Preparation of siloxane-containing vaterite/poly (L-lactic acid) hybrid microbeads with silicate and calcium ions-releasing ability

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Siloxane-containing vaterite (SiV)/poly (L-lactic acid) (PLLA) hybrids have the releasing-ability of silicate and calcium ions for gene-activation. In the present paper a new types of beads were prepared using the hybrids via an electrospraying method. The diameters of the beads were controlled in the range of ~65 to ~180 μm by increasing the feeding rate from 0.1 to 0.9 ml/min. Depressed areas could be observed on the surface of the beads. The SiV particles with concentration of 10 and 20 wt % in the beads were completely enclosed by the PLLA matrix. These beads showed the controlled release of silicate and calcium ions in Tris buffer solution.

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1. Introduction

Third generation biomaterials are those which are biocompatible, bioresorbable and bioactive (osteoconductive and osteoinductive).¹⁾ Bioactive materials possess the ability to form bond to bone and some even enhance bone formation by stimulating genes in cells to proliferate and differentiate into bone cells. These properties of those materials are due to the controlled releases of silicate and calcium ions that have been reported to enhance the osteogenesis through the gene activation of the osteoblast cells.²⁾⁻⁴⁾

Sautier et al.⁵⁾ reported that the enhanced proliferation of rat chondrocytes and the improved expressions of the chondrogenic molecular markers such as Sox9 and Runx2 were observed when the cells were co-cultured with granules of bioactive glasses such as Bioglass®. The release of silicate and calcium ions from the bioactive glasses may have enhanced the cartilage formation.

Cartilage is known to have low natural healing capacity. Due to the deficiency of blood supply and the low migration of cells, once they are damaged from an aging-related wear or diseases such as osteoarthritis and rheumatoid arthritis, regeneration rarely occurs, resulting in the loss of their functions.⁶⁾ The transplantation of the autologous cartilage is the common treatment for damaged cartilage;⁷⁾ however this method is invasive and often leads to donor site morbidity. Additionally, supply of autologous cartilage is low and hence need for new materials that possess the ability to regenerate cartilage is present.

The injection of polymer beads suspended in biocompatible solutions has been used for maxillofacial surgeries such as augmentation of nose and chin.⁸⁾⁻¹⁰⁾ This method is also ideal for achieving minimal invasive cartilage reconstruction. An electrospraying method is an effective method for preparing the beads with a narrow distribution of diameters.¹¹⁾⁻¹³⁾ The diameter of the beads is easily controlled by adjusting the physical properties of slurry and also by varying the slurry-feeding rate. It is important

to control the diameter of the beads as it effects the biological reaction of surrounding tissues such as phagocytosis or encapsulation by fibrous tissues.^{14),15)}

In our earlier work, siloxane-containing polymer-inorganic (calcium carbonate-vaterite) hybrids with the releasing ability of silicate ion was prepared.^{16),17)} The materials were shown to enhance proliferation and differentiation of the murine osteoblast-like cells.¹⁸⁾ The siloxane containing inorganic particles (SiV) derived from aminopropyltriethoxysilane (APTES) and vaterite was hybridized with poly (L-lactic acid) (PLLA) through the formation of amide bond between siloxane and PLLA through the amino groups and coordination of calcium ion in the vaterite to the carboxylic group in PLLA (these hybrids were denoted as SiPVHs).¹⁹⁾

The present paper describes the preparation of bead-shaped SiPVH with ion-releasing ability for the application of minimal invasive cartilage reconstruction. The SiPVH beads with 10, 20 and 60 wt % of SiV particles were prepared via the electrospraying method. To evaluate their degradation behavior, the beads were soaked in a Tris buffer solution. The slurry-feeding rate and the SiV content in the slurry were varied and their effect on bead diameter and morphology was investigated.

2. Experimental procedure

2.1 Preparation of SiV particles

SiV particles were prepared using a carbonation method described in our previous report.¹⁹⁾ Briefly, 150 g of $\text{Ca}(\text{OH})_2$ and 60 ml of APTES were added into 2000 ml of methanol, stirred with blowing CO_2 gas for 75 min and the resulting slurry was then dried at 110°C. The dried powders were spherical secondary particles of ~1 μm in diameter (specific surface area; ~70 m^2/g), consisting of vaterite with siloxane containing ~2 wt % of silicon.

2.2 Preparation of SiPVH beads

PLLA (LACEA®; molecular weight, Mw; ~170 kDa) and SiV particles were melt-blended at 200°C to obtain the SiPVH containing 10, 20 and 60 wt % of SiV, hereafter, these hybrids are denoted by SiPVH₁₀, SiPVH₂₀ and SiPVH₆₀, respectively.

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Table 1. Preparation conditions of SiPVH beads

Sample	SiV:PLLA (mass ratio)	PLLA ratio in the slurry [mass %]	Feeding rate [ml/min]	Applied voltage [kV]
SiPVH ₁₀	10:90	5	0.7	17
SiPVH ₂₀	20:80	5	0.1–0.9	19
SiPVH ₆₀	60:40	5	0.5	19

The beads were prepared via an electrospaying method.¹²⁾ For the investigation of SiV particle content on the bead morphology and ion-releasing ability, slurries for the electrospaying was prepared by dissolving 1.1 g of SiPVH₁₀, 1.3 g of SiPVH₂₀ and 2.5 g of SiPVH₆₀ containing 0.1, 0.3 and 1.5 g of SiV particles, respectively into 19.0 g of chloroform. The final PLLA to chloroform of 5% in mass ratio was achieved for the slurries. **Table 1** shows the slurries and processing conditions used to prepare the beads. In order to investigate the effect of the feeding rate on the SiPVH beads diameter, the electrospaying of the SiPVH₂₀ was performed by varying the feeding rate from 0.1 to 0.9 ml/min. The voltage applied to the capillary was adjusted to the minimum value at which a slurry-jet was stably formed. A glass dish filled with ethanol was used as the collector, which was stirred to avoid the aggregation of the beads.

The morphologies of the beads and their fracture faces were observed with a scanning electron microscope (SEM) (JEOL, JSM-6301F). The average diameter and standard deviation of the beads were measured from the SEM images using Image-J software (<http://rsbweb.nih.gov/ij/>) ($n = 50$). The beads were treated as they were spherical during the measurement.

2.3 Evaluation of silicate and calcium ion-releasing behaviors

50 mg of the SiPVH₁₀ beads prepared with the slurry-feeding rate of 0.7 ml/min and the SiPVH₂₀ and SiPVH₆₀ beads prepared with the rate of 0.5 ml/min were soaked in 10 ml of Tris buffer solution of pH 7.4 and maintained at 37°C for 168 h. After the soaking, the beads were filtrated and dried at 50°C for 1 d. The weight-average molecular weights (M_w) of PLLA in the beads after soaking were estimated with a gel permeation chromatograph (GPC) (Shimadzu, RID-10A), equipped with two columns (Shodex, KF-604). Si⁴⁺ and Ca²⁺ ion concentrations in the solutions were evaluated with an inductively coupled plasma atomic emission spectrometer (ICP-AES) (Shimadzu, ICPS-7000). Note that the amount of ionic silicon-species is measured as that of Si⁴⁺ ion.

3. Results and discussion

Figure 1 shows the SEM images of SiPVH beads. Figures 1(a–c) shows the entire shapes of SiPVH beads. The average diameters of the SiPVH₁₀, SiPVH₂₀ and SiPVH₆₀ beads were measured to be ~110, ~150 and ~130 μm, respectively. Depressed areas can be observed on the surface of the beads, while some of the beads have shrunk and remained spherical. During the electrospaying, on the droplet from the jet a layer of stiff shell forms due to the rapid evaporation of solvent. The inside of the beads, however, would still be wet. The beads then collect into the ethanol and the solvent inside of the beads slowly diffuse out, resulting in the depressions and the deformations. The surface irregularity of the obtained beads might be beneficial for cartilage regeneration, as often the migration of injected materials from the regeneration site is reported to be one of the main problems. Therefore these surface roughness may enable

Fig. 1. SEM images of SiPVH beads containing (a, d) 10 wt%, (b, e) 20 wt% and (c, f) 30 wt% SiV particles in PLLA matrix. (d–f) are fracture faces of (a–c), respectively.

entanglement and remain in regeneration site. Figures 1(d–f) shows the SEM images of the fracture face of SiPVH₁₀, SiPVH₂₀ and SiPVH₆₀. In the SiPVH₁₀ and the SiPVH₂₀ beads, SiV particles are embedded and enclosed by the PLLA matrix. Furthermore, the matrix formed in the SiPVH₁₀ beads appeared to contain smaller amount of pores than that in SiPVH₂₀ beads. On the other hand, in the SiPVH₆₀ beads the particles are in close contact and they are only partially covered with a thin PLLA film. This suggests that the mass ratio between the PLLA and the SiV particles effects the embedding of the particles in the PLLA matrix.

Figure 2 shows the average diameters of SiPVH₂₀ bead as a function of slurry-feeding rate. The diameter increased from ~65 to ~180 μm by increasing the feeding rate from 0.1 to 0.9 ml/min. The figure also shows that the size distribution of the beads widens at high feeding rates. During the electrospaying, a highly charged solution forms a conical shape (Taylor cone) at the tip of the capillary. At the apex of the Taylor cone a fine liquid jet forms, which breaks up to fine droplets.¹³⁾ Increasing the feeding rate increases the volume of materials in the jet. This results in the formation of the large-sized droplets.

Figure 3 shows Si⁴⁺ and Ca²⁺ ions concentrations in the Tris buffer solutions after soaking the beads for given length of time. The SiV content in the beads plays an important role in the amount and rate of ions released, where the higher release is found in the samples containing larger amount of SiV. In the SiPVH₁₀ and SiPVH₂₀ beads, continuous slow releases of the ions were observed after the first 12 h of soaking. From the SiPVH₁₀ beads, 0.3–0.7 mg/L of Si⁴⁺ and 1.2–2.9 mg/L of Ca²⁺

Fig. 2. Average diameters of SiPVH₂₀ beads as a function of slurry-feeding rate during electrospraying.

Fig. 3. Amount of (a) Si⁴⁺ and (b) Ca²⁺ ions released from the beads into a Tris buffer solution after the given period of soaking.

ions were released per day, while 0.4–3.8 mg/L of Si⁴⁺ and 1.7–10.9 mg/L of Ca²⁺ ions were released from the SiPVH₂₀ beads in the same period. The SiPVH₆₀ exhibited rapid ion dissolution in

Fig. 4. Weight-average molecular weights (M_w) of PLLA in the SiPVH beads after soaking in Tris buffer solution.

the first 12 h of soaking. The amount of Si⁴⁺ and Ca²⁺ ions dissolved from the beads were measured to be 59 and 81 mg/L, respectively. After the first 12 h, the controlled dissolution of both the ions was found. The ionic silicon-species were suggested to be released as silicate ions from the beads. **Figure 4** shows M_w of PLLA in the SiPVH beads as a function of soaking in a Tris buffer solution. The M_w values measured with GPC are believed from our preliminary experiments to include their error range within 30 kDa. Almost no change in M_w could be observed, indicating that the PLLA did not degrade in a Tris buffer solution.

In the case of conventional polymer composites having releasing ability of drugs, the releases are facilitated by their degradation. From the SiPVH beads, however, the ions were released from the materials before the onset of the M_w decrease. Thus, the ions were released independent of polymer degradation. Navarro et al.²⁰⁾ reported the formation of apatite in SBF on a PLA/calcium phosphate glass particle composite where the bioactive calcium phosphate phase was enclosed by the PLA matrix. They proposed that the aqueous solutions penetrate through the polymer and reacts with the glass particles to form apatite. In the SiPVH₁₀ and SiPVH₂₀ beads, dissolution of the SiV particles probably occurs by the slow diffusion of the Tris buffer solution which penetrates through the PLLA matrix. The silicate and calcium ions released from the particle also penetrate through the matrix into the solution in a control manner, and this gave rise to the slow and continuous release of these ions. Furthermore, the particles in the SiPVH₁₀ beads were much more enclosed than those in the SiPVH₂₀ beads, which will suppress the diffusion of a Tris buffer solution, resulting in the slowest dissolution. In the SiPVH₆₀ beads, the SiV particles were not entirely covered by PLLA and the beads were also observed to be porous both inside and outside. Therefore they will dissolve immediately into a Tris buffer solution after the soaking. That is, the releasing behaviors of the ions are regulated by the embedding structure of the SiV particles.

4. Summary

The SiPVH beads were successfully prepared by an electrospraying method and the diameter of the beads could be manipulated from ~65 to ~180 μm by changing the feeding rate and the SiV content in the beads. SiPVH₁₀ and SiPVH₂₀ beads showed the sustained release of silicate and Ca²⁺ ions, while SiPVH₆₀ showed rapid initial dissolution. This variation in

ion dissolution behavior originates largely from the differences in the embedding and enclosing of SiV particles in the PLLA matrix.

Cell culture tests and other investigations are under way to test the beads for the application to an injectable scaffold with controlled ion-releasing ability.

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