

Preparation of Poly(lactic acid)/Si-doped Vaterite Hybrid Beads by Electro spraying

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Abstract. Poly (lactic acid)/Si-doped vaterite hybrid beads of 20 to 130 μm in diameter were prepared by electro spraying. Molecular weights of the PLLA in the hybrid beads significantly decreased after soaking in a dilute acetic acid solution. Biodegradability of the PLLA was expected to be enhanced by mixing of SiV particles. The hybrid surfaces were covered with hydroxyapatite after soaking in a simulated body fluid. The hybrid beads may be applied as an injectable biomaterials achieving minimally invasive surgery for cartilage defects.

Introduction

Cartilagenous tissue has a poor natural healing capacity due to the lack of nerve and vascularized tissue. Recently, injectable beads-shaped biomaterials have been researched for applications to minimally invasive surgery in cartilage repairing to replace autologous transplantation. Poly(lactide-co-glycolide) microspheres enhanced formation of native-like cartilage when co-injected with chondrocytes into the subcutaneous space of mice [1]. Porous poly(lactic-co-glycolic acid) microspheres induced homogenous regeneration of neocartilage inside of the microspheres [2].

In the present work, poly(L-lactic acid) (PLLA)/Si-doped vaterite (SiV) hybrid beads were prepared by electro spraying. CO_3^{2-} ions derived from the SiV particles may buffer the pH around the beads, while Ca^{2+} ions may play an important role in the formation of new bones under the regenerated cartilage. Furthermore, the releasability of ionic silicon-species was also given to the beads. When rat chondrocytes were cultured with Bioglass[®] 45S5 granules, cartilaginous nodule formation was enhanced in the vicinity of granules after 5 days of culture [3]. The cartilage formation may be enhanced by ionic silicon-species. Si-doped vaterite particles were mixed to the PLLA in order to achieve the release of these ions, such as CO_3^{2-} , Ca^{2+} and Si^{4+} , from the beads at once. On the assumption of implantation into inflammatory tissues, the beads were soaked in the acetic acid solution (initial pH = 4) to evaluate their degradation behavior. The hybrid beads were soaked in simulated body fluid (SBF) to provide bioactivity by forming hydroxyapatite (HA) on its surface.

Materials and Methods

SiV particles were provided from Yabashi Industries. Co. Ltd. The average particle size of the SiV was about 1 μm and the Si contents were estimated to be around 3 wt%. The SiV particles and PLLA (molecular weight, $M_w = 160$ kDa) were melt-blended with a bench kneader (Irie Shokai, PBV-01) at 200 °C to obtain the PLLA/SiV hybrid including 20, 40, and 60 wt% of SiV. The PLLA was heat-treated in a drying oven at 200 °C for 30 min to degrade its

molecular weight. The hybrids or the PLLA after the heat-treatment were dissolved in the chloroform to obtain their slurry. The microbeads were prepared from the slurries by electrospraying. The preparing conditions of the beads are indicated in Table 1.

Table 1. Preparation conditions of the beads.

Sample	Si-V : PLLA (wt ratio)	PLLA ratio in the slurry [wt%]	Applied voltage [kV]	Feeding rate [ml/min]
S0	0 : 100	5	15	0.500
S20	20 : 80	5	19	0.500
S40	40 : 60	5	19	0.500
S60	60 : 40	5	19	0.500
S60s	60 : 40	3	17	0.050

The shapes of the beads were observed with a scanning electron microscope (JEOL, JSM-6301F : SEM). 50 mg of S0, S20, S40, and S60 beads were soaked in the acetic acid solution, which was adjusted to pH 4, and then stored at an incubator (36.5 °C) for 12 – 168 h. After the incubation, the beads were filtrated and dried. The Ca²⁺ ion concentrations of the solutions were measured with an inductively-coupled plasma atomic emission spectrometer (Shimadzu, ICPS-7000 : ICP-AES). The molecular weights of PLLA in the beads after the incubation were evaluated with a gel permeation chromatograph (Shimadzu, GPC), equipped with two columns (Shodex, KF-604). 10 mg of S60 and S60s beads were soaked in a simulated body fluid (1.5SBF) and then incubated at 36.5 °C for 1 d.

Results and Discussion

Figure 1 shows SEM images of the obtained hybrid beads. The beads with a diameter of around 20 - 130 μm were achieved by controlling the conditions. The diameters of the beads increased with increasing in the PLLA content in the slurry and a fast feeding rate during the electrospray. In the method, high voltage was applied to the slurry. An electrostatic atomization of the slurry affects the formation of the beads [4, 5]. The viscosity of the slurry increases with increasing PLLA content. As a result, the atomization is suppressed, to increase the diameters of the beads. The SiV particles with diameters around 1 μm are distributed homogenously on the surface of the beads, as shown in the Fig. 1 (B).

The molecular weights of the S20, S40 and S60 beads decreased after the soaking. The molecular weights have a tendency to degrade with increasing the SiV content in the beads. The interface area between the PLLA matrix and the SiV particles increases with increasing the particle content in the beads. The beads after the soaking have a porous structure, derived from the dissolution of the SiV particles. The acetic acid solution penetrates through the pores formed inside of the beads. The increase in the interface area may induce the decrease in the molecular weight. The structural change of the PLLA to an amorphous phase during the melt-blending also may cause the degradability. Further work for understanding these phenomena is in progress.

Fast dissolutions of Ca²⁺ ions were observed in the S40 and S60 beads within 12 h of soaking, while relatively slow release of the ion was observed in S20 beads during 168 h of soaking. In the S20 beads, SiV particles were embedded and isolated in the PLLA matrix and the dissolution of the particles may be suppressed. On the other hand, SiV particles were contacted

one another in the S40 and S60 beads. As a result, a fast dissolution of the SiV particles is suggested to occur.

Diameters of the S60s and S60 beads are about 20 and 130 μm , respectively. S60s and S60 beads have the smallest and largest diameter in all of the prepared beads in this work. After soaking in 1.5SBF, dense formation of HA was observed on the surface of the S60 beads. On the other hand, HA formed sparsely on the surface of S60s beads. In the S60s beads, having a larger specific surface area than S60 ones, phosphoric ion in 1.5SBF was supposed to be insufficient for a large amount of the HA formation on the beads surfaces under the present condition. S60s beads aggregated through the HA after the soaking. Small-sized spaces formed among the beads in 1.5SBF accumulating in the bottom of the vessel. HA is suggested to form among the beads due to the localized increase of Ca^{2+} ion concentration at the spaces.

Conclusions

PLLA/SiV hybrid beads were prepared via electrospraying. The degradability of the resulting beads and HA-forming ability on their surface were evaluated. The diameter of the beads can be controlled in the range of 20 to 130 μm . Significant M_w decreases were observed in the beads after the soaking in a dilute acetic acid solution of pH 4. HA formed on the surface of the beads after soaking in simulated body fluid.

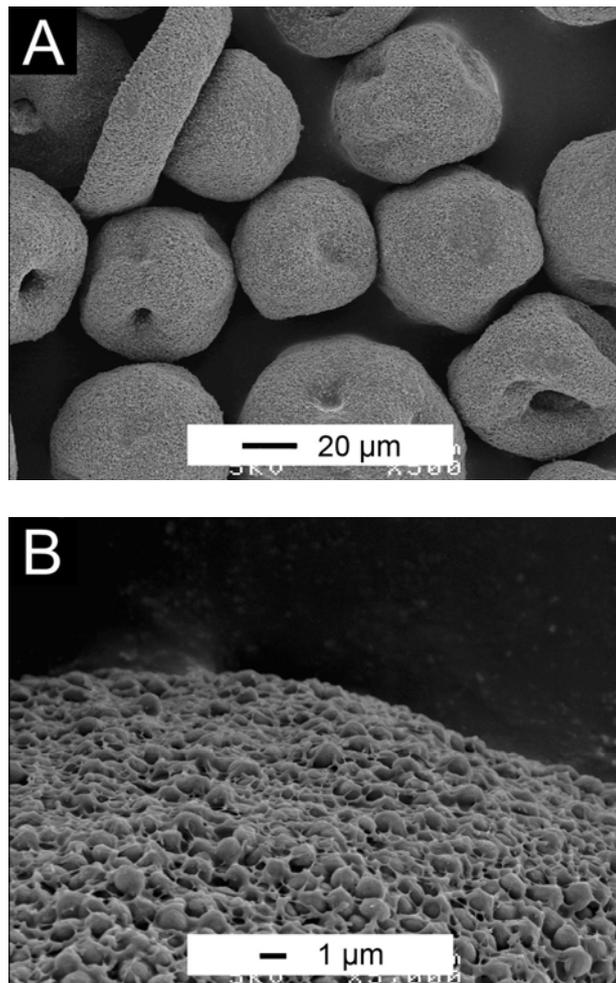


Fig. 1. SEM images of PLLA/SiV hybrid microbeads. (B) is the magnified image of the bead surface.

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