Fabrication of PLGA-Collagen Hybrid Sponge

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A hybrid sponge of biodegradable poly(DL-lactic-co-glycolic acid) (PLGA) and collagen was fabricated by forming microsponges of collagen in the pores of PLGA sponge. Observation of the hybrid sponge by scanning electron microscopy (SEM) showed that microsponges of collagen with interconnected pore structures were formed. The hybrid structure was further confirmed by detecting elemental nitrogen using scanning electron microscopy-electron probe microanalysis (SEM-EPMA). Elemental nitrogen was detected in the microsponges of collagen and on the pore surfaces of PLGA, but not in the cross-sections of PLGA regions. These results indicate that microsponges of collagen were formed in the pores of the PLGA sponge and that the pore surfaces were also coated with collagen.

Scaffolds made from biodegradable materials play an important role in tissue engineering as the temporary scaffold for transplanted cells.[1-4] They are usually fabricated as porous structures to provide adequate space for cell seeding.[5,6] Transplanted cells adhere to the scaffold, proliferate, secrete their own extracellular matrices (ECM) and stimulate new tissue formation. During this process the scaffold gradually degrades and is eventually eliminated. The generally used biodegradable materials include naturally derived collagen and synthetic polyglycolic acid (PGA), poly(L-lactic acid) (PLLA), and their copolymer of poly(DL-lactic-co-glycolic acid) (PLGA).[7] Collagen has the potential advantage of specific cell interactions, but offers limited versatility in designing a scaffold with specific physical properties such as mechanical strength. On the other hand, PGA, PLLA and PLGA-derived scaffolds lack cell-recognition signals, but provide macrostructure, mechanical properties and degradation time that can easily be controlled and manipulated. In the present study, a hybrid sponge of PLGA and collagen was fabricated by forming microsponges of collagen in the pores of the PLGA sponge. The hybrid structure was confirmed by detecting elemental nitrogen with scanning electron microscopy-electron probe microanalysis (SEM-EPMA).

A PLGA sponge was prepared by a particulate-leaching technique using sieved sodium chloride particles.[8] Briefly, sieved NaCl particles (9.0 g), ranging in diameter from 355 to 425 μm, were added to a PLGA (75:25) 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 h and followed by 24 h of vacuum drying. The PLGA/NaCl composite was detached from the aluminum pan, and NaCl was leached out by washing the composite with distilled water. The PLGA sponge was formed after the composite was dried.

The hybrid sponge of PLGA and collagen was fabricated as follows. The PLGA sponge was immersed in a type I collagen acidic solution (pH 3.2) under vacuum so that the sponge pores would be filled with collagen solution. The collagen solution-containing PLGA sponge was then frozen at -80 °C for 12 h, and lyophilized under a vacuum of 0.2 Torr for additional 24 h to form the hybrid sponge. The hybrid sponge was further cross-linked by treating it with glutaraldehyde vapor saturated with 25% glutaraldehyde aqueous solution at 37 °C for 4 h. Then, the hybrid sponge was washed with distilled water and lyophilized. The structures of the PLGA sponge and hybrid sponge were observed by scanning electron microscopy (SEM) and SEM-EPMA.

SEM photomicrograph (Figure 1(a)) shows that the PLGA sponge had uniformly distributed and interconnected pore structures and that the pore size was the same as those of the salt particles used. Then type I collagen acidic solution was induced into the pores of the PLGA sponge, frozen, lyophilized and cross-linked. Microsponges of collagen with interconnected pore structures were formed in the pores of the PLGA sponge, as shown in Figure 1(b). The preferable concentration of collagen solution was in the range from 0.1 to 1.5 (w/v)%. It was difficult to form collagen microsponges in the PLGA sponge pores when the concentration of collagen solution was lower than 0.1 (w/v)%. On the other hand, collagen solution higher than 1.5 (w/v)% could not infiltrate into the pores of the PLGA sponge because of its high viscosity.

The hybrid structure was further confirmed by detecting elemental nitrogen with SEM-EPMA (Figure 2). Nitrogen element was detected in the microsponges of collagen and the pore

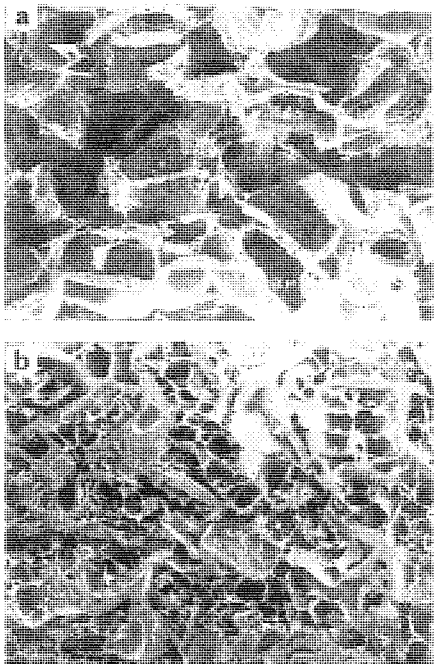

Figure 1. SEM photomicrographs of cross sections of PLGA sponge (a) and PLGA-collagen hybrid sponge prepared from PLGA sponge and 1.0% type I collagen acidic solution (b).

surfaces of PLGA, but not detected in the cross-sections of PLGA regions. This result indicates that microsponges of collagen were formed in the pores of the PLGA sponge and that the pore surfaces were also coated with collagen.

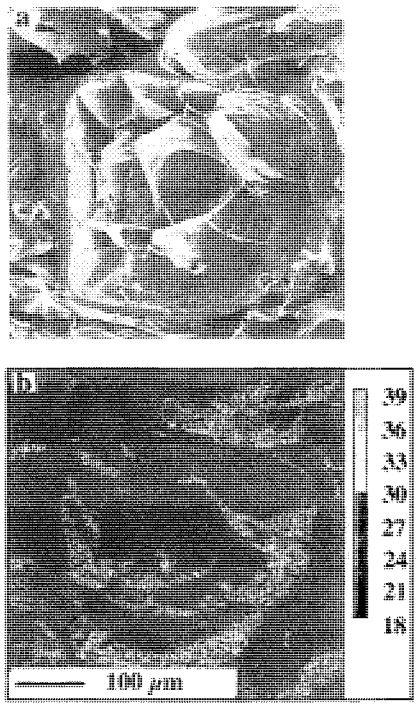

Figure 2. SEM (a) and SEM-EPMA (b) photomicrographs of cross section of PLGA-collagen hybrid sponge as shown in Figure 1(b).

Mouse fibroblast L929 cells were seeded in the hybrid sponge and cultured in Dulbecco's modified Eagle's medium containing 10% (v/v) fetal bovine serum under 5% CO_2 atmosphere at 37 °C. The cells cultured for 1 and 5 days were observed by SEM. L929 cells readily adhered to the microsponges of collagen in the hybrid sponge, spread and proliferated to cover the surfaces of collagen microsponges. These results suggest good biocompatibility of the hybrid sponge. Besides good biocompatibility, formation of collagen microsponges in the pores of the PLGA sponge also increased the surface area of the hybrid sponge for cell attachment. Coating PLLA sponge with collagen or embedding parallel collagen fibers within a PLA matrix has been used to improve the biocompatibility or mechanical properties of these scaffolds.[9,10] However, their surface area/volume ratios remained unchanged.

In summary, a hybrid sponge of biodegradable PLGA and collagen was fabricated by forming microsponges of collagen in the pores of the PLGA sponge. The hybrid structure was confirmed by SEM-EPMA observation. PLGA sponge as a skeleton facilitated formation of the hybrid sponge into designed shapes with high strength, while collagen microsponges gave it good biocompatibility and high surface area/volume ratio. The hybrid sponge could serve as an innovative 3-dimensional biomaterial for tissue engineering.

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