Bonelike Apatite Coating on Skeleton of Poly(lactic acid) Composite Sponge

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A novel sponge, coated with bonelike apatite (b-HA) on its skeleton surface, was prepared using a particle-leaching technique combined with a biomimetic processing. A powder mixture consisting of calcium carbonate/poly(lactic acid) composite (CCPC) and sucrose was hot-pressed and then the resulting compact was soaked in the simulated body fluid at 37 °C. Within the first hour, the sucrose was completely dissolved out, resulting in the formation of large-sized pores in the compact, and subsequently, after 3 hours of soaking, b-HA formed on the skeleton consisting of CCPC. On the other hand, on a pore-free CCPC, the apatite started to form after 12~18 hours. The induction period for b-HA formation on the skeleton of the CCPC sponge prepared using a particle-leaching technique is significantly shorter than that of the pore-free CCPC. The short period is suggested to originate from that a large amount of Ca²⁺ ion is rapidly supplied into the compartment space ( pore) from the CCPC skeleton. The formed sponge has numerous, large pores of 450~580 μm in diameter, which are connected with channels having a diameter in the range of 70~120 μm, as well as a high porosity of 75%. Animal test using rats showed that the sponge has osteoconduction. The sponge is expected to be one of the promising candidates for osteoconducting fillers or tissue-engineering scaffolds.

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1. Introduction

Engineering living tissue for reconstructive surgery requires an appropriate cell source, optimal culture conditions, and a biodegradable scaffold as the basic elements. A scaffolding material is used either to induce the formation of bone from the surrounding tissue or to act as a carrier or template for implanted bone cells or other agents. To serve as a scaffold, the material must be biocompatible, osteoconductive, and have a macroporous structure. Calcium phosphate ceramics such as hydroxyapatite or tricalcium phosphate, which have a osteoconductivity, were reported to be applied to scaffolds for bone tissue engineering.1-3)

Recently, much attention has been paid to bonelike hydroxycarbonate apatite (b-HA) as a novel biomaterial, since b-HA is very similar to the apatite in terms of living bone in its chemical composition and structure4) and shows effective compatibility in cell attachment, proliferation, and differentiation on the material,5) i.e., osteoconductivity, as well as good bioresorbability.6) We expect that the sponges composed of b-HA skeleton can be applied to bone-fillers or scaffolds for tissue engineering. However, in general, ceramics have brittleness and low resistance against an impact loading. Such ceramic sponge materials have a serious risk to be broken in normal handling during operations. To eliminate this risk, it has been reported that the composites were fabricated using the polymer sponge coated with bioactive materials such as hydroxyapatite or Bioglass.7,8)

We reported earlier that a compact of calcium carbonate (vaterite)/poly(lactic acid) composite (CCPC) was reported to form b-HA on its surface even after 3 hours of soaking in SBF at 37 °C.9) The rapid formation of the b-HA was suggested to originate from the integration of PLA having carboxy groups bonded with Ca²⁺ ions for b-HA nucleation and a large amount of calcium carbonate (vaterite) having an ability to effectively increase the supersaturation of b-HA due to the fast dissolution of the nano-sized vaterite. We believe that various novel biomaterials can be prepared using CCPC.

In the present work we biomimetically prepared the novel sponge composed of PLACcomposite skeleton coated with b-HA utilizing CCPC for applications to osteoconducting, bioresorbable bone-fillers or tissue engineering scaffolds.

2. Experimental Procedure

We have already reported that the CCPC containing ~30% calcium carbonate has an excellent mechanical properties with a high HCA-forming ability in SBF.10) The composite containing 30% calcium carbonate shows bending strength of 40~55 MPa, Young’s modulus of 3.5~6 GPa, and significant ductility. In the present work the weight ratio of CaCO₃/PLA was determined to be 1/2.

Calcium carbonates consisting of vaterite were prepared by a carbonation process in methanol.11) CO₂ gas was blown for 3 hours 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, resulting in fine-sized powders. 2.0 g of PLA (with a molecular weight of 160±20 kDa, determined by gel permeation chromatography) was dissolved in 20 mL of methylene chloride at room temperature. The calcium carbonate powders were added to the PLA solution and then the mixture was stirred to prepare a PLA slurry including the calcium carbonate powders.

The sponge was prepared using a conventional particle-leaching technique. In the present work sucrose was used as a sacrificial phase. Sucrose particles, which were sieved with the opening from 0.5~1.0 mm, were added to the PLA slurry. The nominal weight ratio of CCPC/sucrose was 1/6. The slurry mixture was stirred and then cast into a stainless steel die, and subsequently dried in air for solidification. After that, the product in the die was heated at 180 °C and uniaxially hot-pressed at the temperature under a pressure of 40 MPa to

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prepare a CCPC/sucrose composite (denoted by sample A). After the hot-pressing, the specimen was cut in methanol with a diamond saw.

The hot-pressed sample was soaked in 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$_2$OH)$_2$CNH$_2$ and 45.0 mM of HCl at pH 7.4 at 37°C. After soaking, the sample was removed from SBF, gently washed with distilled water, and dried at room temperature. Our strategy for the preparation of the sponge composed of CCPC skeleton coated with b-HA is to leach out the sucrose phase and simultaneously to form b-HA on the composite skeleton.

The crystalline phases in the sponge were identified by X-ray diffraction analysis (XRD). The morphology of the sponge was observed by scanning electron microscopy (SEM). The pore size distribution of the sponge was measured by mercury porosimetry. The compressive strength of the sponge (7 mm × 7 mm × 10 mm) prepared by soaking in SBF for 3 days was estimated by a compressing test at a loading rate of 1 mm/min. Concentrations of Ca$^{2+}$ and P$^{5+}$ ions after soaking sample (10 mm × 10 mm × 5 mm) in SBF were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). A compact of CCPC containing no sucrose powders (denoted by sample B), with the composition of CaCO$_3$/PLA = 1/2 in weight ratio, was used as a control material to compare with sample A (sponge).

The sponge (2-mm diameter × 10-mm thickness) prepared by soaking in SBF for 3 days was implanted into a femur of a 12-week-old Wister rat (male, weight; 265~285 g). The sponge was harvested and used for histological analysis at 4 weeks after implantation. The specimen was fixed, decalcified, embedded in paraffin, and stained with hematoxylin and eosin (HE). The specimen was observed with optical microscope.

3. Results & Discussion

Figure 1 shows XRD patterns before and after soaking the compact of CCPC/sucrose mixture in SBF. The XRD pattern before soaking shows that the hot-pressed compact consists of crystalline phases such as PLA, vaterite, aragonite and sucrose. After 1 hour of soaking, the peaks corresponding to sucrose and vaterite disappear; those to PLA and aragonite are seen. Sucrose was found to be completely dissolved even after dissolution of the sucrose particles, the thicknesses of b-HA, is seen with the peaks due to PLA and aragonite.

Figure 2 shows the SEM photographs of the sample after 3 days of soaking in SBF. The SEM photograph shows that the sponge has numerous, large pores of 450~580 μm in diameter and large interconnected channels of 70~120 μm. The sponge has continuous open foams with a 3D interpenetrating network of struts and pores. As shown in Fig. 2(b), the surface of the CCPC skeleton is covered with the numerous deposits, that is b-HA, judged from the XRD pattern and the morphology. Further experiments showed that apatite formation starts to occur after 3 hours of soaking.

Figure 3 shows the pore size distribution of the sponge prepared by soaking in SBF for 3 days. The median pore size of the sponge is 125 μm. There exist almost no pores below several tens micrometers in diameter. When a compact of the powder-mixture consisting of CCPC and sucrose was hot-pressed at 180°C, the sucrose particles melted (it begins to melt at 160°C), leading to adjacent particles connected each other. As a result, both the sucrose and CCPC phases were unified into an interconnecting three-dimensional network. The porosity was estimated from the measurement to be ~75%. The macroporous structure of the sponge prepared using sucrose may be likely to allow the migration of cells into the interior of the sponge. On the other hand, for comparison, when the sponge was prepared using sodium chloride, which were sieved with the opening from 0.5 to 1.0 mm, the median pore size in the sponge was estimated to be 65 μm. There exist numerous pores several tens micrometers in diameter. Since no sodium chloride melts during hot-pressed at 180°C, large-sized, interconnected particles as the sacrificial phase would not form.

In order to investigate apatite formation on CCPC in SBF after dissolution of the sucrose particles, the thicknesses of b-HA particles formed in SBF on the skeleton of sample A (the sponge derived from the compact of CCPC/sucrose mixture) and sample B were measured from cross-sectional SEM observation of their fracture face. The skeleton surface of sample A (sponge) was covered with numerous b-HA deposits after 3 hours of soaking (Fig. 2(b)). The b-HA formation did not occur within 2 hours. On the other hand, the surface of sample B was completely covered with b-HA after 18 hours of soaking. No b-HA formed on sample B within 12 hours. Figure 4 shows the thicknesses of b-HA layers formed on the skeleton surface of sample A or the surface of sample B as a function of the soaking time in SBF. The thickness of
b-HA particles on these substrates was measured using at least five points in SEM micrographs of their fracture faces and the mean thickness of b-HA particles and the standard deviation were shown. The induction period for b-HA formation on sample A is shorter than that of sample B. However, the crystal growth rate on sample B is higher than that on sample A.

When the sample A is soaked in SBF, the surface area of CCPC increases after the rapid dissolution of sucrose particles. This is suggested to be increased in the adsorption amount of Ca$^{2+}$ and P$^{5+}$ ions in SBF on the sponge skeleton. Figure 5 shows the amount of Ca$^{2+}$ and P$^{5+}$ ions after soaking sample A or sample B in SBF for various periods. When sample A is soaked, the Ca$^{2+}$ ion amount in SBF increases slightly at the initial stage, and subsequently decreases gradually. It is proposed that the amount of the dissoluble vaterite particles, which come into contact with water, increases after dissolution of sucrose, resulting in the increase in the amount of Ca$^{2+}$ ion released in SBF. On the other hand, after sample B is soaked in SBF, the Ca$^{2+}$ ion amount decreases gradually. The P$^{5+}$ ion amount in SBF...
decreases gradually after soaking sample A or B. The phosphorous element in SBF is suggested to adsorb on CCPC as a phosphate ion.

The reduction of the induction period for b-HA formation on the CCPC sponge is suggested to be influenced by the porous structure. When the sample A is soaked in SBF, the surface area of CCPC increases due to the dissolution of the sucrose particles. As a result, a large amount of vaterite in the CCPC sponge skeleton rapidly dissolved. The pores in the sponge are considered as compartmental spaces with channels. In comparison with the surface of sample B, the supersaturation concerning b-HA around the surface of pores in the sponge (sample A) is suggested to increase rapidly. As a result, the induction period for b-HA formation on sample A is reduced. Figure 2(a) shows that the thickness of the sponge skeleton is in a range of 50~100μm. Almost all of vaterite is dissolved within 1 hour after soaking (Fig. 1); almost no Ca$^{2+}$ ions can be newly released from CCPC since 1 hour of soaking. As a result, the crystal growth rate on the sponge skeleton (sample A) may be suppressed. The b-HA growth rate may be also attributed to the surface area. The amount of carboxy group, which is known to induce b-HA nucleation, increases with increase in surface area of the PLA portion due to the dissolution of sucrose and vaterite during the preparation of the sponge. The adsorption amount of the phosphate ion per unit surface area on the sponge skeleton may be smaller than that on the surface of sample B. As a result, the b-HA growth rate would be strictly suppressed.

Figure 6 shows a typical stress-strain curve measured by a compressive tester for the sponge prepared by soaking in SBF for 3 days. The sponge shows the compressive strength of ~1.5 MPa and the maximum strain for the fracture of the sponge is over 10%, which is two orders of magnitude larger than that of a dense hydroxyapatite ceramic. The curve also shows that the fracture proceeds gradually beyond the maximum stress; the sponge leads to ductile fracture. The sponges in the present work are not broken in normal handling during operations.

Figure 7 shows the decalcified sections stained with HE at 4 weeks postimplantation; newly bone formed in the pore. This figure shows that newly formed bone continues to be present in direct contact with the sponge without intervening soft tissues. No adverse tissue response and inflammation reaction were seen around the implant. The present osteoconducting sponge is also expected to have bioreosorbability. Further histological investigation is in progress.

4. Conclusion

Coating with b-HA on CCPC sponge skeleton surface can be easily prepared in a short time utilizing a sucrose-particle-leaching technique combined with a biomimetic processing. The induction period for b-HA formation on the CCPC sponge skeleton is significantly shorter than that of a CCPC compact without sucrose. The sponge prepared using sucrose has numerous, large pores of 450~580μm in diameter, which are connected with channels having a diameter in the range of 70~120μm, as well as a high porosity of 75%. The sponge is expected to allow the migration of cells into the interior of the sponge. It is not broken in normal handling during operations. Animal test using rats showed that the present material has a osteoconductivity. The sponge is one of the great potential candidates as bone-fillers or scaffolds for tissue engineering.

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REFERENCES

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