Dissolution and Bio-compatibility of ZnO Doped Bioactive Glasses after In Vitro Experiment

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ABSTRACT

This work presents the study of 60SiO₂-(36-x)CaO-4P₂O₅-xZnO (x=1, 3, 5 mol.%) glasses synthesized by a modified sol-gel method based on a hydrothermal reaction. The thermal morphology of the glass systems was evaluated by the Thermogravimetric analysis-differential scanning calorimetry (TG-DSC) method. The in vitro assay for dissolution was evaluated by the Inductively coupled plasma-optical emission spectroscopy (ICP-OES) method. Biocompatibility testing was performed with L-929 fibroblast cells. The results show that the ZnO-doped glass systems show good bioactivity and biocompatibility.

Keywords: Bioactive glasses, Hydroxyapatite, Thermal analysis, Bioactivity, Biocompatibility.

1. Introduction

Bioactive glass or bioactive glass material is a new generation biomedical material used as an artificial bone material. The first time bioactive glass was found by Professor Larry Hench in 1969 [1]. The idea of bioactive glass material came, when he saw soldiers returning from the war, having their arms and legs amputated because their bodies refused to have a metal bone graft. Larry Hench has researched, tested and put into application a material used as artificial bone, called bioactive glass with symbol 45S5 with composition 45SiO₂-24.5CaO-24.5Na₂O-6P₂O₅ (wt.%) with the trade name Bioglass or Novabone. The 45S5 glass was synthesized by melting the precursors at high temperature (above 1300°C) (melting method). At high temperature, the oxides produced such as SiO₂, CaO, Na₂O and P₂O₅ do not exist separately but combine together to create a network of amorphous structure of glass.

Besides the melting method, the sol-gel method has been widely applied because it has many advantages over the melting method. The sol-gel method can synthesize bioactive glass systems at low temperatures, preventing the loss of end products because of high temperature P₂O₅ volatilization. Moreover, the sol-gel method can synthesize glass systems with porous structure and high specific surface area, which enhances the biological activity of the composites [2]-[3].

When applying biomedical glass materials in implants, the control of the bioactivity of the material system is important to determine the time that the artificial graft can connect with the natural bone. Therefore, biologically beneficial elements such as Mg, Sr, Al, Cu, Fe, Ag, and Zn are often added to the composition of synthetic glass systems by the sol-gel method. Among the elements mentioned above, Zn plays an important role in bone formation because of its ability to inhibit the activity of osteoclasts and increase the differentiation ability of osteoblasts [4]-[8]. Depending on the content of doped Zn as well as the composition of the synthetic system, the biomedical glass systems exhibit different properties. The content of doped Zn can affect the structure, bioactivity and compatibility of the resulting material systems. Through the research of reference materials, many ZnO-doped
biomedical glass systems have been studied and synthesized by sol-gel method in which the synthesis process uses strong acids or harmful strong bases as catalysts for hydrolysis. Therefore, the non-acid synthesis 'green synthesis' is of great significance in the synthesis of safe glass systems that can not only be used as artificial bone materials, but also can be used as artificial bone materials as additives in toothpaste or in cosmetics.

In the framework of this study, we synthesized a three-component glass system SiO$_2$-CaO-P$_2$O$_5$ doped with Zn in the form of ZnO with ratios of 1, 3 and 5% (mol) by green synthesis method on the basis of a hydrothermal reaction. The synthesis process, the influence of ZnO doping on the thermal properties, in vitro dissolution and in vitro biocompatibility of the glasses were studied and evaluated.

2. Experiment and Method

2.1. Synthesis

Bioactive glasses 60SiO$_2$-(36-x)CaO-4P$_2$O$_5$-xZnO (x=1, 3, 5 mol.%) were prepared by the modified sol-gel method without using acid or base catalysis. The composition of the bioactive glasses was selected according to the bioactive glasses that were previously synthesized by the sol-gel method [9]-[11]. The chemicals used for the synthesis of bioactive glass are tetraethyl orthosilicate (TEOS- Sigma Aldrich), triethyl phosphate (TEP- Sigma Aldrich), calcium nitrate tetrahydrate [Ca(NO$_3$)$_2$.4H$_2$O- Merck ], and zinc nitrate hexahydrate [Zn(NO$_3$)$_2$.6H$_2$O-Merck]. Firstly, the starting precursors were successively added at 30 min intervals to a reaction vessel containing distilled water under continuously stirring conditions.

The molar ratio of H$_2$O/TEOS was investigated and selected as 60. Then, the reaction mixture was transferred to a hydrothermal system and calcined at 160°C for 24 h. The resulting gel was dried at 100°C for 12 h and calcined at 700°C for 3 h to obtain a synthetic glass.

2.2. Characterized Methods

The dried gel samples of the glass systems were thermally analyzed by the TG-DSC method. The analysis allows to choose the right temperature and find the most stable temperature to create the biomedical glass system. ‘In vitro’ experiments were conducted by immersing the glass powder in a solution simulating human humoral Simulated body fluid (SBF) to investigate the possibility of new bone mineral formation. The solubility of glass was assessed by the ICP-OES method. The vitreous system was also tested in cell culture to evaluate the biocompatibility of the composites. The fibroblast line (L-929 fibroblast) was cultured in standard medium at 37°C, with 5% CO$_2$ and 95% humidity. Cell line survival was determined by 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric method.

3. Results and Discussion

3.1. Thermal Behaviour

TG-DSC thermal analysis results of 60SiO$_2$-(36-x)CaO-4P$_2$O$_5$-xZnO dry gel samples (x=1, 3, 5 mol.%) are shown in Figs.1-3. Thermal analysis plot showed three mass loss intervals on the TG curves. The first mass loss ranges from 30 to 200°C with corresponding endothermic peaks at 142.73; 140.52 and 123.75°C, typical for water removal [12]-[13]. The second mass loss ranges from 200 to about 400°C with exothermic peaks observed at
295.23; 264.98; 259.15; 324.76°C is considered to be the dehydration of ethanol because some ethanol may still be present inside the pores of the dry gel [13].

![Fig.1. TG-DSC analysis of glass sample 60SiO$_2$-35CaO-4P$_2$O$_5$-1ZnO (x = 1 mol.%)](image1)

The third mass loss ranges from 400 to 600°C with endothermic peaks observed at 512.33; 530.98; 538.24°C is believed to be the decomposition of NO$_3$- groups in the dried gel sample [14]. Besides endothermic effects, in the 900°C region, exothermic peaks were observed with no loss of mass. This observation is consistent with the phase transition for CaSiO$_3$ mineral formation by the incorporation of oxides in the network of glass structures such as SiO$_2$, CaO [14]. From the TG-DSC thermal analysis diagram, the suitable temperature for calcining the glass samples was selected around 700°C.

![Fig.2. TG-DSC analysis of glass sample 60SiO$_2$-33CaO-4P$_2$O$_5$-3ZnO (x = 3 mol.%)](image2)
3.2. In vitro dissolution

The ionic changes in the SBF solution related to the physico-chemical reactions between the bioactive glass containing Zn and the physiological environment were analyzed by ICP-OES method as depicted in Fig.4. The original SBF liquid contained 100 ppm of Ca, 0 ppm of Si, 31 ppm of P, and 0 ppm of Zn. After the in vitro experiment, the ion exchange phenomena were quite similar for all bioactive glasses. The observation mentioned that the Ca concentration increases, and then decreases. The concentration of Si and Zn gradually increased while the concentration of P gradually decreased. The increased Ca concentration is due to the rapid exchange between the Ca$^{2+}$ ions of the glass networks and the H$^{+}$ ions in the physiological fluid [15].

Thereafter, the Ca concentration decreased sharply to reach saturation after 5 days of soaking. The decrease in Ca concentration is related to its consumption to form HA mineral on the surface of bioactive glasses [16]. As well as a decrease in Ca concentration, P concentration was also noted to decrease as it is consumed to form the apatite mineral layer [16]-[17].

As can be seen, the consumption of Ca and P to form HA layer of bioglass (x = 1 mol.%) is the most, followed by bioglass (x = 3 mol.%), and bioglass (x = 5 mol.%). The Si concentration increased rapidly on the first day of immersion, then increased moderately for successful saturation at day 5.

The increase in Si concentration was explained by the dissolution of the glass lattice through discharge of Si(OH)$_4$ acids while the saturation corresponds to the self-assembly reactions of the above acids to form the silica SiO$_2$ layer [17]-[18]. The amount of Zn released increased in the order of bioglass (x = 1 mol.%), bioglass (x = 3 mol.%), and bioglass (x = 5 mol.%), consistent with the added ZnO content of the synthetic glass. According to the literature [19]-[20], a component of the released Zn ions will form a Zn(OH)$_2$ precipitate, which prevents the deposition of HA on the glass surface. This is consistent as the precipitation amount of HA decreases as the concentration of ZnO added in the synthetic glass increases.
3.3. In vitro biocompatibility

The 60SiO$_2$ - (36-x)CaO - 4P$_2$O$_5$ - xZnO (x = 1, 3, 5 mol.%) glass system samples were tested for biocompatibility in cell culture medium. The viability of fibroblast cells (L-929 fibroblast) directly exposed to extracts from bioactive glass powder at different concentrations for 24 h is shown in Fig.5. Cell viability without exposure to bioactive glass was chosen as a control (100%) [21]. According to ISO 10993-5 (Section 5: Cytotoxicity tests, in vitro methods 2009), cell viability was calculated as a percentage compared to control. In cases where the average cell viability is less than 70%, the material is cytotoxic. The results obtained for the sample x=1 mol.% showed that the cell viability was 124, respectively; 119; 110; and 99% for the 25%, 50%, 75% and 100% extracts. The 20% extract showed the highest cell viability values, while the 60% and 100% extracts showed little difference. Experimental results show that the bioactive glass 60SiO$_2$-35CaO-4P$_2$O$_5$-1ZnO (x = 1 mol.%) synthesized by hydrothermal method without acid-catalysed exhibits good biocompatibility in the environment. Cell viability values for bioactive glass in this study are comparable to those of previously studied glass systems, such as Bioglass® 45S5.
synthesized by melting, 77S glass and 58S glass were synthesized by sol-gel method [22]. Cytotoxicity test results for samples of 60SiO2-(36-x) CaO-4P2O5-xZnO (x = 3, 5 mol.%) gave similar results as sample x=1 mol.%, confirming the biocompatibility of the Zn-doped glass systems in this study.

Fig.5. Biocompatibility of glass materials 60SiO2-(36-x)CaO-4P2O5-xZnO (x = 1, 3, 5 mol.%) in cell culture mediums

4. Conclusion

Bioactive glasses 60SiO2-(36-x)CaO-4P2O5-xZnO (x = 1, 3, 5 mol.%) were prepared by a hydrothermal reaction-based green synthesis method. Thermal analysis showed the effect of ZnO addition on the thermal morphology of the material sample. The in vitro experiment highlights the effect of ZnO addition on the bioactivity of the synthetic glass. The biological activity of glass was arranged in the following order: sample x = 1 > sample x = 3 > sample x = 5. Glass material system 60SiO2-(36-x)CaO-4P2O5-xZnO (x = 1, 3, 5 mol.%) were tested for biocompatibility in cell cultures. The results show that the bioactive glass material in this study has good biocompatibility with the fibroblast cell line L-929 fibroblast. With promising initial results, the glass materials in this study could have applications as artificial bone substances, or as an additive in toothpaste.
Declarations

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Competing Interests Statement

The authors declare no competing financial, professional and personal interests.

Consent for publication

Authors declare that they consented for the publication of this research work.

Availability of data and material

Authors are willing to share the data and material according to relevant needs.

Authors’ Contributions

All authors equally contributed in data analysis and paper drafting.

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