Research Article

Preparation of CaO-SiO₂ Glass-Ceramic Spheres by Electrospraying Combined with Sol-Gel Method

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CaO-SiO₂ glass-ceramic spheres were prepared by an electrospray method using hydrolyzed silicon alkoxide containing calcium nitrate. Crystalline calcium silicates, such as Ca₂SiO₄ and β-CaSiO₃, formed around the surface of the spheres after heat treatment. The dissolution of the crystal phase of the spheres caused the release of Ca²⁺ and Si⁴⁺ ions during the initial stage of soaking in Tris-buffer solution, leading to the formation of nanosized pores at the sphere surface. The incorporation of Ca²⁺ ions into the glassy phase of the spheres suppressed the rapid pH increase during the initial stage of soaking in Tris-buffer solution.

1. Introduction

Recently, interest has arisen in the biomaterials field in a new approach that considers the biological interaction between synthetic materials and cells [1–3]. Silicate and calcium ions released from Bioglass were reported to stimulate bone formation on the material via gene activation [4]. One important strategy for rapid regeneration of bone is to deliver and release these ions into bone defects. Calcium silicates are thought to be potential carriers for these ions. In general, crystalline calcium silicates, such as wollastonite (CaSiO₃), show high degradability [5]. However, they also show a tendency to increase in alkalinity due to their dissolution, resulting in the induction of an inflammatory reaction at an early stage after implantation. Therefore, the dissolution behavior of crystalline calcium silicates must be controlled. To improve their chemical instability, the substitution of other elements, such as strontium and zinc, into their structure has been proposed in combination with calcium phosphates such as hydroxyapatite and tricalcium phosphate, which exhibit slower degradability [6–8].

Bioactive glasses based on CaO-SiO₂ systems have a great potential as materials for bone regeneration because they bond to bone and are osteoinductive [9]. Sol-gel-derived

bioactive glasses have been reported to improve bioactivity, as compared to melt-derived glasses with the same composition [10, 11]. For chemical instability of calcium silicates, our material design is to prepare CaO-SiO₂ glass-ceramics, which contain crystalline calcium silicate ceramics as an ion-releasing component in a glass phase with a silica-based network as a matrix. In the case of CaO-SiO₂ glass derived from sol-gel method, calcination at 700°C has been reported to lead to the stabilization of the silica network [12]. The heat treatment at a high temperature plays an important role in the improvement of its chemical stability for the matrix.

We believe that CaO-SiO₂ glass-ceramic particles are applicable as fillers in injectable bone substitutes for releasing silicate and calcium ions and inducing bioactivity. The fillers should show a narrow distribution of diameter size and should simultaneously be highly dispersed within the materials. That is, monodisperse sphere shapes are needed for use as fillers. It is well known that the electrospray method can be used to synthesize polymer spheres with monodispersity [13, 14]. Polymer solution dissolved in an organic solvent is pressed using a syringe under a high voltage, resulting in the formation of spheres with diameters in the range of nanometers to micrometers owing to the intertwining of polymer chains. We have previously succeeded in preparing

FIGURE 1: XRD patterns (a) and SEM micrographs (b) for GC750 and GC900 spheres.

porous spheres containing a large amount of CaSiO₃ with monodispersity using electrospraying combined with the sol-gel method [15].

In the present work, sol-gel-derived glass spheres (80 mol% SiO₂ and 20 mol% CaO as a starting chemical composition) were prepared by electrospray method using a hydrolyzed alkoxide. In general, the sintering temperature of the ceramic precursor with a CaO-SiO₂ system derived from the sol-gel method leads to the formation of a separate crystalline phase [16]. CaO-SiO₂ gel spheres were heat treated at different temperatures to investigate release of inorganic ions with biological effects and suppression for the rapid pH increase during the initial stage of soaking in Tris-buffer solution.

2. Materials and Methods

Tetraethylorthosilicate (TEOS), ethanol, and distilled water (DW) were used as starting materials. Nitric acid acted as an acid catalyst. The molar ratio of TEOS/ethanol/DW/nitric acid was 1:2:4:0.05. Calcium nitrate tetrahydrate was used as a calcium source. The molar ratio of Ca/Si was 1:4. After aging for 12 h at 35°C, the precursor solution of hydrolyzed TEOS containing calcium ions was electrosprayed to prepare gel spheres. The electrospraying system was constructed using a substrate holder, a stainless steel capillary tube (22 gauge), a precursor solution tank, and a high voltage source. The applied voltage was 20 kV. The distance between the substrate and the tip of the capillary tube was 150 mm. The resulting spheres were dried in air at 80°C for 24 h and subsequently stored in a desiccator. Calcination at 550–600°C is necessary to remove nitrates [17]. The spheres were heated

at 750 or 900°C for 1 h for crystallization to control the crystal phase. We determined the heat temperature by an optimization process based on a trial-and-error approach to achieve satisfactory results in terms of precipitating different crystal phases in the spheres. The samples prepared at 750 and 900°C of heat treatment are denoted as GC750 and GC900, respectively. The samples were analyzed using X-ray diffractometry (XRD; PANalytical, X'pert-MPD) using CuK α radiation, operating at 45 kV, 40 mA. The samples were coated with amorphous osmium by plasma chemical vapor deposition and then observed by scanning electron microscopy (SEM; JEOL, JSM-6301F) with 5 kV of acceleration voltage. The sphere diameter was measured for at least 200 points by image-editing software: ImageJ, obtained from National Institutes of Health. The concentrations of ions released from the spheres while soaking in Tris-buffer solution containing 50 mmol/L (CH₂OH)₃CNH₂ and 45 mmol/L HCl at pH 7.5 at 36.5°C in a polypropylene beaker were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Shimadzu, ICPS-7000). The dissolution test was performed using a spheres/Tris-buffer solution ratio of 1:1. At least three tests were evaluated for ion concentrations and pH in Tris-buffer solution after soaking of the samples. A statistical analysis was performed by *t*-test.

3. Results and Discussion

3.1. Crystal Phases and Morphologies of the CaO-SiO₂ Glass-Ceramic Spheres. Figure 1 shows the XRD patterns and SEM micrographs of GC750 and 900 spheres. The XRD pattern for GC750 spheres showed sharp peaks at around

FIGURE 2: The concentrations of Ca^{2+} (circle) and Si^{4+} (triangle) ions released from GC750 (closed marks) and GC900 (open marks) spheres after soaking.

32, 34, and 37° corresponding to Ca_2SiO_4 (number 83-0461) and a halo peak at around 22° corresponding to an amorphous phase. In the XRD pattern for GC900 spheres, new peaks at around 27, 28, and 29° corresponding to $\beta\text{-CaSiO}_3$ (number 84-0655) were seen with peaks corresponding to Ca_2SiO_4 and an amorphous phase. It has been already reported that $\beta\text{-CaSiO}_3$ is synthesized by heat treatment of gel-derived materials at 1000°C using TEOS and hydrated calcium nitrate as starting materials in a solvent of diluted nitric acid or sodium hydroxide solution [18], which agrees with the results of the present work. It was clearly seen in both micrographs that bright and dark portions originate from crystalline and amorphous phases in these samples, respectively. The diameters of the GC750 and GC900 spheres were determined to be $0.6\text{--}1.2\ \mu\text{m}$ (average $0.9 \pm 0.2\ \mu\text{m}$) and $0.7\text{--}1.2\ \mu\text{m}$ (average $0.9 \pm 0.1\ \mu\text{m}$), respectively, in SEM micrographs, independent of the heating temperature.

3.2. Dissolution Tests of the CaO-SiO_2 Glass-Ceramic Spheres in Tris-Buffer Solution. Figure 2 shows the concentrations of Ca^{2+} and Si^{4+} ions released from the spheres after soaking in Tris-buffer solution for various time periods. After soaking of GC750, the concentration of Ca^{2+} ions increased dramatically during the first 12 h and then stabilized. In the case of GC900 spheres, the concentration of Ca^{2+} ions increased dramatically during the first 12 h and then kept increasing gradually. The concentration of Ca^{2+} ions released from GC750 spheres was higher than that from GC900 spheres.

By contrast, the amount of Si^{4+} ions released into the Tris-buffer solution increased rapidly during the first 12 h and then tended to increase gradually. The concentration of Si^{4+} ions released from GC750 spheres into Tris-buffer solution was lower than that from GC900 spheres.

Figure 3 shows the XRD patterns and SEM micrographs for GC750 and GC900 spheres after 72 h of soaking in Tris-buffer solution. In both XRD pattern for GC750 and GC900 spheres, no peaks corresponding to a crystalline phase were observed. Ions released from the samples should be composed predominantly of crystalline calcium silicates. The difference in crystalline phase between the samples depends on the ratio of $\text{Ca}^{2+}/\text{Si}^{4+}$ ions release. Pores that are several tens of nanometers in diameter can be seen at the surfaces of both samples as shown in Figure 3(b). The formation of pores is attributed to dissolution of the crystalline phase of the samples during soaking. This finding implies that surface crystallization at nanometer sizes occurred under these experimental conditions. The diameters of the GC750 and GC900 spheres after the soaking were determined to be $0.6\text{--}1.2\ \mu\text{m}$ (average $0.9 \pm 0.2\ \mu\text{m}$) and $0.7\text{--}1.2\ \mu\text{m}$ (average $1.0 \pm 0.1\ \mu\text{m}$), respectively, in SEM micrographs. There was no significant change in the diameter even after the soaking.

The ion-releasing ratios, which were calculated from the amounts of both ions in the Tris-buffer solution divided by the total amounts of both ions in the samples were estimated to be approximately 60% and 10 ~ 15% for Ca^{2+} and Si^{4+} , respectively. After calcination, some Ca^{2+} ions in the gel derived from the sol-gel process were reported to be incorporated into the disordered glassy structure comprising a silica-based network [19]. The remaining Ca^{2+} ions are proposed to be released gradually with the dissolution of the silica network over a long period.

Figure 4 shows the change in pH of the Tris-buffer solution after soaking of GC750 or GC900 spheres. The pH behavior showed a similar trend for both samples. The pH increased slightly to 7.6 during the first 24 h of soaking and subsequently showed almost constant value without statistical difference. Ca^{2+} ions released from the samples led to an increase in pH of the Tris-buffer solution. The incorporation of Ca^{2+} ions into the amorphous phase of the CaO-SiO_2 -based glass-ceramics plays an important role in suppressing the pH increase during the initial stage. We propose that CaO-SiO_2 glass-ceramic spheres are preferred for use as filler materials in injectable bone substitutes for releasing silicate and calcium ions over time.

4. Conclusion

Silica-based glass-ceramic spheres containing crystalline calcium silicates were prepared by electrospraying combined with the sol-gel method. The calcium silicates formed selectivity at the surface. Ca^{2+} and Si^{4+} ions were released predominantly from the crystal phase of the spheres. The existence of a glassy phase and incorporation of Ca^{2+} ions into the glassy phase suppressed the rapid pH increase during soaking of the spheres in solution. Investigation on the biological effects of the spheres is now in progress.

FIGURE 3: XRD patterns (a) and SEM micrographs (b) for GC750 and GC900 spheres after 72 h of soaking.

FIGURE 4: pH change in Tris-buffer solution after soaking of GC750 and GC900 spheres.

Conflict of Interests

The authors certify that there is no conflict of interests with any financial organization regarding the material disused in the paper.

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References

- [1] A. Hoppe, N. S. Güldal, and A. R. Boccaccini, "A review of the biological response to ionic dissolution products from bioactive glasses and glass-ceramics," *Biomaterials*, vol. 32, no. 11, pp. 2757–2774, 2011.
- [2] J. R. Jones, O. Tsigkou, E. E. Coates, M. M. Stevens, J. M. Polak, and L. L. Hench, "Extracellular matrix formation and mineralization on a phosphate-free porous bioactive glass scaffold using primary human osteoblast (HOB) cells," *Biomaterials*, vol. 28, no. 9, pp. 1653–1663, 2007.
- [3] M.-Y. Shie, S.-J. Ding, and H.-C. Chang, "The role of silicon in osteoblast-like cell proliferation and apoptosis," *Acta Biomaterialia*, vol. 7, no. 6, pp. 2604–2614, 2011.
- [4] I. D. Xynos, A. J. Edgar, L. D. K. Buttery, L. L. Hench, and J. M. Polak, "Ionic products of bioactive glass dissolution increase proliferation of human osteoblasts and induce insulin-like growth factor II mRNA expression and protein synthesis," *Biochemical and Biophysical Research Communications*, vol. 276, no. 2, pp. 461–465, 2000.
- [5] S. Ni, J. Chang, and L. Chou, "A novel bioactive porous CaSiO₃ scaffold for bone tissue engineering," *Journal of Biomedical Materials Research A*, vol. 76, no. 1, pp. 196–205, 2006.
- [6] H. Zreiqat, Y. Ramaswamy, C. Wu et al., "The incorporation of strontium and zinc into a calcium-silicon ceramic for bone

- tissue engineering,” *Biomaterials*, vol. 31, no. 12, pp. 3175–3184, 2010.
- [7] F. Zhang, J. Chang, K. Lin, and J. Lu, “Preparation, mechanical properties and in vitro degradability of wollastonite/tricalcium phosphate macroporous scaffolds from nanocomposite powders,” *Journal of Materials Science*, vol. 19, no. 1, pp. 167–173, 2008.
- [8] H. Du, Z. Wei, H. Wang, E. Zhang, L. Zuo, and L. Du, “Surface microstructure and cell compatibility of calcium silicate and calcium phosphate composite coatings on Mg-Zn-Mn-Ca alloys for biomedical application,” *Colloids and Surfaces B*, vol. 83, no. 1, pp. 96–102, 2011.
- [9] L. L. Hench, “Bioceramics,” *Journal of the American Ceramic Society*, vol. 81, no. 7, pp. 1705–1727, 1998.
- [10] P. Sepulveda, J. R. Jones, and L. L. Hench, “Characterization of melt-derived 45S5 and sol-gel-derived 58S bioactive glasses,” *Journal of Biomedical Materials Research*, vol. 58, no. 6, pp. 734–740, 2001.
- [11] H. Maeda, T. Okuyama, E. H. Ishida, and T. Kasuga, “Preparation of porous spheres containing wollastonite by an electrospray method,” *Materials Letters*, vol. 95, pp. 107–109, 2013.
- [12] S. Lin, C. Ionescu, K. J. Pike, M. E. Smith, and J. R. Jones, “Nanostructure evolution and calcium distribution in sol-gel derived bioactive glass,” *Journal of Materials Chemistry*, vol. 19, no. 9, pp. 1276–1282, 2009.
- [13] D. Fantini, M. Zanetti, and L. Costa, “Polystyrene microspheres and nanospheres produced by electrospray,” *Macromolecular Rapid Communications*, vol. 27, no. 23, pp. 2038–2042, 2006.
- [14] A. Gomez, D. Bingham, L. De Juan, and K. Tang, “Production of protein nanoparticles by electrospray drying,” *Journal of Aerosol Science*, vol. 29, no. 5-6, pp. 561–574, 1998.
- [15] S. Labbaf, O. Tsigkou, K. H. Müller, M. M. Stevens, A. E. Porter, and J. R. Jones, “Spherical bioactive glass particles and their interaction with human mesenchymal stem cells in vitro,” *Biomaterials*, vol. 32, no. 4, pp. 1010–1018, 2011.
- [16] M. A. de La Casa-Lillo, P. Velásquez, and P. N. De Aza, “Influence of thermal treatment on the “in vitro” bioactivity of wollastonite materials,” *Journal of Materials Science*, vol. 22, no. 4, pp. 907–915, 2011.
- [17] J. R. Jones, L. M. Ehrenfried, and L. L. Hench, “Optimising bioactive glass scaffolds for bone tissue engineering,” *Biomaterials*, vol. 27, no. 7, pp. 964–973, 2006.
- [18] P. Siriphannon, Y. Kameshima, A. Yasumori, K. Okada, and S. Hayashi, “Formation of hydroxyapatite on CaSiO_3 powders in simulated body fluid,” *Journal of the European Ceramic Society*, vol. 22, no. 4, pp. 511–520, 2002.
- [19] G. M. Luz and J. F. Mano, “Nanoengineering of bioactive glasses: hollow and dense nanospheres,” *Journal of Nanoparticle Research*, vol. 15, no. 1457, 11 pages, 2013.