Effect of preparation route on the degradation behavior and ion releasability of siloxane-poly(lactic acid)-vaterite hybrid nonwoven fabrics for guided bone regeneration

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Two types of nonwoven fabric, consisting of siloxane-doped vaterite (SiV) and poly(lactic acid) (PLA), for guided bone regeneration (GBR) were prepared by an electrospinning. One of the fabrics, SiV-PLA(M), was derived from PLA mixed with the solution of SiV dispersed in chloroform. Another one, SiV-PLA(K), was derived from a composite prepared by kneading SiV and PLA while heating at 200°C. The SiV-PLA(K) fabric shows higher degradability in dilute NaOH *aq.* than the SiV-PLA(M) fabric. To improve the cellular compatibility of the fabric, the fibers were coated with hydroxyapatite (HA) by soaking in simulated body fluid. The HA-coated SiV-PLA(K) fabric showed the release of silicate ions; the amount was reduced by 1/5 to 1/8 compared with that of the HA-coated SiV-PLA(M) fabric, and the excessive release was controlled. The preparation route of kneading at 200°C led to formation of a fabric with degradation behavior and ion releasability effective for bone regeneration.

Keywords: Electrospinning, Vaterite, Poly(lactic acid), Siloxane, Hybrid

INTRODUCTION

Guided bone regeneration (GBR) is a well-established therapy to repair alveolar bone defects, where in the new bone is reconstructed using a barrier membrane to guard the defective area from invasion by other tissues[1,2]. The barrier membrane for this therapy is called a "GBR membrane". GBR membranes should satisfy several criteria: they should control the intrusion of soft tissue into the bone site, have surface roughness that enables good integration with the surrounding tissues and thus enhances their stability (*i.e.*, tissue integration), have high mechanical strength to maintain the shape of the defect to be reconstructed, and enhance the formation of bone[3,4].

Nondegradable expanded-polytetrafluoroethylene (e-PTFE)[5], biodegradable materials such as collagen[5], and synthetic biodegradable polymers such as poly(lactic acid) (PLA) or poly(lactic-co-glycolic acid) (PLGA)[2], have been used as membrane materials. Although these membranes work as barriers that control the intrusion of soft tissues, they do not show any bone-formation-enhancing ability. GBR membranes are sometimes applied with transplanted autologous or heterogenous bone or implanted together with bioactive materials to achieve enhanced bone formation.

Recently, we have developed a novel hybrid material consisting of PLA and calcium carbonate (vaterite) particles doped with siloxane[6-8]. Hereafter, the material is denoted as SiPVH (siloxane-PLA-vaterite hybrid). The film prepared from SiPVH has been shown to release ionic silicon species (silicate ions) and calcium ions in a controlled manner, which have been reported to stimulate the proliferation and differentiation of murine osteoblast-like cells (MC3T3-E1 cells)[6]. This phenomenon is consistent with reports that have suggested that trace amounts of silicon (or silicate) and calcium ions enhance the mineralization and bone-forming abilities of osteogenic cells[9-12]. Furthermore, we have developed a novel GBR membrane consisting of SiPVH prepared by an electrospinning[7]. It is suggested that the large spaces between the fibers in the electrospun SiPVH nonwoven fabric are effective for the MC3T3-E1 cells to enter into the fabric and proliferate actively[8].

In dentistry, the SiPVH nonwoven fabric is expected to be applied significantly to a GBR membrane for the effective repair of the alveolar bone defect.

In the present work, the effect of the preparation route of the SiPVH fabric on its morphology, chemical structure, biodegradability and ion releasability was examined. Two types of route were discussed: preparation by mixing PLA and SiV in chloroform, and preparation by kneading with heating.

MATERIALS AND METHODS

Preparation of siloxane-doped vaterite (SiV) powders
SiV powders were prepared by a carbonation process with methanol, following the method described in our earlier reports[6,13]. One-hundred and fifty grams of $Ca(OH)_2$, 60 mL of aminopropyltriethoxysilane (APTES) and 2,000 mL of methanol were mixed in CO_2 atmosphere for 75 min at a rate of 2,000 mL/min. The resulting slurry was dried at 110ºC, resulting in the formation of spherical

SiV powders with an average particle size of ~1 µm. The amount of silicon in SiV was estimated to be ~2 wt% by X-ray fluorescence analysis.

Preparation of nonwoven fabrics by electrospinning
PLA (PURASORB®; molecular weight of 200–300 kDa, PURAC, Gorinchem, The Netherlands) was used as a matrix polymer.

PLA-solution mixing method: A solution was prepared by dispersing SiV powders into chloroform using a vacuum emulsifier (T.K. AGI HOMO MIXER®; 2M-03, PRIMIX, Osaka, Japan). The SiV/chloroform weight ratio was 1/6. PLA was dissolved into the SiV-dispersed solution while stirring at the SiV/PLA weight ratio of 3/2. As a result, a mixed solution consisting of PLA and SiV was obtained for electrospinning. The weight ratio of SiV/PLA/chloroform was 3/2/18. Hereafter, this solution prepared by mixing is denoted as SiV-PLA(M).

Kneading method: PLA and SiV powders were blended using a mechanical kneader with heating. The SiV/PLA weight ratio was 3/2. Twenty-eight grams of PLA powder and 42 g of SiV powder were kneaded at 200ºC for 10 min. The resulting composite was dissolved into chloroform at the SiV/PLA/chloroform weight ratio of 3/2/18, the same ratio as for SiV-PLA(M), to form a solution for electrospinning. Hereafter, the solution prepared from the kneaded composite is denoted as SiV-PLA(K).

Two types of nonwoven fabric were prepared by electrospinning using the above solutions under the following conditions: impressed voltage of 20 kV, flow rate of 0.05 mL/min, and distance between the nozzle and the collector of 150 mm. The nonwoven fabrics prepared from SiV-PLA(M) and SiV-PLA(K) are denoted as SiV-PLA(M) fabric and SiV-PLA(K) fabric, respectively.

The structural morphologies of the prepared nonwoven fabrics were observed by scanning electron microscopy (SEM; VE-8800, Keyence, Osaka, Japan). To estimate the degree of crystallinity of PLA composing the nonwoven fabrics, differential scanning calorimetry (DSC; DSC-8230, Rigaku, Tokyo, Japan) of each fabric was conducted under the following conditions: range of temperature of 50–200°C and heating rate of 10°C/min. The degree of crystallinity (Xc) was calculated using

$$Xc = (\Delta Hf - \Delta Hc)/93.7 \ [\text{J/g}] \times 100\%.$$

Here, ΔHf and ΔHc are the enthalpies of fusion and recrystallisation, respectively, calculated from the areas under the curves of recrystallisation and melting peaks[14]. The enthalpy of fusion for 100% crystalline PLA has been reported to be 93.7 J/g[15].

Structural analyses of the resulting nonwoven fabrics were performed using ^{13}C cross-polarization magic angle spinning nuclear magnetic resonance (^{13}C CP/MAS-NMR; Varian UNITY 400 plus, Varian, Palo Alto, CA, USA) and Fourier transform infrared spectroscopies (FT-IR; FT/IR-4100, Jasco, Tokyo, Japan).

^{13}C CP/MAS-NMR spectra were taken with 4.7 µs and 8.0 s recycle delays. The sample spinning speed was 5.0 kHz. The integration frequency was 1,000 times. Chemical shifts were measured relative to that of adamantine.

Measurement of PLA degradability
The degradabilities of PLA in the SiV-PLA(M) and SiV-PLA(K) fabrics were evaluated by measuring the molecular weights of PLA. The acceleration of hydrolysis originated from the degradation of PLA. Each fabric was soaked in 5 mmol/L of NaOH solution. The molecular weights of the PLA matrix in the prepared fabrics were measured by gel permeation chromatography (GPC; LC-20, Shimadzu, Kyoto, Japan) using two columns (Shodex®; KF-604, KF-606M, Showa Denko, Tokyo, Japan). The fabrics were dissolved at a concentration of 10 mg PLA per 5 mL chloroform, and then the resulting solutions were filtered. The measurements were carried out at 40°C at a flow rate of 0.6 mL/min. Polystyrene standards (Shodex®; SM-105, Showa Denko, Tokyo, Japan) were used to establish a calibration curve.

Coating of fibers with HA
The cellular compatibility of materials has been reported to be enhanced after coating with HA by soaking in simulated body fluid (SBF)[16]. Therefore, the fibers of the SiV-PLA(M) and SiV-PLA(K) fabrics were coated with HA by soaking in a modified SBF (denoted as 1.5 SBF) at 37°C for 24 h. The 1.5 SBF, containing 1.5 times the inorganic ion concentration of conventional SBF in order to accelerate the deposition of HA, comprised the following ions: 3.75 mM Ca^{2+}; 213.0 mM Na^+; 2.25 mM Mg^{2+}; 7.5 mM K^+; 223.2 mM Cl^-; 6.3 mM HCO_3^-; 1.5 mM HPO_4^{2-}; and 0.75 mM of SO_4^{2-}. The pH was adjusted to 7.4 by adding $(CH_2OH)_3CNH_2$ and HCl. After soaking, the sample was washed with distilled water and then dried at room temperature. Hereafter, the SiV-PLA(M) and SiV-PLA(K) fabrics after HA-coating are denoted as SiV-PLA(M)/HA and SiV-PLA(K)/HA fabrics, respectively.

Measurement of silicate and calcium ions released from SiV-PLA(M)/HA and SiV-PLA(K)/HA fabrics
Silicon-species and calcium-ion release rates from the SiV-PLA(M)/HA or SiV-PLA(K)/HA fabric were evaluated by immersing the fabric in 4 mL of cell culture medium (alpha minimum essential medium; α-MEM) containing 10% foetal bovine serum (FBS) and incubating it at 37°C in a humidified atmosphere of 95% air and 5% CO_2 for 5 days. Samples of 3.7 mg were used in the release study. The culture medium was changed after 1 and 3 days of soaking. The amounts of silicon and calcium ions released from the sample to the culture medium were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES; ICPS-500, Shimadzu, Kyoto, Japan). The silicate ion was observed as the silicon species.

RESULTS

Characterization of SiV-PLA(M) and SiV-PLA(K) fabrics
Under the present conditions, SiV-PLA(M) was electrospun intermittently, while SiV-PLA(K) was electrospun continuously. The SiV-PLA(M) fabric was composed of fibers with nonuniform diameters of approximately 10 to 40 µm and was brittle. On the other hand, the SiV-PLA(K) fabric was composed of fibers with almost uniform diameters of ~10 µm and was flexible, and could be bent without fracture using a pair of tweezers[7].

Figures 1 and 2 show the SEM micrographs of the surface and the fracture face of the SiV-PLA(M) and SiV-PLA(K) fabrics, respectively. Many holes were observed at the surface of the SiV-PLA(M) fiber, and SiV particles were exposed at the fiber surface (Fig. 1(a)). There existed various SiV particles completely exposed and embedded in the PLA matrix (Fig. 1(b)). On the other hand, in the case of the SiV-PLA(K) fiber, the SiV particles were embedded in the PLA matrix and were uniformly dispersed in the fiber (Fig. 2(b)).

Figure 3 shows ^{13}C CP/MAS-NMR spectra of the SiV-PLA(M) and SiV-PLA(K) fabrics. The peaks at approximately 170 ppm are related to the carboxy groups. It has been reported that when a bivalent ion is coordinated with a carboxy groups, a new peak corresponding to that groups appears at the side of low magnetic field in the band[17], and that, on the composites prepared from PLA and vaterite, a peak indicating the coordination between Ca^{2+} ions originating from vaterite and the carboxy groups originating from PLA appears at approximately 172 ppm[18]. In this work, to identify the existence of the peak at approximately 172 ppm, each broad peak at approximately 170 ppm was deconvoluted into two peaks by using a 2-component Gaussian fitting method. The peak at approximately 172 ppm for the SiV-PLA(M) fabric was larger than that for the SiV-PLA(K) fabric; there exists a greter degree of coordination between Ca^{2+} ions and the carboxy groups in the SiV-PLA(M) fabric.

Figure 4 shows the FT-IR spectra of the SiV-PLA(M) and SiV-PLA(K) fabrics. The weak peak at approximately 1,650 cm^{-1}, which has been reported to originate from the amide bond[7], appeared in both spectra.

The degrees of crystallinity of the PLAs in the SiV-PLA(M) and SiV-PLA(K) fabrics were estimated to be 19% and 16% by DSC, respectively.

The weight-average molecular weight (Mw), number-average molecular weight (Mn), and polydispersity (Mw/
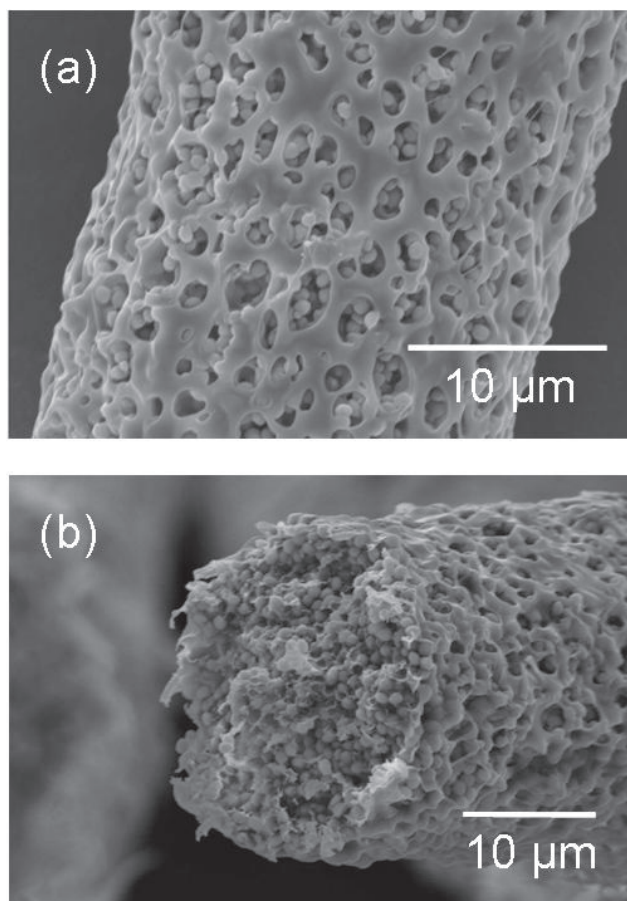

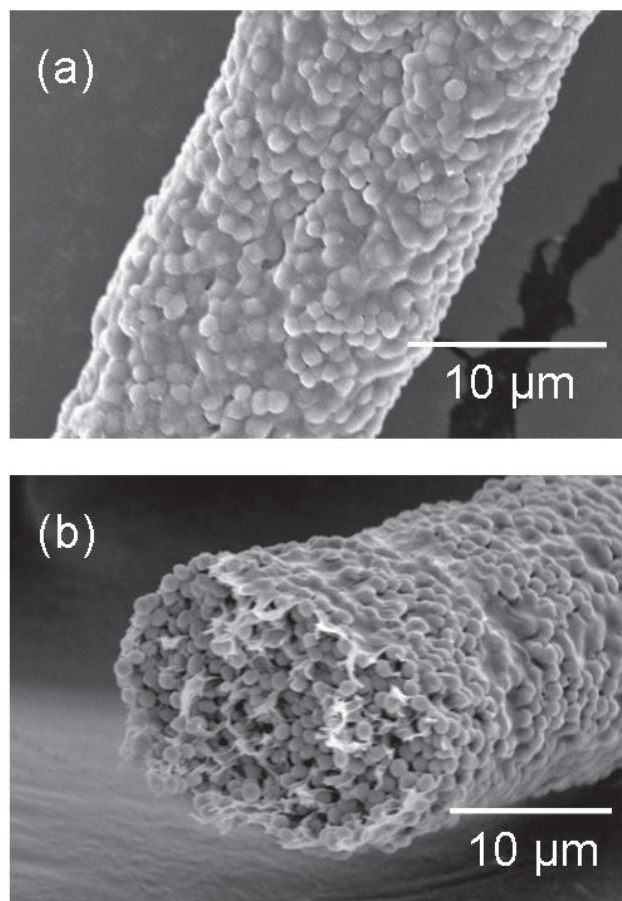Fig. 1 SEM micrographs of SiV-PLA(M) fabric: (a) surface and (b) fracture face of the fiber.

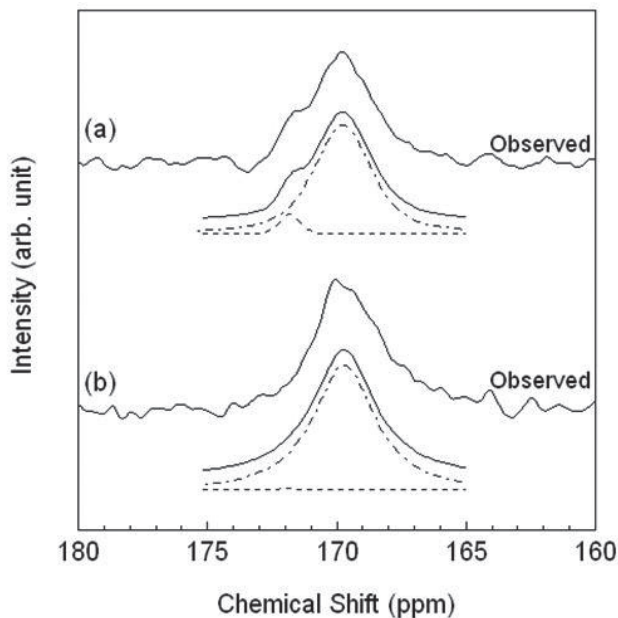Fig. 2 SEM micrographs of SiV-PLA(K) fabric: (a) surface and (b) fracture face of the fiber.

Mn) of PLA in the SiV-PLA(M) fabric were estimated to be 310 kDa, 211 kDa, and 1.5, respectively. On the other hand, these values of the PLA in the SiV-PLA(K) fabric were 265 kDa, 145 kDa, and 1.8, respectively; these molecular weights were lower than those in the SiV-PLA(M) fabric.

Molecular weight changes of PLA after soaking in dilute NaOH solution
Figure 5 shows the molecular weight and polydispersity changes of PLAs in the SiV-PLA(M) and SiV-PLA(K) fabrics after soaking in 5 mmol/L NaOH solution. Almost no changes in the molecular weights of PLA in the SiV-PLA(M) fabric were observed in the experimental period of 28 days after soaking. On the other hand, PLA in the SiV-PLA(K) fabric decreased rapidly within 5 days after soaking and then decreased gradually.

Releasabilities of calcium and silicate ions
The HA at the surfaces of the SiV-PLA composites soaked in the 1.5 SBF at 37°C for 24 h has been reported to have a porous and leaf-like shapes with thickness of approximately 1 μm[7]. Figure 6 shows the amounts of Ca^{2+} and Si^{4+} ions released from the SiV-PLA(M)/HA and SiV-PLA(K)/HA fabrics into a culture medium. Forty mg/L of Ca^{2+} ions was released from the SiV-PLA(M)/HA fabric within the first day, and subsequently, 20 mg/L/day was continuously released. The amount of Ca^{2+} ions

Fig. 3 ^{13}C CP/MAS-NMR spectra of (a) SiV-PLA(M) and (b) SiV-PLA(K) fabrics and their deconvoluted results. In the results, dashed lines, (–·–·–) and (-----), and a solid line show the separated peaks, and the synthesized one, respectively.
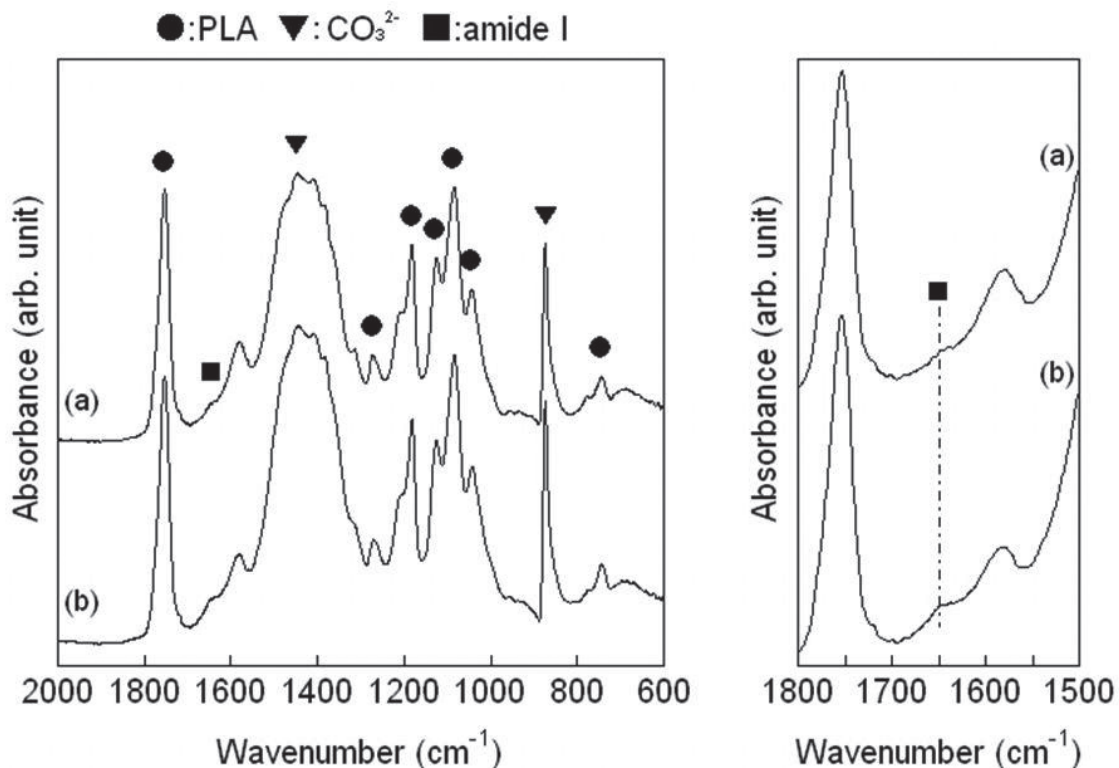

Fig. 4   FT-IR spectra of (a) SiV-PLA(M) and (b) SiV-PLA(K) fabrics.

released from the SiV-PLA(K)/HA fabric was less than that from the SiV-PLA(M)/HA fabric; 35 mg/L of Ca^{2+} ion was released within the first day, and subsequently, 12 mg/L/day was continuously released. The amounts of Si^{4+} ions released from the SiV-PLA(M)/HA fabric were measured to be 3.7 mg/L within the first day, and subsequently, 1.7 mg/L/day of Si^{4+} ions was continuously released. On the other hand, the amounts of Si^{4+} ions released from the SiV-PLA(K)/HA fabric were smaller by 1/5 to 1/8 than those released from the SiV-PLA(M)/HA fabric; 0.68 mg/L of Si^{4+} ions was released within the first day, and 1.6 mg/L/day was subsequently continuously released.

DISCUSSION

The peak at approximately 172 ppm in the ^{13}C CP/MAS-NMR spectra (Fig. 3) indicates the coordination between the Ca^{2+} ion originating from SiV and the carboxy groups originating from PLA; the peak for the SiV-PLA(M) fabric is larger than that for the SiV-PLA(K) fabric. SiV-PLA(M) used for the electrospinning was prepared by mixing PLA with the solution of SiV dispersed in chloroform. It is suggested that the siloxanes
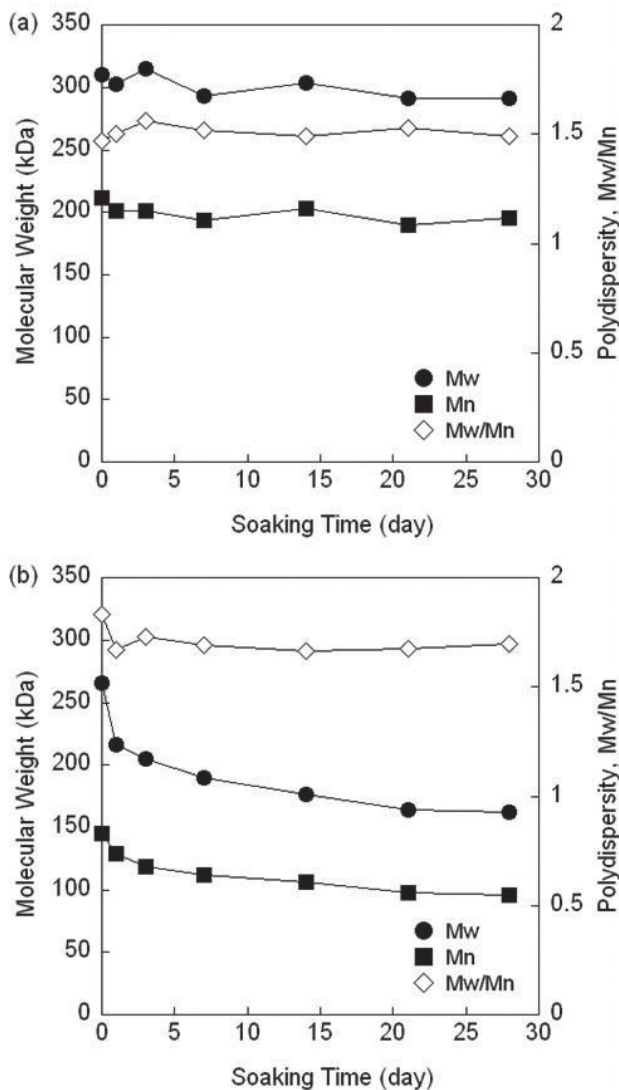

Fig. 5 Molecular weights (Mw and Mn) and polydispersity (Mw/Mn) of (a) PLA in SiV-PLA(M) fabric and (b) PLA in SiV-PLA(K) fabric after soaking in 5 mmol/L NaOH solution.
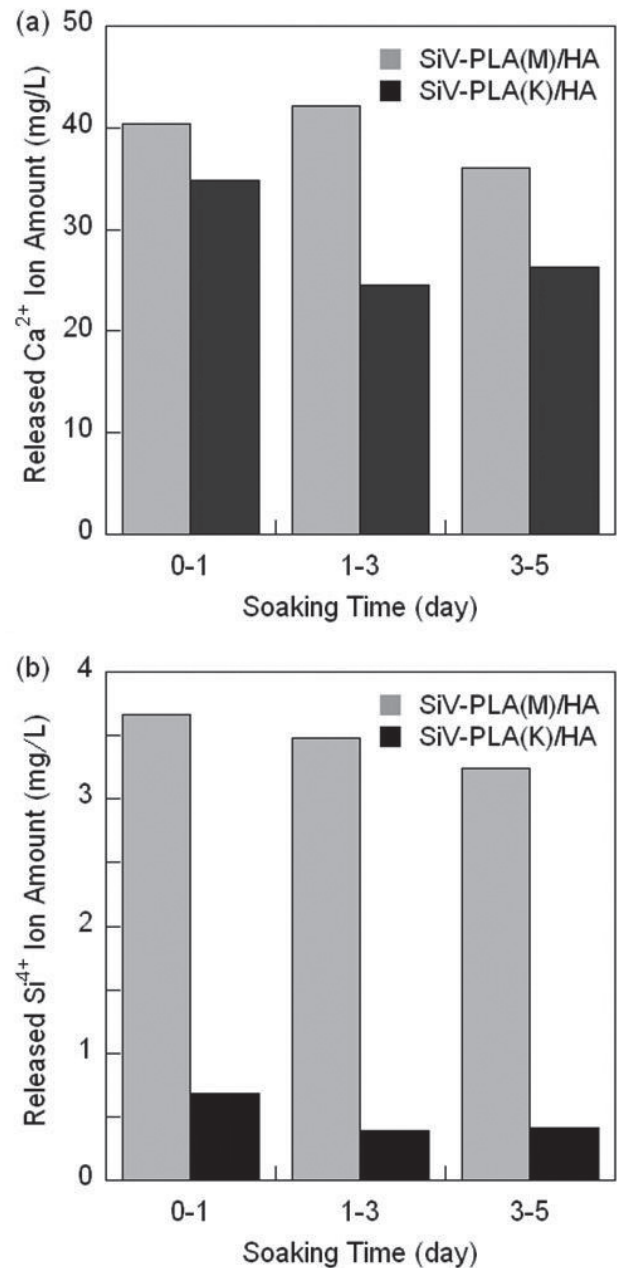

Fig. 6 (a) Calcium and (b) silicon ion concentrations in a culture medium after soaking SiV-PLA(M)/HA and SiV-PLA(K)/HA fabrics.

in SiV were dissolved by mixing with chloroform and the vaterites were exposed. As a result, Ca^{2+} ions around the surface of SiV were coordinated with the carboxy groups in PLA. In general, silanol groups (Si-OH) originating from silane coupling agents, such as APTES, form covalent bonds by the condensation reaction with dehydration between the hydroxy groups on the material surfaces. Silanol groups also bond to each other to form siloxane oligomers by the condensation reaction. These condensation reactions with dehydration are accelerated by heating or desiccating to remove the water or the alcohol, which are by-products of the reaction[19]. Furthermore, it has been reported that silanol and hydroxy groups on the material surfaces form metaloxane bonds (Si-O-M; M indicates a metallic element) by the condensation reaction with dehydration[20]. SiV-PLA(K) was prepared by kneading SiV with PLA at 200°C for 10 min. During kneading, the condensation reactions between the vaterites and the siloxanes in the SiV particles are accelerated and, as a result, the surfaces of vaterites are covered with siloxanes. Therefore, it is suggested that the coordination between the Ca^{2+} ion originating from SiV and the carboxy groups originating from PLA was to a very low degree. As a result, a small peak appeared at approximately 172 ppm.

The peaks at around 1,650 cm^{-1} originating from the amide bond appeared on the FT-IR spectra of both the SiV-PLA(M) and SiV-PLA(K) fabrics. This result indicates that the amino groups in the siloxane, which was dissolved from or remained in the SiV, formed the amide bond with carboxy groups of the PLA.

Figure 7 shows the speculated drawing of the structure around the interfaces between the SiV particle and PLA in (a) PLA-SiV(M) and (b) PLA-SiV(K), from the above discussion.

The amounts of Ca^{2+} and Si^{4+} ions released into the culture medium from the SiV-PLA(M)/HA fabric were larger than those released from the SiV-PLA(K)/HA fabric (Fig. 6). In the case of the SiV-PLA(M) fabric, the dissolution of the siloxane from SiV and the resulting exposure of vaterite were suggested by the ^{13}C CP/MAS-NMR spectra. These results indicate that the silicate ions were released from not only the siloxane dissolved from SiV but also the numerous SiV exposed at the surface of the SiV-PLA(M) fibers, as shown in Fig. 1, and that the numerous exposed SiV led to the release of a larger amount of Ca^{2+} ions.

Although there was almost no difference between the crystallinities of PLAs in the SiV-PLA(M) and SiV-PLA(K) fabrics, 19% and 16%, respectively, the molecular weight of PLA in the SiV-PLA(K) fabric was smaller. Almost no changes in the molecular weight of PLA in the SiV-PLA(M) fabric occurred in the experimental period. On the other hand, the molecular weight of PLA in the SiV-PLA(K) fabric decreased gradually, indicating a higher degradability than that in the SiV-PLA(M) fabric. PLA matrix in the SiV-PLA(K) fabric would be decomposed thermally during kneading and the resulting carboxy groups would form amide bonds with the amino groups in the siloxane released
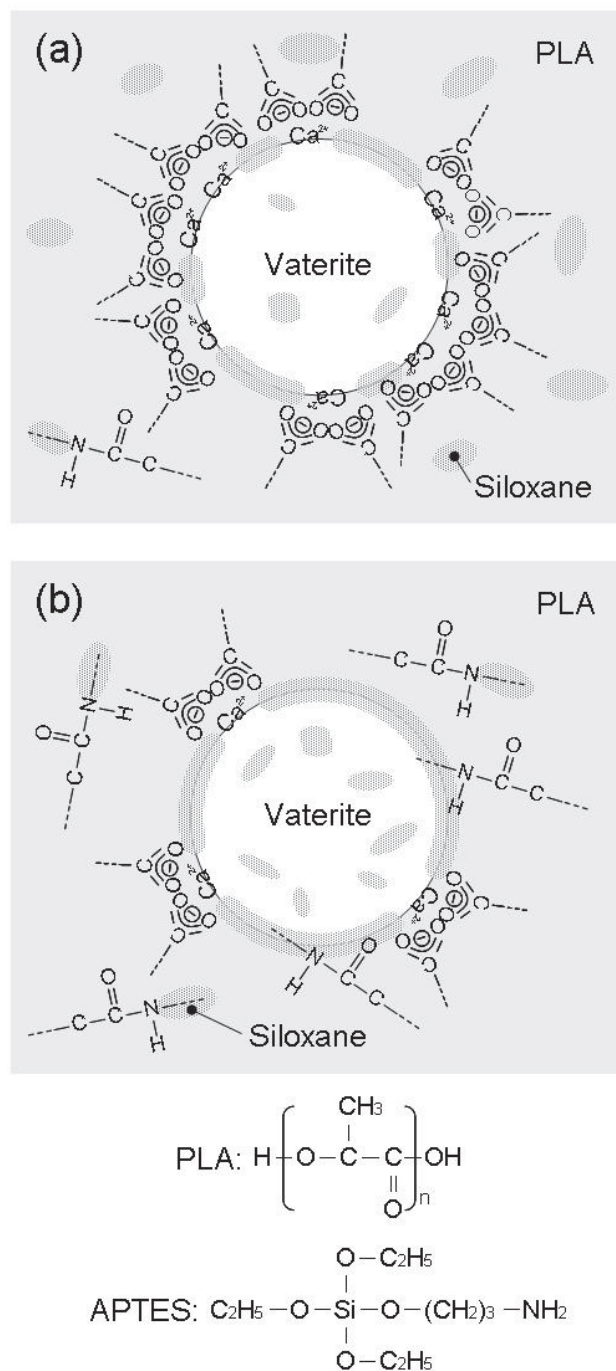

Fig. 7   The speculated drawing of the structure around the interfaces between the SiV particle and PLA of (a) PLA-SiV(M) and (b) PLA-SiV(K). Siloxane containing amino group is derived from APTES. Siloxane is embedded also in the SiV particle. In the case of PLA-SiV(K), during kneading with heating, siloxane is released from SiV particles, to react with carboxy group in PLA matrix.

from SiV. As a result, the molecular weight of the PLA matrix would decrease and the fabric is proposed to show higher degradability. Much attention has been paid to bioresorbable materials for enhancing bone regeneration. The SiV-PLA(K) fabric, which has great potential to be bioresorbed, is expected to continuously release Ca^{2+} and Si^{4+} ions during the biodegradation of PLA. In our previous report, the amounts of Ca^{2+} and Si^{4+} ions released from the HA-coated SiV-PLA composite fabric were noted to be sufficient to enhance their proliferation and differentiation of murine osteoblast-like cells (MC3T3-E1 cells) [6,7].

CONCLUSION

The electrospun SiV-PLA(M) fabric, which was prepared via the PLA-solution mixing method, was composed of porous, nonuniform fibers containing agglomerated SiV and was brittle. The SiV-PLA(K) fabric, which was prepared via the kneading method, was composed of fine fibers containing SiV embedded in the PLA matrix and was flexible. SiV and PLA were found to be mixed more uniformly by kneading with heating than by dissolving with a solvent. Furthermore, the molecular weight of PLA in the SiV-PLA(K) fabric decreased after soaking in dilute NaOH solution, that is, PLA in the SiV-PLA(K) fabric showed the degradability. The higher degradability is suggested to originate from the decrease in the molecular weight of PLA matrix in the fabric during kneading with heating and the formation of amide bonds with siloxane released from SiV particles.

The SiV-PLA(K)/HA fabric can continuously release Ca^{2+} and silicate ions, to enhance bone regeneration, because of their controlled excess dissolution. The kneading process is very important for preparing a bioresorbable GBR membrane with flexibility.

ACKNOWLEDGMENTS

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