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Preparation of a PLGA-collagen-hydroxyapatite Composite Sponge

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A novel composite sponge of poly(DL-lactic-co-glycolic acid)(PLGA), collagen and hydroxyapatite was prepared by introduction of collagen microsponges into pores of PLGA sponge and deposition of hydroxyapatite microparticles on the collagen microsponges. At first, a porous PLGA sponge with an open cellular morphology was prepared by the salt-leaching method. Subsequently, the PLGA sponge was soaked in collagen solution and the collagen solution-containing PLGA sponge was freeze-dried and cross-linked by glutaraldehyde to form a PLGA-collagen sponge nested with collagen microsponges. Finally, the PLGA-collagen sponge was alternately immersed in $CaCl_2$ and Na_2HPO_4 solutions to deposit hydroxyapatite microparticles on the collagen microsponges. The formation of collagen microsponges in the pores of PLGA sponge and the deposition of hydroxyapatite microparticles were confirmed by SEM observation. The deposited microparticles were flake-like and grew big with the increase of alternate immersion cycle. EDS analysis showed that the main elements of the deposited microparticles were calcium and phosphorus. The calcium to phosphorus molar ratio increased from the initial 1.14 to 1.63 after 4 cycles of alternate immersion, which suggests that the deposited microparticles were apatite-like after 4 cycles immersion.

Keywords: PLGA, collagen, hydroxyapatite, three-dimensional scaffold, biodegradable.

1. INTRODUCTION

Tissue engineering can serve as a promising alternative approach for bone repair (1-4). In this approach, a temporary scaffold is needed to serve as an adhesive substrate for the implanted cells and a physical support to guide the formation of the new organ. The scaffold should be biocompatible, biodegradable, highly porous with a large surface/volume ratio, mechanically strong, and malleable into desired shapes. Aliphatic polyesters such as poly(DL-lactic-co-glycolic acid) (PLGA) are biocompatible, degrading to products that can be eliminated by metabolism or renal excretion. Their degradation periods can also be manipulated by controlling the crystallinity, molecular weight, and copolymer ratio of lactic acid to glycolic acid (5, 6). They are easily processed into desired shapes.

In addition to these requirements, scaffolds for tissue engineering of bone should be osteoconductive so that osteoblasts attach and migrate on them. Collagen and hydroxyapatite are two of the most potential biomaterials to construct such kinds of scaffolds, since they are the two primary compositions of bone. And various hydroxyapatite-collagen composites have been developed for bone substitutes (7-9).

In this study, a novel three-dimensional biodegradable porous composite sponge for tissue engineering of bone was prepared by nesting collagen microsponges in pores of PLGA sponge and depositing hydroxyapatite microparticles on the surfaces of collagen microsponges. PLGA sponge was used as a skeleton to facilitate formation of the composite sponge into desired shapes, while hydroxyapatite and collagen should contribute good osteoconductivity.

2. MATERIALS AND METHODS

2.1. Preparation of PLGA sponge

The PLGA sponge was prepared by a particulate-leaching technique using sieved sodium chloride particulates (10). Briefly, sieved NaCl particulates (9.0 g), ranging in diameter from 355 to 425 um, were added to a PLGA solution (5 mL) in chloroform at a concentration of 20 (w/v)%. The dispersion was vortexed and poured into an aluminum pan. The chloroform was allowed to evaporate by air-drying in a draft for 24 hours and followed by 24 hours of vacuum drying. The PLGA/NaCl composite was detached from the aluminum pan, and NaCl was leached out by washing the composite with deionized water until the weight of the dried sponge remained unchanged. Finally, the PLGA sponge was formed after drying.

2.2. Preparation of PLGA-collagen composite sponge

The composite sponge of PLGA and collagen was prepared by nesting collagen microsponges in the pores of the PLGA sponge (11). The PLGA sponge was immersed in type I collagen acidic solutions (pH 3.2, 0.5 wt %) under a vacuum so that the sponge pores filled with collagen solution. The collagen solution-containing PLGA sponge was then frozen at -80 °C for 12 hours, and freeze-dried under a vacuum of 0.2 Torr for an additional 24 hours to allow the formation of collagen microsponges in the sponge pores. The collagen microsponges were further cross-linked by treatment with glutaraldehyde vapor saturated with 25% glutaraldehyde aqueous solution at 37 °C for 4 hours. After washing with deionized water and freeze-drying, the PLGA-collagen hybrid sponge was fabricated.

2.3. Preparation of PLGA-collagen-hydroxyapatite composite sponge

The composite sponge of PLGA, collagen and hydroxyapatite was prepared by depositing hydroxyapatite microparticles on the surfaces of collagen microsponges of the PLGA-collagen sponge. Deposition of hydroxyapatite microparticles was carried out by alternate immersion of PLGA-collagen sponge in $CaCl_2$ and Na_2HPO_4 solutions (12, 13). The PLGA-collagen sponge was first immersed in a 100mM $CaCl_2$ solution in Tris buffer (7.4) at 37 °C for 12 h. After removing from the solution, the sponge was centrifuged at 600 rpm. Subsequently, it was immersed in a 100mM Na_2HPO_4 solution in Tris buffer (7.4) at 37 °C for another 12 h. It was centrifuged at 600 rpm after removing from the Na_2HPO_4 solution (1 cycle). The alternate immersion was continued to 8 cycles. The PLGA-collagen-hydroxyapatite composite sponges after each cycle of alternate immersion were completely washed with distilled water and freeze-dried.

2.4. SEM, SEM-EPMA observation and EDS analysis

The structures of PLGA, PLGA-collagen, PLGA-collagen-hydroxyapatite sponges were observed by scanning electron microscopy (SEM) and SEM-electron probe microanalyser (SEM-EPMA). The sponges were cut with a razor blade. The cross-sections were coated with gold using a sputter coater (Sanyu Denshi Co., Tokyo, Japan). The gas pressure was set at 50 mtorr and the current was 5 mA for a coating time of 180 s. The samples were observed with a scanning electron microscope (JSM-6400Fs; JEOL, Ltd., Tokyo, Japan) operated at 3 kV voltage. For SEM-EPMA (SX100; Cameca S.A., Courbevoie Cedex, France) and energy dispersive spectroscopy (EDS) analysis, the sponges were coated with carbon to a depth of 200 Å using a vacuum evaporator (JEE-4C; JEOL, Ltd.). The distribution of elemental nitrogen in the PLGA-collagen sponge was analyzed by SEM-EPMA. The deposited hydroxyapatite microparticles on collagen microsponges were characterized with EDS.

3. RESULTS and DISCUSSION

SEM observation of the PLGA sponge demonstrated that it had a uniformly distributed an

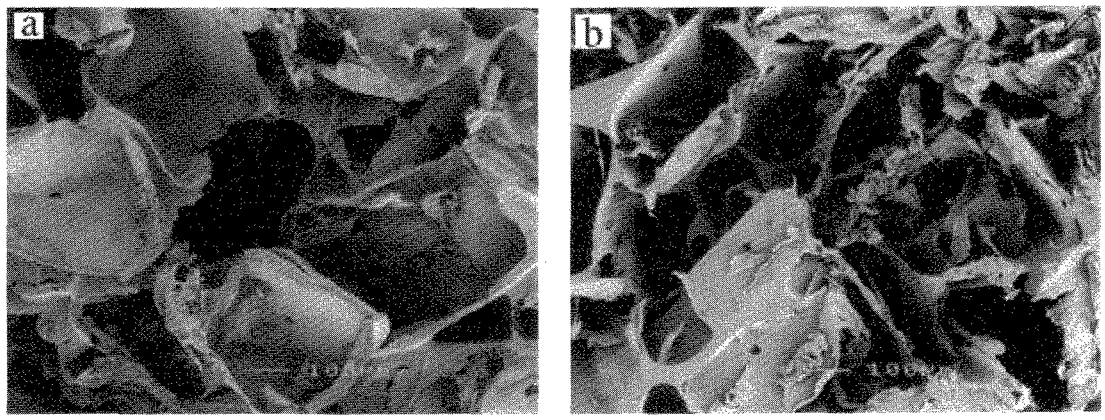

Figure 1. SEM photomicrographs of cross sections of PLGA sponge (a) and PLGA-collagen sponge (b) at original magnification×100.

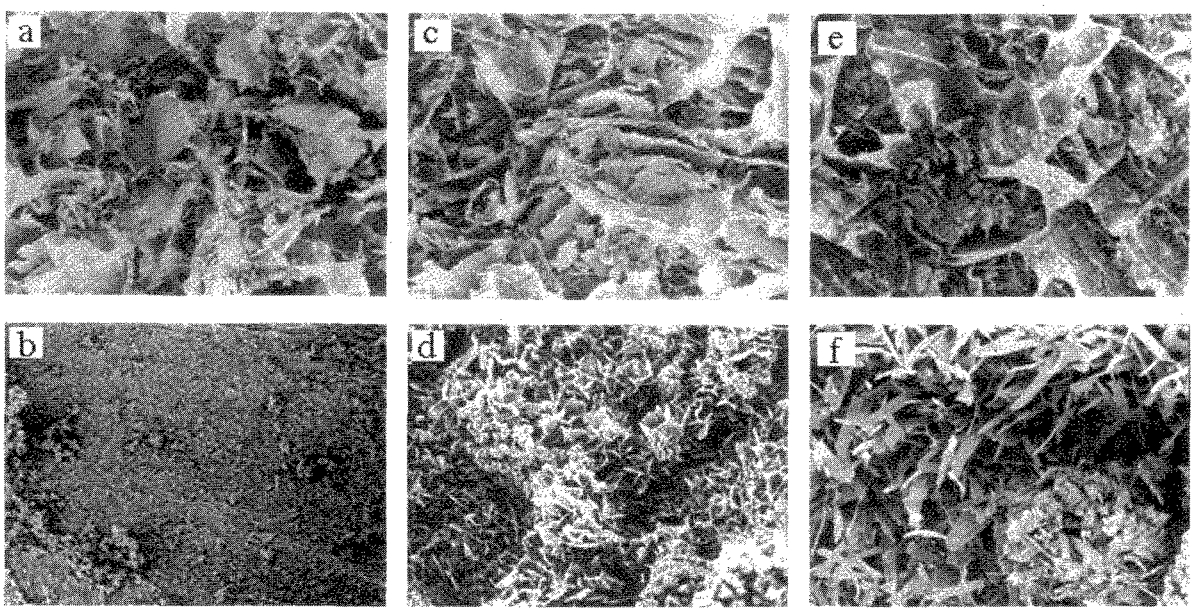

Figure 2. SEM photomicrographs of cross sections of PLGA-collagen-hydroxyapatite composite sponge after 1 cycle of alternate immersion at original magnification×100 (a) and ×5000 (b), 4 cycles at original magnification×100 (c) and ×5000 (d), and 6 cycles at original magnification×100 (e) and ×5000 (f).

interconnected pore structure (Fig.1a). The pore size was equal to the size of the NaCl particulates used. The porosity was approximately equal to the initial sodium chloride weight fraction. Microsponges of collagen with interconnected pore structures were formed in the pores of the PLGA sponge (Fig.1b). The hybrid structure of the PLGA-collagen sponge was further confirmed by detecting elemental nitrogen, which exists in collagen but not in PLGA copolymer, with SEM-EPMA. Nitrogen element was detected in the microsponges of collagen and the pore surfaces of PLGA pores, but not in the cross-sections of PLGA regions. This indicates that microsponges of collagen were formed in the pores of PLGA sponge and that the pore surfaces were also coated with collagen. The porosity decreased slightly after the introduction of collagen microsponges.

Hydroxyapatite microparticles were uniformly deposited on the collagen microsponges by alternate immersion of PLGA-collagen sponge in $CaCl_2$ and Na_2HPO_4 solutions (Fig. 2). The deposited

microparticles were flake-like and grew big with the increase of immersion cycle. The weight of deposited hydroxyapatite microparticles increased with the alternate immersion cycle. EDS analysis showed that the main elements of the deposited microparticles were calcium and phosphorus. The calcium to phosphorus molar ratio was 1.14 after 1 cycle immersion and increased to 1.63 after 4 cycles immersion. This result suggests that the deposited microparticles were apatite-like after 4 cycles of alternate immersion.

Three-dimensional biodegradable porous scaffolds for tissue engineering of bone should not only maintain the desired shapes while reserving sufficient free space for the formation of new tissue via cell proliferation and extracellular matrix secretion, but also be osteoconductive to stimulate the formation of new organ. Use of the PLGA sponge as a skeleton facilitated easy formation of the composite sponge into the desired shapes. The collagen-hydroxyapatite composite nested in the pores of PLGA sponge would contribute the PLGA-collagen-hydroxyapatite composite sponge good osteoconductivity. The composite sponge could serve as a promising three-dimensional biodegradable porous scaffold for tissue engineering of hard tissues.

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