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Preparation of Poly(lactic acid) Composite Hollow Spheres with an Open Channel

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Abstract. Novel hollow spheres for bone fillers incorporating cells were prepared using composites consisting of poly(lactic acid) and calcium carbonates. An open channel of ~800 μm in diameter was easily formed using a chemical etching method to provide a pathway to the interior of the sphere. Cells could migrate through the open channel into the interior of the sphere. Bonelike apatite coating on the surface of the sphere was prepared by soaking in calcium chloride solution to supply excess Ca^{2+} ions on the surface and subsequently by soaking in simulated body fluid. The hollow spheres with an open channel may be one of the great potential candidates as novel bone fillers combined with a cell-delivery system.

Introduction

Materials with a high bioactivity or biodegradability play an important role in the recovery of the part of bone defects. In our earlier work a composite consisting of bioresorbable calcium carbonate (vaterite) and biodegradable poly(lactic acid) (PLA) was reported to form bonelike apatite (b-HA) on its surface after 1 d of soaking in SBF[1,2]. The rapid formation of the b-HA was supposed to originate from the integration of PLA having carboxy groups bonded with Ca^{2+} ions for the b-HA nucleation and a large amount of vaterite with an ability to effectively increase the supersaturation of b-HA. The composite was reported to show osteoconductivity and bioresorbability[3,4]. Novel bone fillers using this material are now being investigated.

Various porous blocks for bone fillers are reported. Some cases may require particle-shaped fillers. Our approach is to fill the bone defects with hollow spheres with open channels incorporating cells. This type of bone filler, if developed, would be expected to induce bone formation and to enhance the ingrowth of bone tissue to the interior of the hollow-shaped fillers.

We have already reported to prepare novel hollow spheres of ~1.0 mm in diameter consisting of calcium carbonate and poly(lactic acid) (PLA) using an oil-in-water emulsion method[3]. The hollow in the sphere was supposed to be formed by numerous CO_2 gas generated from decomposition of the nanometer-sized calcium carbonate powders.

An open channel in the shell of the sphere may provide a pathway to allow the migration of cells into the interior of the sphere. The sphere material is expected to have an advantage in that the incorporated cells on the inside surface can be protected against damages due to handling during operation. In the present work we prepared the sphere with an open channel in the shell. b-HA shows an excellent cell-compatibility[6,7]. b-HA coating on the surface of the sphere is believed to play an important role in the attachment of cells. The present work also involves the b-HA coating on the surface.

Materials and Methods

The preparation procedures of vaterite were described in our previous papers[1,2]. CO_2 gas was blown for 3 h at a flow rate of 300 mL/min into the suspension consisting of 7.0 g of $Ca(OH)_2$ in 180 mL of methanol at 0 °C in a Pyrex® beaker. The resultant slurry was dried at 70 °C in air to prepare fine-sized powders. The BET surface area was measured to be ~40 m^2/g. The molecular weight of the received PLA was determined to be 160 ± 20 kDa by gel permeation chromatography. 0.5-g of PLA was dissolved in methylene chloride to form a 1/20 w/v polymer solution. The calcium carbonate powders were added to the PLA solution and then the mixture was stirred. The weight ratio of $CaCO_3$/PLA was 1/2. The sphere was produced by an oil-in-water emulsion solvent evaporation method. The resulting slurry was drop-wise added to aqueous solution including 1 % poly(ethylene glycol) as a surfactant with stirring for 8 h. The spheres were isolated by vacuum filtration, washed with ethanol, and dried at 40 °C.

An open channel in the shell was prepared by a chemical etching method. The spheres were soaked for 3 min in 50 % solution of methylene chloride diluted with acetone to prepare an open channel in them.

To supply excess Ca^{2+} ions on the surface of the spheres for b-HA coating, they were soaked in 1 M of calcium chloride solution for 1 d prior to soaking in simulated body fluid (SBF) consisting of 2.5 mM of Ca^{2+}, 142.0 mM of Na^+, 1.5 mM of Mg^{2+}, 5.0 mM of K^+, 148.8 mM of Cl^-, 4.2 mM of HCO_3^-, 1.0 mM of HPO_4^{2-}, and 0.5 mM of SO_4^{2-} that included 50 mM of $(CH_2OH)_3CNH_2$ and 45.0 mM of HCl at pH 7.4 at 37 °C for 3 d.

The morphology of the sphere after the chemical etching and soaking in SBF was observed by scanning electron microscopy (SEM) incorporating an energy dispersive spectrometer (EDS). The sphere before and after soaking in SBF was examined by laser Raman spectroscopy.

Results and Discussion

Figure 1 shows an SEM photograph of the sphere after the chemical etching in the present work. A sphere with an open channel can be easily formed in the shell. The channel size is ~800 μm in diameter. The sphere before the chemical etching has a shell with a thickness in the range of 50~300 μm. An open channel can be made at the thinner portion in the shell due to dissolution of PLA in a dilute methylene chloride. XRD analysis showed that the sphere consists of crystalline PLA and calcium carbonates consisting mainly of aragonite. A large amount of vaterite in calcium carbonates as the starting material disappeared after the formation of the spheres. No hollow spheres were prepared using a PLA composite containing calcite powders instead of the vaterite powders as the starting material. It is suggested that the present nanometer-sized vaterite powders with high solubility play an important role in the formation of hollow spheres. Figure 2 shows an SEM photograph of MC3TC-E1 cells on the inside surface of the shell after incubation for 5 d. An *in vitro* evaluation of osteoblast-like cells showed that numerous cells, which were seeded on the spheres with an open channel, attached even to the inside surface. The open channel size is believed to be enough to allow the migration of cells into the interior.

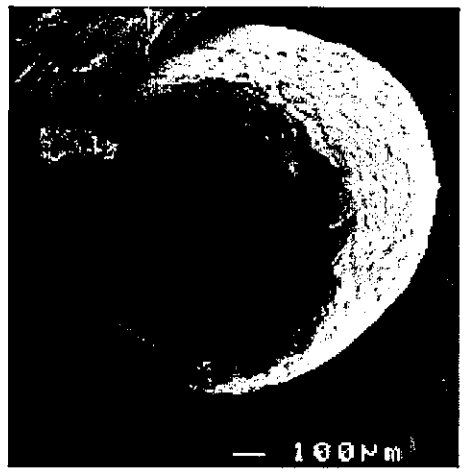

Fig. 1. SEM photograph of a hollow sphere with an open channel in the shell.

Figure 3 shows an SEM photograph of the inside surface of the sphere with an open channel after soaking in SBF. SEM observation shows that the inside surface of the sphere is covered with the leaf-like deposits after soaking in SBF as well as the outside surface. When the spheres were not soaked in calcium chloride solution, no newly deposits formed on the inside surface after soaking in

SBF for 3 d. Figure 4 shows EDS spectra of the inside surface of the sphere with an open channel before and after soaking in SBF. EDS spectrum before soaking in SBF show that the sphere includes Cl with Ca. The sphere is suggested to adsorb both calcium and chlorine ions on the surface after soaking in calcium chloride solution. On the other hand, EDS spectrum after soaking in SBF shows that the deposits include Ca and P without Cl. The deposits are calcium phosphate crystals. It is suggested that, when the spheres were soaked in SBF, calcium chloride that had been adsorbed on their surface was dissolved in SBF and supersaturation of the calcium phosphate increased.

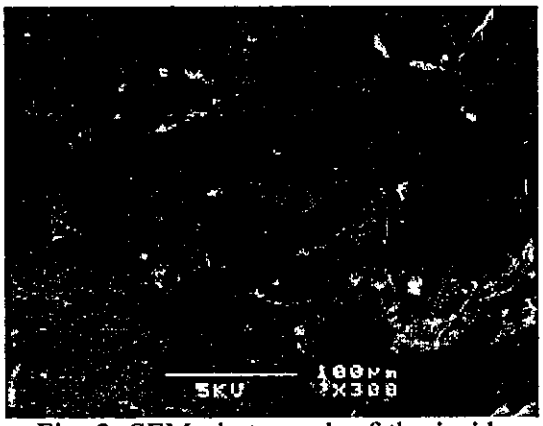

Fig. 2. SEM photograph of the inside surface of the hollow sphere with an open channel after 5 d of incubation of MC3T3-E1 cells.

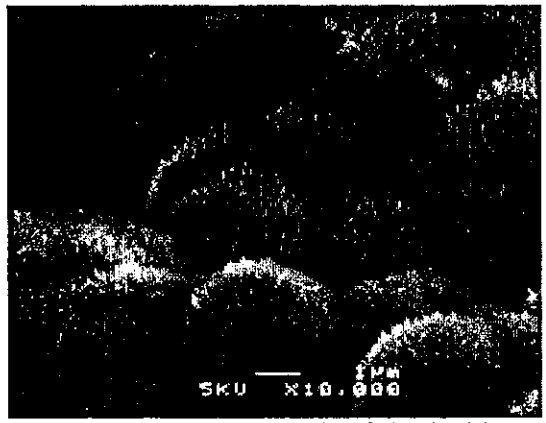

Fig. 3. SEM photograph of the inside surface of the hollow sphere with an open channel after soaking in SBF.

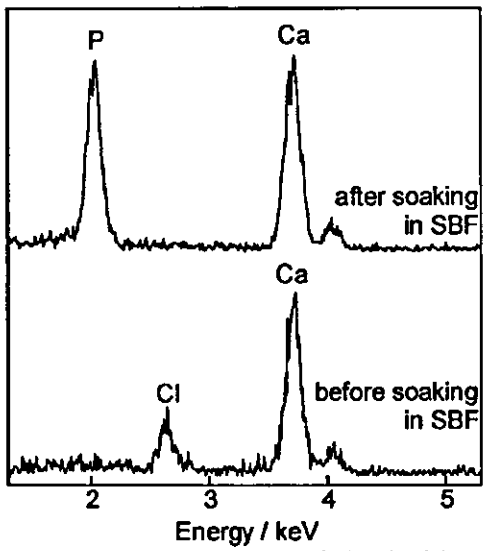

Fig. 4. EDS spectra of the inside surface of the hollow sphere with an open channel before and after soaking in SBF.

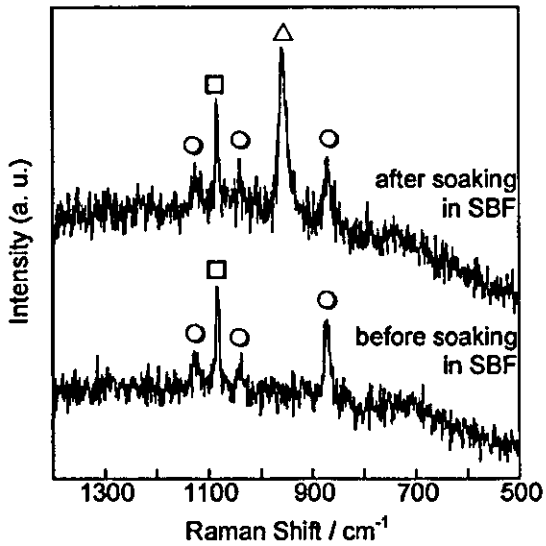

Fig. 5. Laser Raman spectra of the inside surface of the hollow sphere with an open channel before and after soaking in SBF. (□) CO_3^{2-}, (○) PLA and (△) PO_4^{3-}.

Figure 5 shows laser Raman spectra of the inside surface of the sphere with an open channel before and after soaking in SBF. In a laser Raman spectrum after soaking in SBF, a peak at ~960 cm^{-1} due to PO_4^{3-} is seen. The newly formed deposits on the surface are b-HA, judged from the morphology and the Raman spectrum. The inside and outside surface coated with b-HA of the sphere are expected to enhance cell-compatibility.

Summary

Poly(lactic acid) composite hollow spheres with an open channel was prepared. A sphere with an open channel was ~800 μm in diameter. An *in vitro* evaluation using osteoblast-like cells showed that the open channel size is enough to allow the migration of cells into the interior. The inside and outside surface of the sphere was coated with b-HA by soaking in calcium chloride solution and subsequently in SBF. The hollow spheres coated with b-HA having an open channel may be one of the great potential candidates as novel bone fillers incorporating cells.

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