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A Biodegradable Composite of PLGA Mesh Sandwiched in Salmon Collagen Sponge

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A biodegradable composite of PLGA mesh and salmon collagen sponge sheet was prepared by embedding PLGA mesh in salmon collagen sponge sheet. SEM observation showed that collagen sponge with interconnected micro-pore structures was formed in the interstices of the PLGA mesh. The PLGA mesh and the collagen sponge sheet were alternately chained. The thickness of the salmon collagen sheet could be manipulated. The composite mesh showed high mechanical strength. Human skin fibroblasts adhered more and therefore proliferated more quickly on PLGA-collagen composite mesh than on PLGA mesh. The fibroblasts spread well on the PLGA-collagen composite mesh and proliferated to become completely connected in layer structure after cultured for 14 days. The composite mesh could serve as an effective biomaterials for wound dressing.

Keywords: biodegradable, composite, PLGA mesh, salmon collagen sponge, dressing.

1. INTRODUCTION

The meshes of aliphatic polyesters like poly(lactic acid) (PLA) and poly(DL-lactic-co-glycolic acid) (PLGA), and collagen sponge sheet are always used as temporary scaffolds for tissue engineering of skin, tendon, ligament, etc. (1-7). PLA and PLGA meshes are mechanical strong, but their hydrophobic properties and the mesh interstices hinder successful cell seeding. On the other hand, collagen has the potential advantages of specific cell interactions and hydrophilicity, and collagen sponges have micro-pore structures, which facilitate cell seeding. However, collagen offers limited versatility in designing a scaffold with specific physical properties such as mechanical strength (8).

In this study, we prepared a new kind of biodegradable composite of PLGA mesh sandwiched in collagen sponge sheet by hybridization of PLGA mesh with salmon collagen sponge sheet. The hybrid structure was confirmed by SEM observation. Fibroblasts from human skin were cultured in the PLGA-collagen composite mesh.

2. MATERIALS AND METHODS

2.1. Preparation of PLGA-collagen composite mesh

The PLGA-collagen composite mesh was prepared by embedding a PLGA mesh in salmon collagen sponge sheet. A Vicryl knitted mesh of polylactin 910 was immersed in a salmon collagen acidic solution (pH 3.2, 0.5 wt%), and then the solution was frozen at -80 °C and freeze-dried to allow the formation of collagen sponge sheet. The collagen sponge was further cross-linked by treatment with glutaraldehyde vapor saturated with 25% glutaraldehyde aqueous solution at 37 °C for 4 hours (9). After cross-linking, the sponge was treated with 0.1 M glycine aqueous solution to block non-reacted aldehyde groups. After washing with deionized water and freeze-drying, the PLGA-collagen composite mesh was formed.

2.2. SEM observation

The hybrid structure of PLGA-collagen composite mesh was observed by scanning electron microscopy (SEM). The meshes were cut with a razor blade. The cross-sections were coated with gold using a sputter coater. The gas pressure was set at 50 mtorr and the current was 5 mA for a coating time of 180 s. And then the cross-sections of the meshes were observed with a scanning electron microscope operated at 3 kV voltage.

2.3. Mechanical tests

20 mM HEPES buffer-soaked PLGA and PLGA-collagen composite meshes were used for static tensile mechanical tests (TMI UTM-10T; Toyo Baldwin Co., Ltd., Tokyo, Japan) to measure their mechanical strength in the direction parallel to the orientation of knits of the meshes. The mechanical strength of salmon collagen sponge sheet was also measured. Their Load-deformation curves were obtained from a chart recorder. Their static Young's moduli were determined from the load-deformation curves and the dimensions of each sample. The average values were calculated from 5 measurements.

2.4. Cell culture

Fibroblasts from human foreskin were subcultured in 106S medium supplemented with 2 (v/v)% fetal bovine serum (FBS), 10 ng/mL recombinant epidermal growth factor (rEGF) and 3 ng/mL recombinant fibroblast growth factor-basic (rb-FGF). The fibroblasts and medium were purchased from Kurabo Co. Ltd. (Osaka, Japan).

The PLGA and PLGA-collagen meshes were circularly cut into the same size as the wells of 24-well cell culture plate. After sterilized with ethylene oxide gas, they were placed in the wells of a 24-well plate. The subcultured fibroblasts were harvested using HEPES buffer containing 0.025 (w/v)% trypsin (2,000 units/g) and 0.01 (w/v)% ethylenediaminetetraacetic acid. After washing with the serum medium, the cells were suspended in the serum medium and seeded on the meshes (2×10^4 cells/piece). The cells were cultured in 106S serum medium under a 5% CO_2 atmosphere at 37℃. Medium was replaced every 2 days.

After culturing for various periods of 6 hours, 1, 3 and 5 days, the cells attached to PLGA or PLGA-collagen mesh were detached from the meshes by treated with HEPES buffer containing 0.025 (w/v)% trypsin (2,000 units/g) and 0.01 (w/v)% ethylenediaminetetraacetic acid and counted by a hemocytometer. For SEM observation, the cells cultured on the composite mesh for 5 or 14 days were fixed with 0.25% glutaraldehyde solution in PBS at room temperature for 1 h. After rinsing three times with PBS and once with deionized water, the samples were freeze-dried. After coated with gold using a sputter coater, the samples were observed by a scanning electron microscope operated at 3 kV voltage.

3. RESULTS and DISCUSSION

Fig. 1 shows the photographs of PLGA knitted mesh and its composite with salmon collagen sponge sheet. SEM observation showed that the collagen sponge with interconnected micro-pore structures was formed in the interstices of the PLGA mesh. The PLGA mesh and salmon collagen sponge sheet were alternately chained (Fig. 2). The thickness of the collagen sponge sheet surrounding PLGA mesh could be manipulated by adjusting the volume of the salmon collagen solution.

The mechanical strength of the composite mesh was much higher than that of collagen sponge sheet, and almost the same as that of the PLGA mesh (Fig. 3). The PLGA mesh serving as a skeleton reinforced the composite mesh.

Human skin fibroblasts were seeded onto the

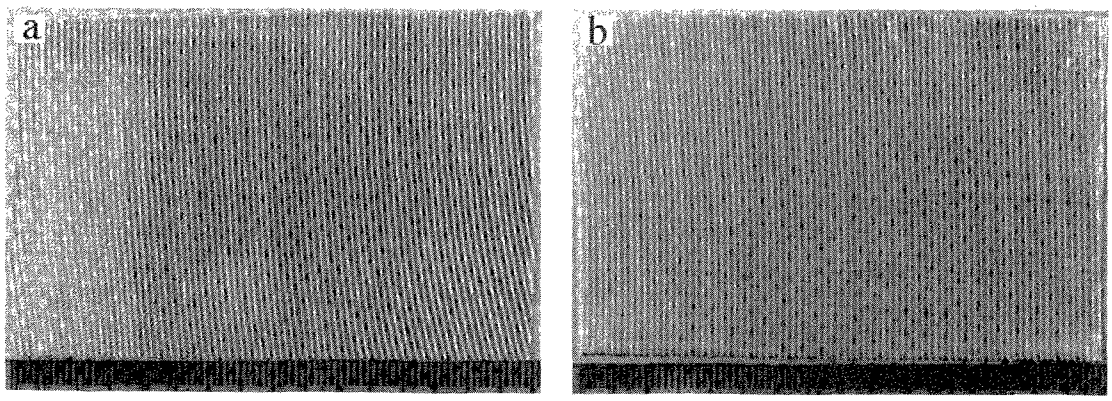

Figure.1 Photographs of PLGA mesh (a) and PLGA-collagen composite mesh (b)

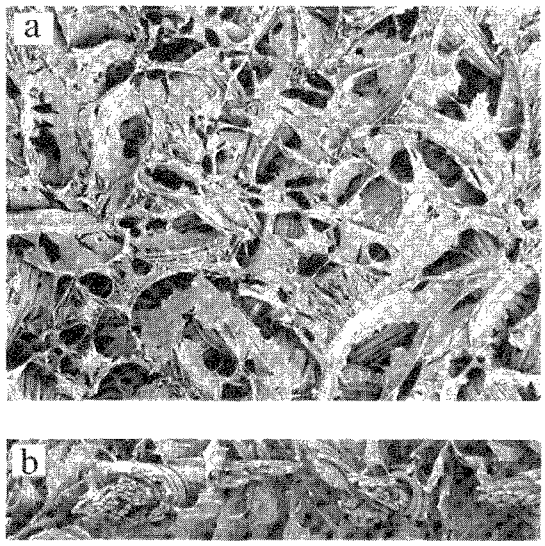

Figure 2. SEM photomicrographs of horizontal (a) and vertical (b) cross sections of PLGA-collagen composite mesh.

PLGA and PLGA-collagen meshes and cultured under a 5% CO_2 atmosphere at 37℃. The cells adhered more and therefore proliferated more quickly on PLGA-collagen composite mesh than on PLGA mesh (Fig. 4). The number of fibroblasts adhered on PLGA-collagen composite mesh after 6 hours culture was 9 times of that adhered on PLGA mesh. Hybridization with salmon collagen sponge facilitated seeding of fibroblasts onto the composite mesh. SEM photomicrographs show that fibroblasts adhered and spread well on the PLGA-collagen composite mesh after cultured for 5 days, and

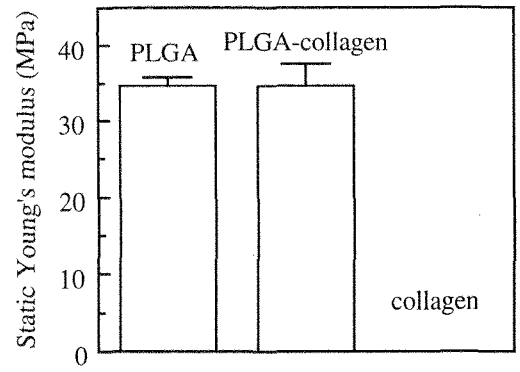

Figure 3. Static tensile strength of PLGA mesh collagen sponge sheet and PLGA-collage composite mesh.

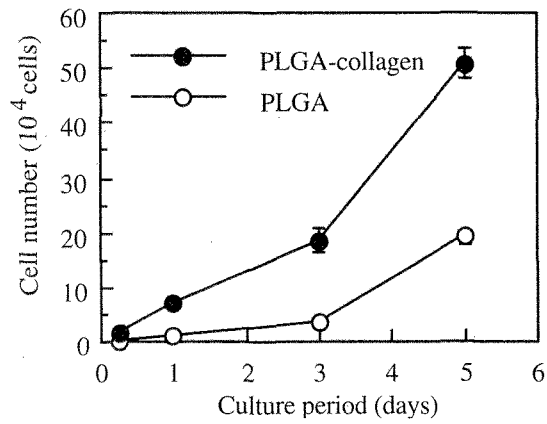

Figure 4. Proliferation of fibroblasts NHDF cell cultured on PLGA and PLGA-collagen meshes.

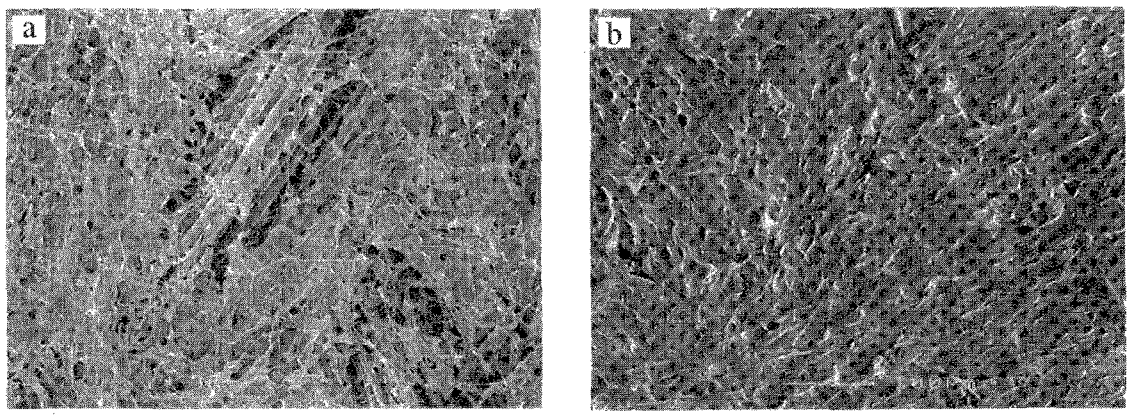

Figure 5. SEM photomicrographs of fibroblasts NHDF cells cultured on PLGA-collagen composite mesh fo 5 (a) and 14 days (b).

proliferated to become completely connected in layer structure after cultured for 14 days (Fig. 5). These results suggest that the PLGA-collagen composite mesh would be an effective biomaterials for wound dressing.

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