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Effect of Zn Addition on Sol-Gel Derived Apatite/Wollastonite Glass-Ceramics Scaffolds

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Abstract

Bioactive glasses and glass-ceramics consisting of CaO-MgO-P_2O_5-SiO_2 system have been shown to be bioactive, resorbable and to exhibit appropriate mechanical properties for bone tissue engineering applications. Bioactive glass-ceramics scaffolds consisting of zinc doped-apatite/wollastonite (AW) glass-ceramics were prepared by coating polyurethane foams with sol-gel derived glass gels. Density and compressive strength of the scaffolds decreased with increased in zinc contents. In vitro bioactivity of Zn-doped AW glass-ceramics in a simulated body fluid (SBF) showed the formation of apatite layer on the surface within 7 days of soaking. However, increasing Zn addition increased the chemical durability of the AW glass-ceramics, resulting in a decrease in the rate of apatite formation in a simulated body fluid. With observation by SEM technique microstructure of pores showed interconnected macropores in the range of 300-500 μm as depended on the type of sponge. Average pore size increased with increasing the zinc additions. Macropores and interconnect size can be tailored by controlling the zinc concentrations.

Key words: Zinc, Glass-Ceramics, Scaffolds, Apatite-Wollastonite, Sol-gel, Bioactive glass scaffolds

Introduction

Bioactive glass-ceramics are polycrystalline materials produced by controlled crystallization of glass. Most bioactive glass-ceramics are based on compositions which are similar to those of Bioglass© but they have very low contents of alkali oxides. Apatite-wollastonite glass ceramic (AW-GC) is the one of the most extensively studied glass-ceramic for use as a bone substitute material. AW-GC exhibits substantially superior mechanical properties with respect to bioactive glass and forms an apatite layer on its surface which allows it to bond directly to the bone. The development of glass-ceramic scaffolds is to overcome the limitations of glass scaffolds in terms of mechanical properties and to widen their application beyond small defect sites and non-loading bearing applications. However, the architectural design of a scaffold is still a great challenge: a sufficient mechanical competence and allow new bone in-growth within its own structure. Therefore, scaffold properties should be carefully designed on the basis of the final clinical use. In this sense, the choice of a proper method of fabrication plays a key role. There is a great variety of methods for scaffold processing that leads to porous body formation with different structure, architectural design, pore size and interconnection. Sponge replication can be a good candidate in this sense due to its easiness, effectiveness, versatility and low cost. It was demonstrated that high strength trabecular scaffolds can be successfully obtained by carefully setting the processing parameters.(1)

The structure and chemistry of glasses can be tailored at a molecular level by varying either compositions or thermal and environmental processing history. Sol-gel derived glass is possible to design glasses with degradation properties specific to a particular application of bone tissue engineering.(2-3) Several glass ceramic scaffolds have been successfully prepared by sol-gel technique and showing a high bioactivity.(4-6) In the past 15 years, many research works carried out to demonstrate the ion released by bioactive glasses which can exert a gene control regulation. Silicon,(7) zinc(8-9) and magnesium(10) ions were found to promote osteoblastic cells proliferation,

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differtiation, and thus bone mineralization. Therefore, the genetic design of bioactive scaffolds is a fascinating and attractive field of research able to open new perspectives of tissue regeneration. Many bioactive glass compositions have been investigated as possible zinc ion delivery vehicles.(11-12) It has been proposed that bioactive glass allows a tailored modulation of zinc release in the biological system due to the incorporation of zinc ions in the forming hydroxyl-carbonate-apatite layer. In this study aims to study the effect of zinc doping concentration on the characteristic of A-W glass ceramic scaffold and the bioactivity in vitro of samples prepared by the sol-gel method.

Materials and Experimental Procedures

In this work, glass-ceramic macroporous scaffolds were obtained by sol-gel process as repeated follow by Deepak K., 2009.(13) The chosen glass-ceramic belongs to the system SiO_2-P_2O_5-CaO-MgO-CaF_2 and has the following weight composition: 34.2% SiO_2, 16.3% P_2O_5, 44.9% CaO, 4.6 %MgO and 0.5% CaF_2. The glass-ceramic was referred to as AW-GC. Briefly, approximately 10 g AW-GC was prepared by mixing between aqueous solution of 18.81 g calcium nitrate tetrahydrate $Ca(NO_3)_2.4H_2O$, Ajax.) and 2.91 g magnesium nitrate hexahydrate ($Mg(NO_3)_2.6H_2O$, Ajax) in 12.5 ml and 2.8 ml de-ionized water, respectively, to form a inorganic precursor. 6 ml Methanol (Merck) and 12.90 ml tetra ethoxy orthosilane (TEOS, Acros) were then mixed together into a beaker and then CaF_2 (Carlo) particles were dispersed in this solution by constant stirring to form an organic precursor. Inorganic solution was added by drop wise to the organic precursor with continuous stirring followed by addition of H_3PO_4, HCl and zinc nitrate hexahydrate ($Zn(NO_3)_2$. $6H_2O$, Ajax) as a dopant in doping concentration of 1 and 5 mol%, respectively. This solution was heated up to 35°C for 20 min, and then aged for an hour at room temperature. To prepare porous samples, the polyurethane sponges (1 x 1 x 2 cm in dimension) were immersed in the mixture solution and compressed to squeeze the excess solution out. Then the impregnated foams were aged in oven at 60°C and dried at 130°C at least 3 days. To remove the polyurethane foams and get the porous scaffolds, the impregnated foams were sintered in a furnace at 900°C for 3 hours, and then the specimens were cooled down in the furnace. To determine the density and measure the compressive strength of samples, gel was casted into plastic mould in 1 cm diameter and 2 cm height and then allowed to dry and heat-treatment as same as the scaffold samples. Apparent density and apparent porosity of the samples were determined by Archimedes' method. Bioactivity of the Zn doped A-W glass ceramic scaffolds was evaluated by immersing in SBF with an ionic composition almost equal to human plasma.(14) Tris-HCl used as buffer to maintain a constant pH value of 7.4. Scaffold specimen was immersed with surface area to volume equal to 0.1 cm^{-1}(14) contained in a sealed plastic vial without solution replacement. The plastic vials were placed in an incubator at 37°C for 3 and 6 hours, 1, 3, 7 and 14 days, respectively. At predetermined periods of time, the specimens were removed from SBF and washed gently with distilled water and then air dried at room temperature. After soaking in SBF, SEM (JSM-5800LV, JEOL) observation and chemical analyses (FT-IR spectroscopy) were carried out to assess the formation of a silica-rich layers and the precipitation of hydroxyapatite layer on the specimen surface.

Results and Discussion

Figure 1a shows the morphology of the polyurethane sponge used as a template. The sponge has a 3D porous interconnection structure with pores ranging from 300 up to 500 μm and it is similar to trabecular bone structure. During immersion of the sponge in the gel was processing for an hour, the higher ZnO doping concentration, the higher viscosity gel obtained. The sol-to-gel transition consists of clusters growing through condensation reactions until these clusters collide producing a gel. The sudden change in rheological behavior near the transition seems to be the most accurate way to evaluate the gel time.(15) This behavior was also found in the gelation of fluoride-doped silica sols(16) which the gelation times for sols were varied by changing the fluoride ion content. Thus, good impregnation of the sponge can be obtained in specimens for high ZnO doping concentration in AW-GC scaffolds, as in Figures1b-1d. Therefore, to obtain the good impregnation of sponge, the ageing time and immersion time for high ZnO doping concentration should be adjusted. Most of pores were still open due to the optimized characteristics of gel solution and proper removal of the excess gel. The pore struts thickness was approximately 30-50 μm. Most of pores of ZnO doped AW-GC scaffold were open and 300-500 μm diameter.

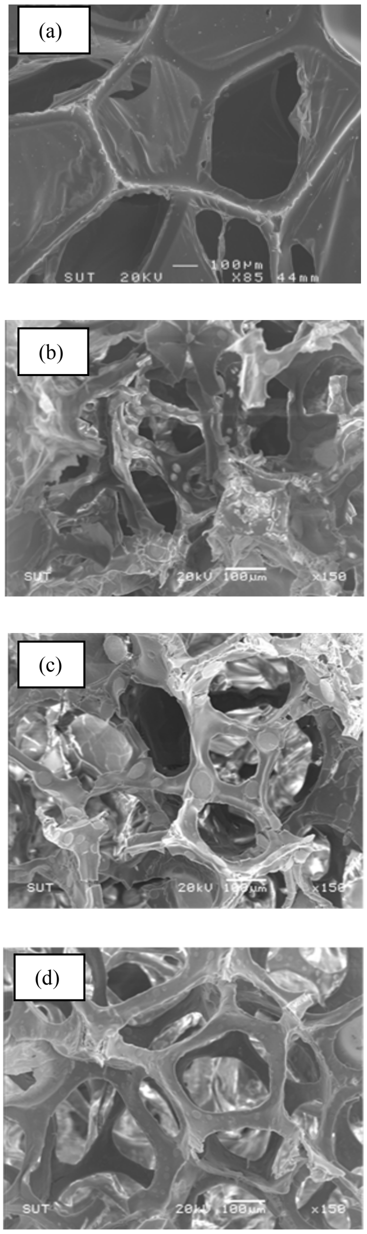
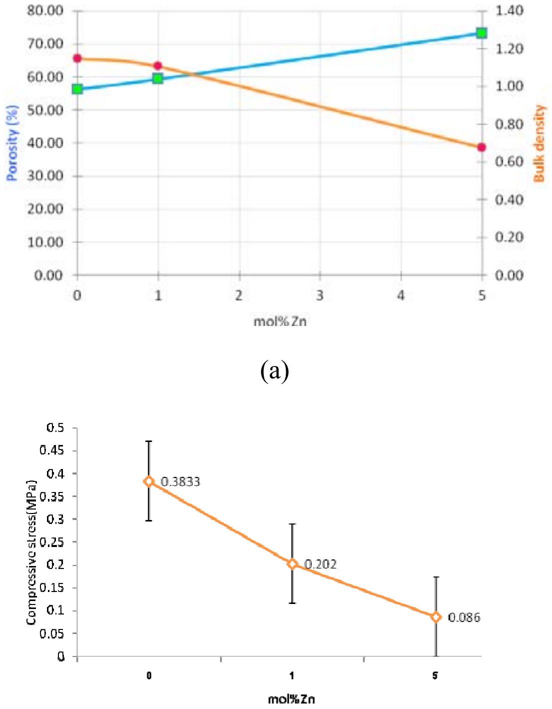


Figure 1. SEM micrographs of (a) sponge and of AW-GC scaffolds (b) undoped with ZnO, (c) doped with 1 mol% ZnO and (d) doped with 5 mol% ZnO

Figure 2(a) shows the relationship of porosity and apparent density of AW-GC scaffolds with different ZnO concentration. When increasing the ZnO doping concentration, the porosity of sample increased as corresponding with decreasing of the apparent density of samples. This was possible that gel with high ZnO doping concentration had short aging time so that the gels became to higher viscosity and obtained many pores after gel casting or the sponge immersing. The compressive strength of samples decreased with increasing of ZnO doping concentration, as can be seen in Figure 2(b).



(a)

(b)

Figure 2. ZnO concentrations as a function of (a) porosity and apparent density of AW-GC and (b) compressive strength of the AW-GC

Figure 3 shows the FTIR spectra of the surface of the AW–GC and AW-GC with 1, 5 mol% Zn soaked for 7 and 14 days in SBF. Presence of PO_4^{3-} asymmetric stretching mode of vibration was identified by a strong, complex band in the 1000–1150 cm^{-1} range and a medium intensity band at about 960 cm^{-1} due to symmetric stretching vibration. The bending vibration of PO_4^{3-} was characterized by bands located at 560–610 cm^{-1}. A broad OH band was also present in the 3400–3500 cm^{-1} region which together with the PO_4^{3-} peaks confirmed the presence of apatite in the spectra. The peaks at 1450–1700 cm^{-1} show the existence of a Ca–O phase in the structure while the Mg–O stretching vibration was observed in the range of 750–800 cm^{-1}. As similar result, CaO was replaced by ZnO in the different glass composition.(14) Si–O stretching vibration was observed at 1000–1100 cm^{-1}. After soaking in SBF, peak PO_4^{3-} and CO_3^{2-} became to sharper for samples after soaking for 7 and 14 days. The band in between 1400–1500 and 875 cm^{-1} were assigned to the CO_3^{2-} peaks for AW-GC and the band in between 1400–1500 cm^{-1} was assigned to the CO_3^{2-} peaks for AW-GC with 1mol% ZnO. With comparison to the spectra pattern of the undoped AW-GC scaffold, it was quite evident that, during the soaking in SBF, the 930 cm^{-1} shoulder tended to disappear due to leaching of cations from the materials. At the same time, the sharp peak at 1000-1100 cm^{-1} became to broaden as indicated the growing contribution of a vibrational component of phosphate species.(14) It is evidenced that, with increasing the ZnO doping concentrations, Zn ion substituted in the glass ceramic structure and new apatite layer formed on the surface.

Kamitakahara M. et al reported that silicon released from the glass-ceramics significantly decreased with increasing ZnO content. Therefore, the suppression of the formation of silanol groups leads to the suppression of apatite-formation on the glass-ceramics. However, for small content of ZnO, the AW-GC scaffold still showed apatite-forming ability. This suggests that the biodegradability of AW-GC scaffold was able to be controlled without losing its bioactivity.

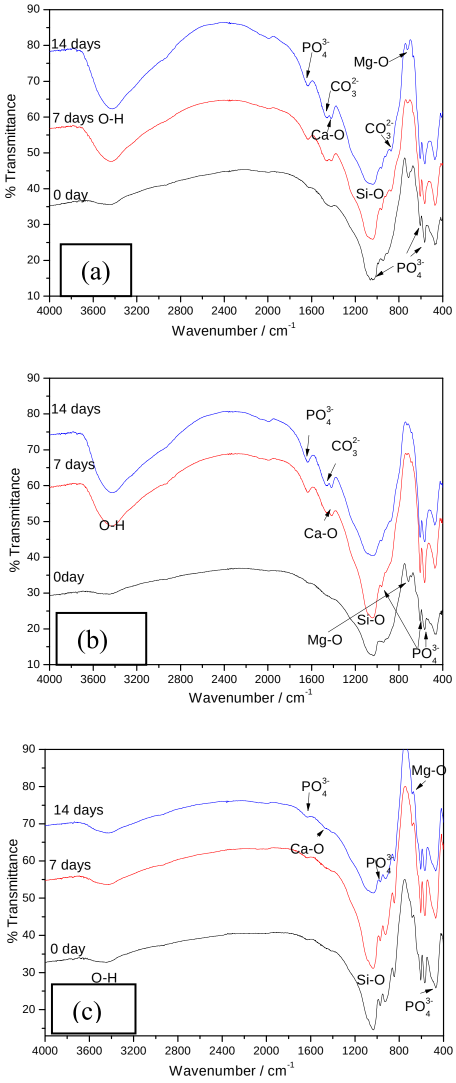


Figure 3. FT-IR spectra of (a) AW-GC, (b) AW-GC with 1 mol% ZnO and (c) AW-GC with 5 mol% ZnO after soaking in SBF

Figure4 shows the SEM micrographs of the surface of AW-GC with different ZnO concentrations after soaking in SBF solution at 37°C for 7 and 14 days, respectively. It was formed that the roughness of surface of AW-GC was developed being smoother with increasing ZnO concentration. The spherical globules, which were new apatite layers as corresponded with the FT-IR result, were formed on the surface of the AW-GC and AW-GC with 1 mol% ZnO within 7 days of soaking in SBF as seen in Figures 4(b), 4(c), 4(e) and 4(f). Thickness of these globules increased with longer immersion time and decreased with more Zn. The high zinc released from AW-GC with 5 mol% ZnO causes a drastic reduction apatite on AW-GC.

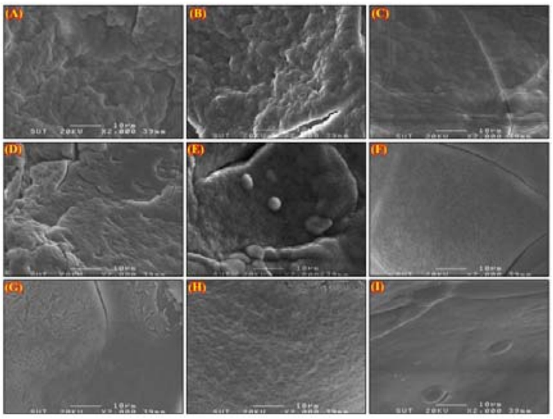


Figure 4. SEM micrographs of (a) AW-GC, (b) AW-GC soaked in SBF for 7 days, (c) AW-GC soaked in SBF for 14 days, (d) AW-GC with 1 mol.% Zn, (e) AW-GC with 1 mol.% Zn for 7 days, (f) AW-GC with 1 mol.% Zn for 14 days (g) AW-GC with 5 mol.% Zn, (h) AW-GC with 5 mol.% Zn for 7 days and (i) AW-GC with 5 mol.% Zn for 14 days.

Conclusion

Bioactive glass ceramic scaffold in the system of SiO_2-P_2O_5-CaO-MgO-CaF_2 as a function of ZnO doping concentration was prepared by the duplication of polyurethane sponge of sol-gel glass and heat treatment at 900°C. Bioactive studies performed in SBF showed that the apatite formation is mainly dependent on amount of ZnO incorporation which appears a good durability in SBF solution and only a small quantity of apatite-like particles can be formed on the AW-GC scaffold surface after soaking in SBF solution for 14 days. With increasing the ZnO concentrations, the porosity increase due to higher viscosity of gel during immersion the sponge, resulting in the compressive strength of scaffolds to decrease.

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