Stereoscan S 360, Cambridge) equipped with energy dispersive X-ray analysis (EDXA) and operated at accelerating voltage of 20 kV. For SEM, the samples were dried, and coated with a thin layer of gold before SEM examination.

Releasing Ca and Zn Ions and Weight Loss Test

Dissolution behavior of the cements in a physiologic medium was examined by measuring concentration of Ca and Zn ions released from the samples into Ringer's solution at defined time intervals. The release studies were carried out by soaking disk-shaped specimens (3 mm in height and 10 mm in diameter) in Ringer's solution at a concentration of 1 mg sample per mL of the solution at 37°C for intervals from 1 to 21 days. After each time interval, the Ringer's solution was extracted for determining the content of Zn and Ca ions and the samples were immediately fed with fresh Ringer's solution. Concentration of the Zn and Ca ions was determined using ICP atomic emission spectroscopy technique. Furthermore, the loss in weight of each specimen (Δw) was calculated at the end of the evaluation period (21 days) as follows:

$$\Delta w = \frac{w_1 - w_2}{w_1} \times 100 \quad (2)$$

where w_1 and w_2 are weight of dried specimens before and after soaking, respectively.

TABLE II. Setting Time and Compressive Strength of the Calcium Sulfate Cements with Various Amounts of Zn-Doped

Cements	Initial Setting Time (min)	Compressive Strength (MPa)
Zn (0)	17 ± 3	3.2 ± 1.1
Zn (1)	9 ± 1	5.5 ± 1.3
Zn (2.5)	8 ± 2	4.7 ± 0.9
Zn (4)	6 ± 3	6.1 ± 1.5
Zn (5.5)	6 ± 1	5.5 ± 1.6

MTT Assay and ALP Activity

The proliferation of osteoblast cells next to the calcium sulfate specimens was measured by MTT assay. The sterilized specimens were immersed in culture medium, a 1:9 v/v mixture of fetal bovine serum (FBS) and modified Eagle's medium (MEM) containing 3×10^4 cell/mL G-292 osteoblast cells (human osteosarcoma, NCBI C 116 national cell bank of Iran). The cell/calcium sulfate constructs were cultured in humidified incubator at 37°C with 5% CO_2 and 95% air for 1, 3, and 7 days. After each period, the samples were removed from the wells and 10 μL of a 5 mg/mL solution of MTT, 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, solution was added in each well. The optical density (OD) was measured at the wavelength of 590 nm using a multiwell microplate reader (ICN, Switzerland).

The osteoblast activity was determined by measuring ALP production from the G-292 cells. The cells were seeded on the samples under the same culturing condition described above and the level of ALP activity were determined on days 1, 3, and 7. The G-292 cells lysates were frozen and thawed three times to disrupt the cell membranes. ALP activity was determined at 405 nm using *p*-nitrophenyl phosphate in diethanolamide buffer as chromogenic substrate. Five specimens of each calcium sulfate composition were tested.

Statistical Analysis

Quantitative data were presented as mean ± standard deviation (SD) for at least four specimens. Statistical analyses were assessed using SPSS. Student's *t*-test was performed to determine the statistical significance between experimental groups. A value of $p \leq 0.05$ was considered to be statistically significant.

RESULTS

Table II presents the initial setting time and mechanical properties of the calcium sulfate specimens with or without Zn doped. The setting time decreased when zinc sulfate was added to the cement composition. No significant change was observed between the setting time values of the samples with different amounts of zinc sulfate, except that the value of the Zn (5.5) was significantly lower than that of Zn (1). For mechanical strength however, the results was different. The compressive strength values of zinc sulfate-added (zinc-doped) calcium sulfate cements were significantly higher than that of pure calcium sulfate. However, all zinc sulfate-added cements showed almost the same compressive strength value regardless of the $ZnSO_4$ concentration and the differences between the mean values were not statistically significant.

Figure 1 illustrates XRD patterns of various calcium sulfate cements incubated for 24 h [Figure 1(a)] and

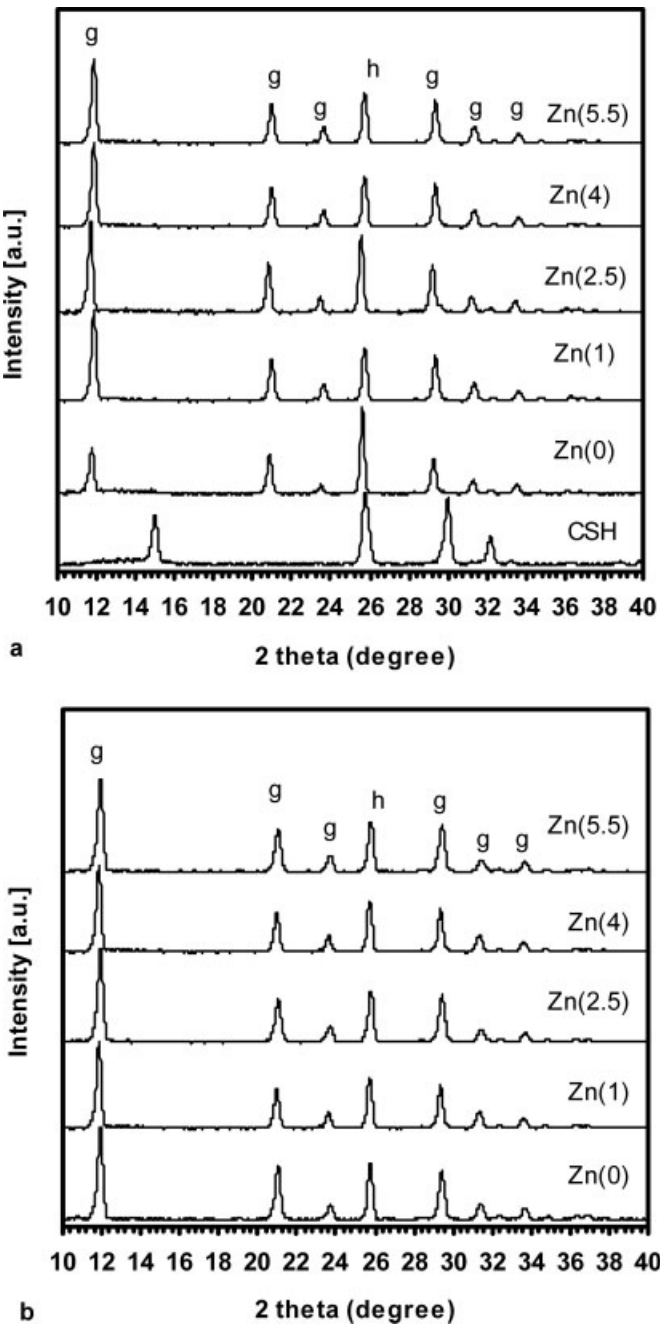

Figure 1. The XRD patterns of various calcium sulfates: (a) 24 h after incubation and (b) 1 day after immersion in Ringer's solution. (g: gypsum, h: calcium sulfate hemihydrate).

TABLE III. Quantity of Gypsum Phase in the Composition of Calcium Sulfate Cements Incubated for 24 h

	Zn (0)	Zn (1)	Zn (2.5)	Zn (4)	Zn (5.5)
Amount of gypsum phase in the cement (%)	40 ± 5*	65 ± 4	69 ± 7	62 ± 6	66 ± 4

* Mean ± standard deviation ($n = 5$).

immersed in Ringer' solution for 1 day [Figure 1(b)]. The pattern of the powder phase of the cement, i.e., calcium sulfate hemihydrate is also presented. The patterns were recognized with X'Pert HighScore software. The lacks of zinc compounds (such as zinc sulfate) in the patterns of Zn-containing specimen support this hypothesis that Zn^{2+} ions have been incorporated into the calcium sulfate lattice. In the patterns of the incubated Zn-doped cements, gypsum was the main phase (PDF-Number 33-0311) next to some un-reacted hemihydrate phase, while CSH was the predominant phase in the composition of the incubated pure calcium sulfate. The quantitative values of gypsum phase in all specimens have been presented in Table III. When the samples were immersed in Ringer's solution, the hemihydrate phase was considerably converted to dihydrate one, so which gypsum was the predominant phase, even in the pure calcium sulfate specimen.

The FTIR spectra of some incubated calcium sulfate specimens are shown in Figure 2. The spectra showed the vibration bands associated to the functional groups of gypsum, which are labeled in the corresponding Figure. The spectra of zinc-containing cements were completely similar to that of pure calcium sulfate cement, indicating lacks of zinc phase impurities (such as zinc sulfate) in the cement composition.

SEM micrographs of the samples with different amounts of zinc ions have been shown in Figure 3. Entanglements of rod-shaped crystals were observed in the micrographs of all specimens with well-grown and more elongated crystals for those cements doped with Zn ions [Figure 3(b,c)].

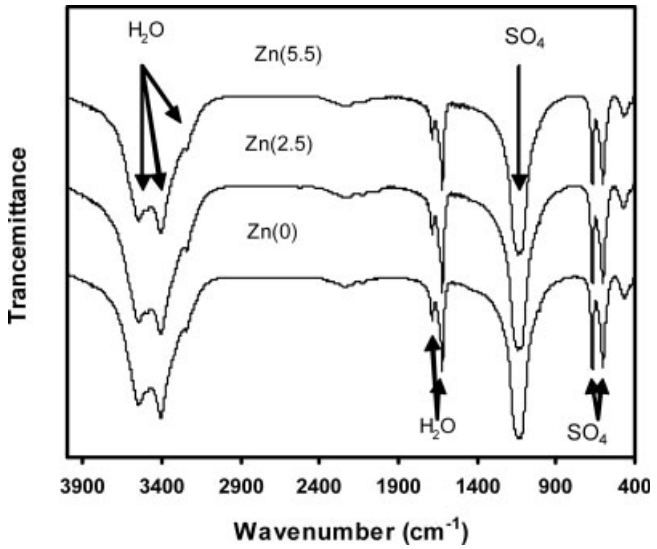

Figure 2. FTIR spectra of Zn-doped calcium sulfates.

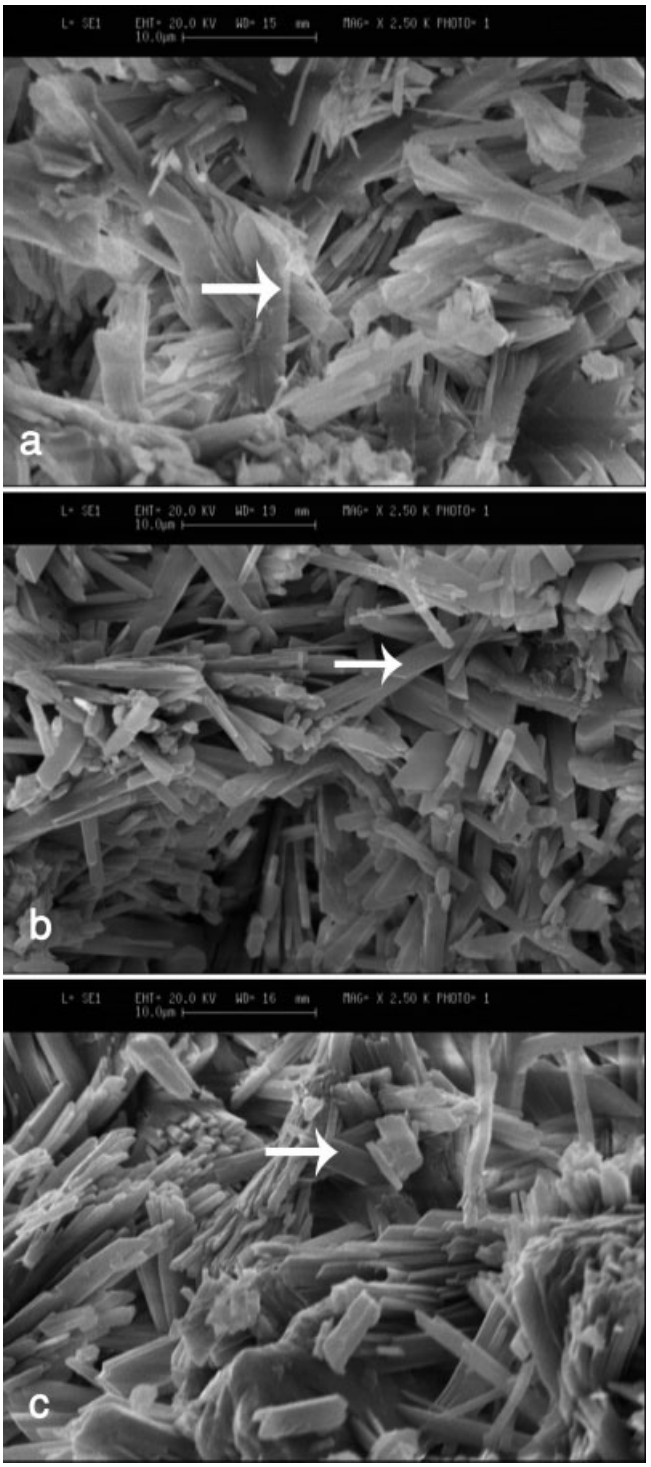

Figure 3. SEM micrographs of incubated calcium sulfate specimens: (a) Zn (0), (b) Zn (2.5), and (c) Zn (5.5).

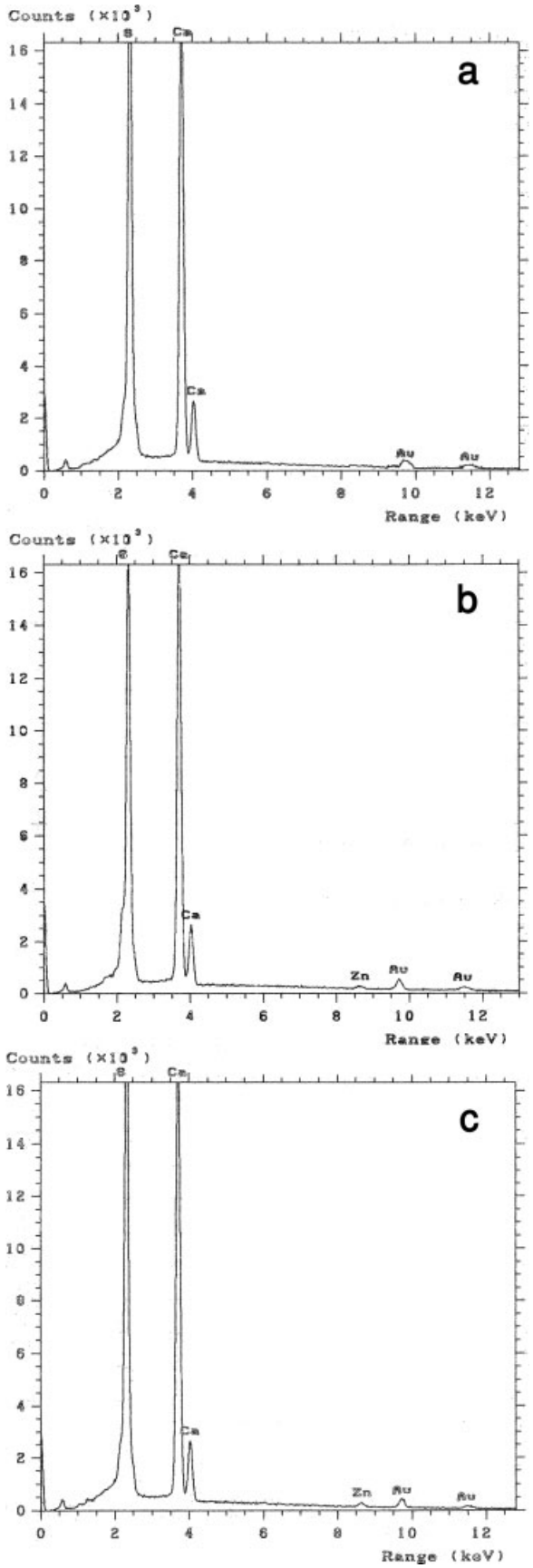

Figure 4. EDXA of the calcium sulfate cements at the point showed by arrows in the SEM micrographs of Fig. 3: (a) Zn (0), (b) Zn (2.5), and (c) Zn (5.5).

Figure 4 shows the EDXA patterns, corresponding to SEM micrographs shown in Figure 3. Ca and S ions were clearly recognized in the EDXA pattern of pure calcium sulfate and presence of Zn ions was also found the patterns of those specimens doped with Zn ions. Since no other zinc compounds were found in the composition of the Zn-containing calcium sulfates, the appearance of these peaks confirms incorporation of this cation in the lattice structure of the gypsum phase produced during the cement hydrolysis.

Figure 5 shows the cumulative concentration of both Ca [Figure 5(a)] and Zn [Figure 5(b)] ions released from the various calcium sulfate specimens into Ringer's medium. The high concentration of Ca ions in the Ringer's solution relates to high solubility of calcium sulfate, which make it as a bioresorbable material. The higher amount of Ca ions released from the Zn (0) specimen indicates the higher solubility of pure calcium sulfate compared to Zn-doped ones. Although the release behavior of Ca ions from all Zn-doped calcium sulfates specimens were almost the same, the rate of Zn release was higher for those samples con-

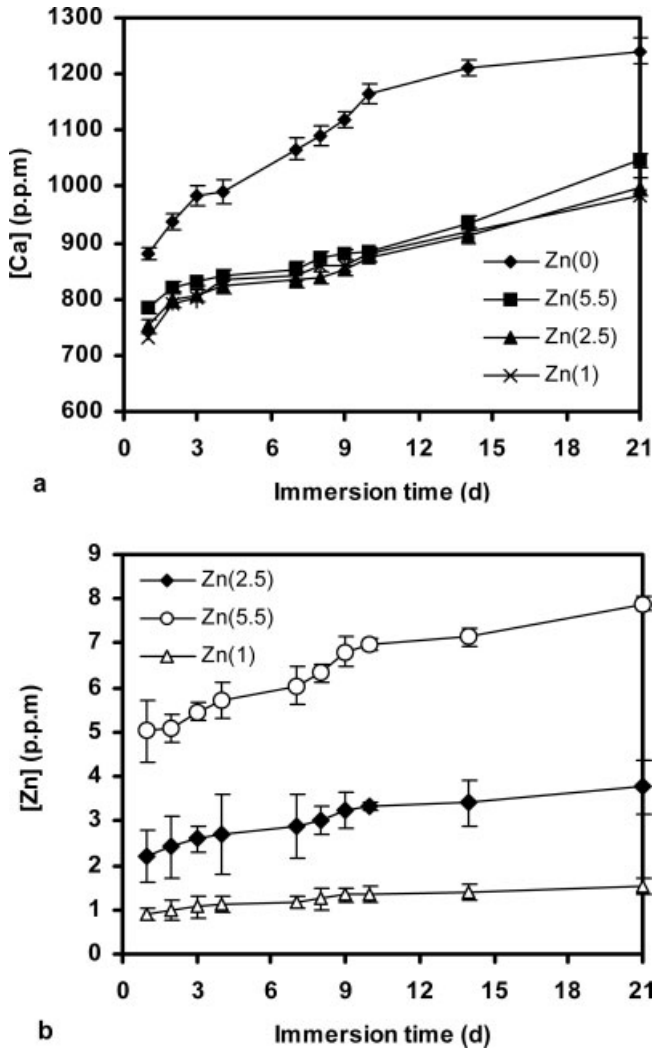

Figure 5. Concentrations of Ca (a) and Zn (b) ions found in Ringer's solution after different evaluating times.

TABLE IV. Loss in Weight of Calcium Sulfate Cements After Soaking the Samples in Ringer's Solution for 21 days

	Zn (0)	Zn (1)	Zn (2.5)	Zn (4)	Zn (5.5)
Δw (%)	7.2 ± 1.3	3.5 ±1.5	4.1 ± 1.2	5.1 ± 0.9	4.4 ± 2.1

tained more Zn content. The loss in weight of calcium sulfate specimens soaked in Ringer's solution for 21 days have been shown in Table IV. The maximum value was experience for pure calcium sulfate cement (control) and the weight loss significantly decreased by incorporating Zn ions into the cement composition.

The results of the MTT test of calcium sulfate cements with different amounts of doped Zn were shown in Figure 6. The OD values were significantly higher for Zn (0), Zn (1), and Zn (2.5) than other groups, indicating promoted cell growth.

When the concentration of Zn ions increased beyond 1.97% [Zn (2.5) specimen], the OD value tends to decrease as culturing time increased. It reflects the inhibitory (toxic) effect of the samples on cell growth.

According to the results of ALP activity in Figure 7, at day 1, the low level of ALP production was related to little osteoblastic phenotype. After 7 days, the level of ALP increased significantly on Zn (0) ($p < 0.05$), Zn (1) ($p < 0.005$), and Zn (2.5) ($p < 0.05$) specimens with more relevant changes in Zn (1) and Zn (2.5). No significant changes were observed in the level of ALP produced by the cells subjected to Zn (4) and Zn (5.5) samples ($p < 0.05$).

DISCUSSION

Although known bioceramics such as hydroxyapatite, apatitic calcium phosphate cement, and bioglasses are highly

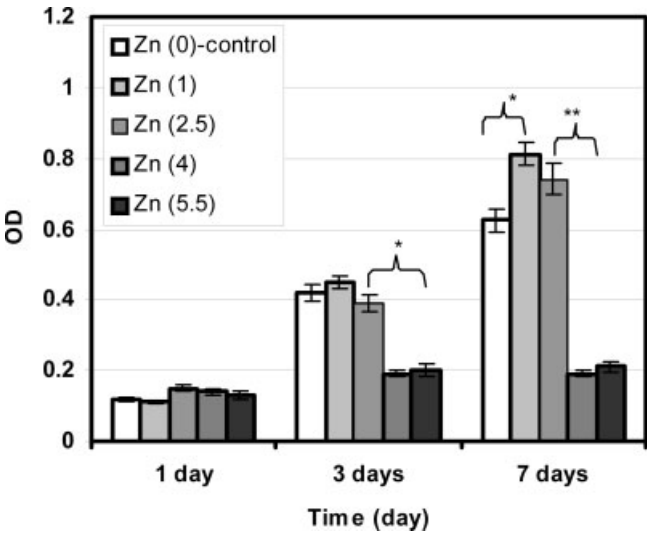

Figure 6. Proliferation conditions of the osteoblasts cells (as optical density of the cultured cements) in contact with calcium sulfate samples containing various amounts of Zn-doped (*$p < 0.05$, **$p < 0.005$).

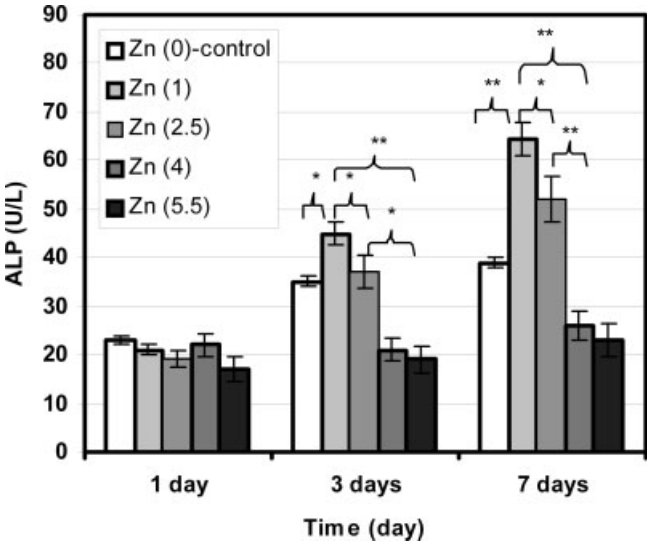

Figure 7. ALP activity for osteoblast G-292 cells on various calcium sulfate samples after different culturing times (*$p < 0.05$, **$p < 0.005$).

osteoconductive and bioactive materials, clinicians usually prefers to use biodegradable and resorbable substitutes in the treatment of bone defects.

Calcium sulfate is a highly biocompatible, bioresorbable, and osteogenic material *in vivo*, which alters osteoblastic activity to promote bone formation through inducing molecular activation.[17] This study revealed that the physical and biological properties of the calcium sulfate cement drastically changed with incorporating Zn ions into the cement composition.

The setting times of the Zn-containing cements were significantly lower than that of pure calcium sulfate. The setting reaction of calcium sulfates is consequence of a dissolution-precipitation process. At room temperature, CSH is partially dissolved in water to form Ca^{2+} and SO_4^{2-} ions. Once the suspension is supersaturated with respect to $CaSO_4$ñ$2H_2O$, gypsum reprecipitates taking 1.5 mole H_2O (according to Eq. 3), because gypsum is thermodynamically more stable than CSH[18]:

$$CaSO_4 \cdot 0.5H_2O + 1.5H_2O = CaSO_4 \cdot 2H_2O \qquad (3)$$

The SO_4^{2-} ions additionally supplied by $ZnSO_4$ play an important role in the precipitation rate of initial gypsum crystals by shortening the time required for suspension to be supersaturated with respect to gypsum. In fact, they make suspension highly supersaturated with respect to gypsum and accelerate precipitation of gypsum nuclei. The mechanism of setting reaction of calcium sulfate cement is very close to that of CPC.[19] Thus, the rate of gypsum precipitation (R) can be expressed by the following Equation used also for the precipitation of hydroxyapatite in CPC[20–22]:

$$R = K\exp\left(\frac{-F}{(\mathrm{Ln}S)^2}\right) \quad (4)$$

where, S is the supersaturation, K is Boltzmann constant, and F is a function of absolute temperature, nuclei shape, interfacial energy between the gypsum and solution and the contact angle between the solution and precipitates. The solution supersaturation with respect to gypsum is expressed as follows[23]:

$$S = \frac{(Ca^{2+})(SO_4^{2-})}{K_s} \quad (5)$$

where K_s is solubility of product.

There is strong relationship between the quantitative amount of setting reaction product (gypsum) and the compressive strength of the samples, i.e., the higher amount of the gypsum product, the higher compressive strength values. It is clear that higher amounts of $Zn^{2+}$and SO_4^{2-} ions are introduced into reaction medium using higher amounts of $ZnSO_4{\cdot}7H_2O$ precursor. In this study, what seems that affects the mechanical strength of calcium sulfate cement is the lack or presence of $SO_4{}^{2-}$ ions in the reaction medium. The mechanical strength of calcium sulfate cement comes from interlocking of gypsum crystals in the cement microstructure and can be influenced by the quantity and microstructural features of these crystals. The XRD and related quantitative analysis showed that higher amount of gypsum could be recrystallized in the calcium sulfate cements made with zinc sulfate solutions, regardless the concentration of SO_4^{2-} (or Zn^{2+}). Thus, these cements had a higher compressive strength than the cement made with distilled water. In other words, in all cement specimens made with zinc sulfate solutions, approximately equal quantities of gypsum were found. It can explain the lack of differences between mechanical strength of specimens with various amounts of Zn content. Such mechanical behavior has been also reported for hydroxyapatite cements that had been made with the same powder phase and various solutions of PO_4^{3-}.[24]

The rate of the CSH hydrolysis (Eq. 3) influences the morphology of the precipitated crystals too. Gypsum precipitated in the presence of zinc sulfate additive had crystals with a well known morphology of thin, elongated, and rod-shaped because of the rapid growth in the [020] faces. In contrast, more agglomerated crystals were observed in the SEM image of pure calcium sulfate cement as a result of a deceleration in the growth of the [020] faces, which consequently resulted in a plate-like crystals.

Incorporation of Zn ions into the lattice of gypsum influenced its dissolution behavior too. The bioresorption of bioceramics is usually assessed by determining the level of ions dissolved from the specimen into the simulated physiological fluid.[25,26]

In this study, it suggests that both Ca^{2+} and Zn^{2+} ions have a reciprocal effect on the release behavior of each other. Incubated calcium sulfate cements had been composed of Zn-doped gypsum and remained hemihydrate phase. When this sample is immersed in Ringer's solution Ca^{2+} and Zn^{2+} ions begin to release until the solution reached to a supersaturated level with respect to gypsum. The Ca^{2+}, SO_4^{2-}, and Zn^{2+} ions contribute to form Zn-doped gypsum through hydrolysis of hemihydrate phase, which precipitates onto the surfaces of previously formed crystals. This can retard the release rate of Zn ions. Introduction of Zn^{2+} ions into the solution medium and its migration into lattice structure of gypsum can be continued through dissolution and precipitation processes until the whole of hemihydrate phase be converted to gypsum. The gradual increase in concentration of Zn^{2+} ions reveals that the rate of release is higher than the rate of migration. However, another important point that should be considered is the *in vitro* resorbability of the zinc-doped calcium sulfate cements. The cumulative release of Ca^{2+} and weight loss tests revealed that dissolution and consequently resorption rate of the cement decreased when $ZnSO_4$ was added to its composition. There are some suggestions for this, including: (i) Inhibitory effect of Zn^{2+} and SO_4^{2-} ions presented (released) in Ringer's solution on dissolution of gypsum[27] and (ii) Increasing effect of sulfate ions on formation of gypsum product (note that gypsum is less soluble than CSH).

To determine the effect of Zn ions incorporated into calcium sulfate structure on the biological properties of the cement, the tests were focused on cell proliferation and cell activity using osteoblasts, the target cells for bone filler materials.

How bioceramics regulate cellular functions is the focus of considerable biomaterials researches.[28] Recent *in vitro* and *in vivo* studies[29–31] have demonstrated biological sensitivity to presence of Zn ions and the level of these ions within the ceramic structures, because Zn directly determines the type of cellular response in contact with materials. In this study, various amounts of Zn ions were incorporated into calcium sulfate lattice structure to estimate the optimum concentration, stimulating osteoblast activities. In ceramic-based biomaterials, the optimum level of zinc content for stimulating cell proliferation and totally cell responses depends on various parameters such as the type of the medium used for cell culture, the type of the cells and specially the type of the materials.[12] A zinc content of 1.2 wt % doped in tricalcium phosphate/hydroxyapatite composites showed the best stimulating effect on MC3T3-E1 osteoblast-like cells,[10] while in this study, the best G-292 cell responses was observed for the calcium sulfate cement contained 0.74 wt % Zn. In addition, compared to other zinc-doped ceramics such as tricalcium phosphate and tricalcium phosphate/hydroxyapatite composites,[10] higher amount of Ca and Zn ions released from the calcium sulfate specimens into physiologic medium. It is not far from the mind, because calcium sulfates are more soluble materials than calcium phosphates. The rapid

resorption can promote osteogenic activity, leading to the induction of new bone formation. *In vivo* mechanism of calcium sulfate action has not been completely understood, but several explanations have been recently forwarded by authors. For instance, Walsh et al.[32] suggested that the local acidity during calcium sulfate resorption was the possible mechanism for acting this material. Palmieri et al.[33] stated that calcium sulfate causes overtranslation of several genes such as BMP1 and 7, some hormones like PTH and CALCA and some receptors like FGFR1, acting on bone formation. Lazary et al.[34] demonstrated that calcium sulfate dehydrate (gypsum) with special crystal structure may act as osteoinductive material due to releasing high calcium content in the extracellular matrix that plays an important role in the regulation of bone cells.

Destruction of Zn-doped calcium sulfate lattice by the Ringer's solution leads to introducing Ca and Zn ions in the culture (or physiologic) medium. However, it should not be assumed that the Zn ions are always effective in cellular responses. In this study, Zn ions doped in calcium sulfate structures have been shown to affect osteoblastic activity, mainly at optimum concentration of 0.74 wt %. The stop in proliferation and activity of G-292 cells cultured in contact with the Zn (4) and Zn (5.5) specimens reflects the deleterious effect of high level of Zn ions released from these specimens. In other words, in all culturing time intervals, the Zn (1) specimen showed higher proliferation rate than others. ALP activity also indicates osteoblstic activity and determines bone turnover and bone remodeling. ALP is believed to have an important in hydrolysis of pyrophosphatase and ATP to orthophosphate, which is used to form the nascent calcium phosphate mineral.[35] Thus, an increase in ALP production *in vitro* correlates to bone formation *in vivo*. The increase in ALP activity indicated that the cells had ceased to proliferate and had begun to differentiate, especially for Zn (1) and Zn (2.5) samples.

CONCLUSIONS

It can be concluded from this study that the addition of zinc ions to the composition of calcium sulfate cements alters the physical and morphological properties of the cement and improves proliferation and differentiation of osteoblast cells. The amount of the dopant is critical, because too much Zn release would result in adverse reactions such as cytotoxicity.

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