Fabrication of Non-woven Mats of Gelatin/Poly(L-lactic acid) Composites by Electrospinning and Their Application for Scaffold of Cell Proliferation

Young Sun Moon,[1] Hiroshi Uyama,*[1] Sachiko Inoue,[2] and Yasuhiko Tabata[2]
[1]*Department of Applied Chemistry, Graduate School of Engineering, Osaka University, Suita, Osaka 565-0871*
[2]*Department of Biomaterials, Field of Tissue Engineering, Institute for Frontier Medical Sciences, Kyoto University, 53 Kawara-cho Shogoin, Sakyo-ku, Kyoto 606-8507*

(Received March 7, 2006; CL-060275; E-mail: uyama@chem.eng.osaka-u.ac.jp)

Nanofiber non-woven mats of gelatin/poly(L-lactic acid) composites were fabricated by electrospinning. The mechanical strength of the composite mats was much larger than that of the gelatin nanofiber mat. Mesenchymal stem cells adhered and proliferated well on the present composite mats.

There has been much interest in electrospinning as a convenient and straightforward process to fabricate non-woven mats of ultrafine fibrous polymers.[1] The diameter of the electrospun fibers is often in the sub-micron range; in contrast, conventional polymer fibers are in the range of more than micron size in diameter. The small fiber diameter and non-woven morphology (apparent porous structure) give rise to large specific surface area. This is advantageous in various applications such as wound dressings, artificial blood vessels, matrices for drug delivery systems, high-performance filters and membranes, and reinforcements in composite materials.

Another characteristics of electrospinning is to conveniently fabricate fibers of polymeric composite materials; the selection of common solvents for more than two polymers as medium of the electrospinning will enable the production of the composite non-woven mats. So far, various composite nanofibers have been fabricated: silk/poly(ethylene glycol) (PEG),[2] polyurethane/PEG,[3] chitin/poly(glycolic acid),[4] chitosan/poly(vinyl alcohol),[5] collagen/chondroitin sulfate,[6] gelatin/poly(ε-caprolactone),[7] and so on.

Recently, the nanofiber non-woven mats have extensively been applied for scaffolds of tissue engineering,[8] since the nanoscaled non-woven fibrous structure is similar to that of native extracellular matrix. The proliferation of various cells such as smooth muscle cell, breast epithelial cell, osteoblastic cell, and endothelial cell has been examined on the electrospun mats of bioabsorbable polymers.

This study deals with fabrication of nanofiber non-woven mats of polymer composites consisting of bioabsorbable gelatin and poly(L-lactic acid) (PLLA) and their application for scaffold to proliferate mesenchymal stem cells (MSC). Gelatin is a good material to adhere and proliferate cells, but shows low mechanical properties. Cell adhesion for PLLA is sometimes not strong, although its mechanical strength is enough for biomaterial use. Thus, the combination of gelatin and PLLA will provide the composite materials with good cell adhesion and mechanical properties. The present composite nanofiber mats are fabricated from polymers approved as materials for human body in Japan. Biomaterials for use of human body are strictly limited by laws. Long term is generally required for approval of new materials to confirm biosafety in human body. Thus, use of approved biomaterials as scaffold is strongly demanded for tissue engineering in clinic.

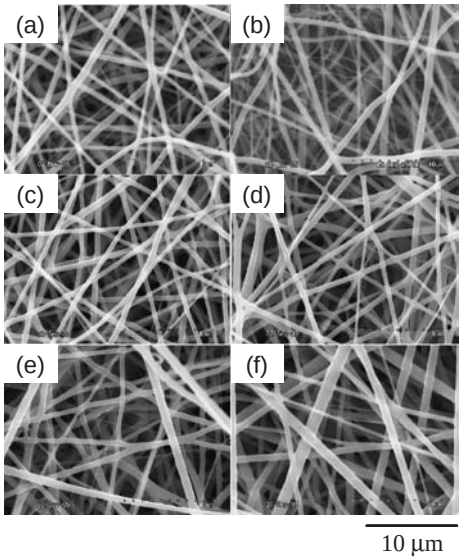

Figure 1. SEM photographs of electrospun gelatin/PLLA composite nanofibers with the gelatin/PLLA ratio of (a) 100/0, (b) 85/15, (c) 70/30, (d) 50/50, (e) 15/85, and (f) 0/100.

In this study, gelatin with an isoelectric point of 5.0 derived from bovine bone was used. The electrospinning of a mixture of gelatin and PLLA with the different mixing ratio was carried out in using 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) as solvent, a good solvent for both polymers.[9] The surface morphology of the resulting mats was observed by scanning electron microscope (SEM).

Figure 1 shows SEM images of the resulting fibers. As shown in Figure 1b–1e, the diameter of the composite fibers was around 600 ± 300 nm and did not strongly depend on the mixing ratio of gelatin and PLLA. The composite fibers were finer than the solo fibers of PLLA (500–1400 nm).

Figure 2 shows strain–stress curves of the non-woven mats of gelatin, PLLA, and their composites. Gelatin showed low mechanical properties: low elongation (7.5%) and low tensile stress at break (1.3 MPa). On the other hand, the elasticity was greatly improved by the addition of PLLA. The elongation of the 1:1 mixed mat was 46%. The composite mat of gelatin/PLLA = 15/85 showed good mechanical properties: elongation = 110%; tensile stress at break = 2.4 MPa, although these values were somewhat lower than those of the PLLA mat. These data clearly show that the electrospinning of gelatin and PLLA fabricated the composite nanofiber mats with good mechanical properties.

MSC, isolated from bone marrow of rats, was selected as

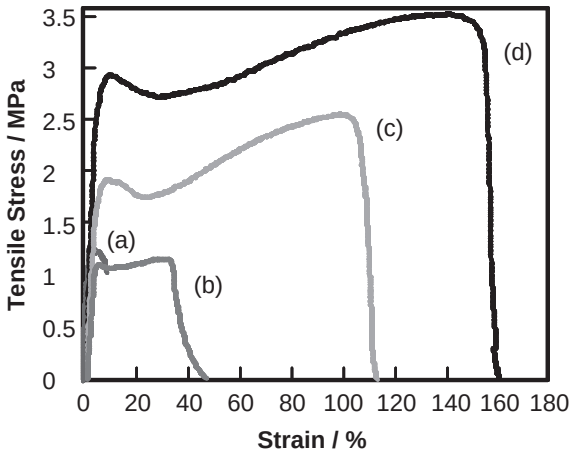

Figure 2. Strain–stress curves of electrospun gelatin/PLLA composite nanofibers with the gelatin/PLLA ratio of (a) 100/0, (b) 50/50, (c) 15/85, and (d) 0/100.

model substrate to examine adhesion and proliferation behaviors of cells on the present composite mats. Since MSC possesses an inherent potential for differentiation to various types of cell lineage, control of proliferation and differentiation of MSC on bioabsorbable scaffold is important for development of tissue engineering.[10]

MSC isolation and culture were carried out according to the literatures.[10,11] Thereafter, MSC was seeded and cultured on the present non-woven mat as well as the corresponding films for reference.[12] MSC well elongated and spread on the non-woven mats, and the cells attached exhibited flat morphology (Figure 3). When the film was used as scaffold, on the other hand, the cell adhesion was inferior to that on the non-woven mat and the shape of MSC attached was spherical. The cell number of MSC attached on the non-woven mat of the 85% gelatin composite, determined by DNA assay,[11] was larger than that on the film; the cell numbers cultured on the mat and film for 5 days were 4.6×10^5 and 2.3×10^5/scaffold, respectively. These data indicate that the nano-scale fibrous structure of the non-woven mats is suitable for proliferation of MSC.

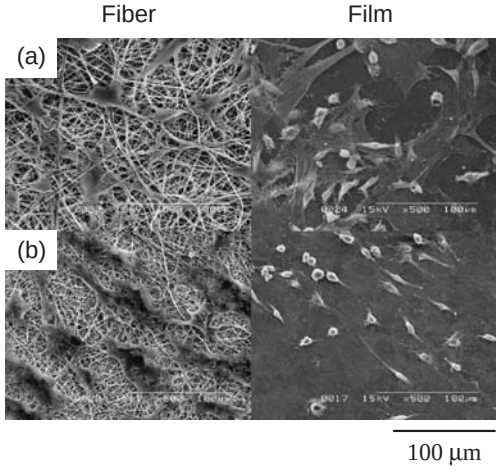

Figure 3. SEM photographs of MSC cultured for 2 weeks on electrospun gelatin/PLLA nanofibers and films with the gelatin/PLLA ratio of (a) 85/15 and (b) 15/85.

In conclusion, the nanofibrous non-woven mats of gelatin/PLLA composites were successfully fabricated by the electrospinning using HFIP as solvent. The mechanical strength of the composite mats was much superior to that of the gelatin mat. MSC proliferated more effectively on the composite nanofiber mats than the corresponding films. The present nanofibers are composed of biomaterials approved for human body in Japan; and hence, their non-woven mats have large potential for scaffold of tissue engineering in clinic. Further investigations including differentiation of MSC on the present composite mats are under way in our laboratories.

References and Notes

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9 A HFIP solution of gelatin/PLLA was placed in a 10 mL of glass syringe with a 18 gauge needle, which was connected to a high voltage generator. The glass syringe was horizontally mounted in a syringe pump. The flow rate of the delivery system and the distance between the needle tip and the drum collector were fixed as 3 mL/min and 10 cm, respectively. A grounded rotating metal drum served as a counter electrode. A voltage of 15 kV was applied to the solution and the jet emerging from the solution was collected on the drum. The morphology of the obtained non-woven mat was observed by SEM.
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12 The electrospun nanofibers and films of gelatin/PLLA composites, which crosslinked at 140 °C for 48 h and cut into circle of 1.4 cm diameters, were placed on the bottom of a 24-well plate dish. Then, they were sterilized with 70% ethanol, followed by washing with PBS. MSC was seeded (2×10^4 cells/well) and cultured in αMEM +15% FCS at 37 °C in 5% CO_2 with the medium change of every 3 days.

Published on the web (Advance View) April 25, 2006; doi:10.1246/cl.2006.564